14 9 152
1749
1-21
Library of the Museum
OF
COMPARATIVE ZOÖLOGY,
AT HARVARD COLLEGE, CAMBRIDGE, MASS.
Founded by private subscription, in 1861.
Deposited by ALEX. AGASSIZ.
No. 7527
September 6 1870 - March 16 1896.

1449
1-21

QUARTERLY JOURNAL

OF

MICROSCOPICAL SCIENCE.

LIBRARY
MUS. COMP. ZOÖLOGY
CAMBRIDGE, MASS.

EDITED BY

E. RAY LANKESTER, M.A., LL.D., F.R.S.,
Linacre Professor of Comparative Anatomy, Fellow of Merton College, and Honorary Fellow of Exeter College, Oxford; Corresponding Member of the Imperial Academy of Sciences of St. Petersburg, and of the Academy of Sciences of Philadelphia; Foreign Member of the Royal Bohemian Society of Sciences.

WITH THE CO-OPERATION OF

ADAM SEDGWICK, M.A., F.R.S.,
Fellow and Lecturer of Trinity College, Cambridge;

AND

W. F. R. WELDON, M.A., F.R.S.,
Jodrell Professor of Zoology and Comparative Anatomy in University College, London; late Fellow of St. John's College, Cambridge.

VOLUME 38.—NEW SERIES.

With Lithographic Plates and Engravings on Wood.

LONDON:
J. & A. CHURCHILL, 11, NEW BURLINGTON STREET.
1896.

LIBRARY
MUS. COMP. ZOÖLOGY,
CAMBRIDGE, MASS.

CONTENTS.

CONTENTS OF No. 149, N.S., AUGUST, 1895.

MEMOIRS : PAGE

On the Variation of Haliclystus octoradiatus. By Edward T. Browne, B.A., University College, London. (With Plate 1) 1

The Collar-cells of Heterocœla. By George Bidder. (With Plate 2) 9

The Metamorphosis of Echinoderms. By Henry Bury, M.A., F.L.S., late Fellow of Trinity College, Cambridge. (With Plates 3—9) 45

A Criticism of the Cell-Theory ; being an Answer to Mr. Sedgwick's Article on the Inadequacy of the Cellular Theory of Development. By Gilbert C. Bourne, M.A., F.L.S., Fellow of New College, Oxford 137

CONTENTS OF No. 150, N.S., NOVEMBER, 1895.

MEMOIRS:

On the Distribution of Assimilated Iron Compounds, other than Hæmoglobin and Hæmatins, in Animal and Vegetable Cells. By A. B. Macallum, B.A., M.B., Ph.D., Associate-Professor of Physiology, University of Toronto. (With Plates 10—12) . 175

On the Structural Changes in the Reproductive Cells during the Spermatogenesis of Elasmobranchs. (From the Huxley Research Laboratory, Royal College of Science, London.) By J. E. S. Moore, A.R.C.S. (With Plates 13—16) . . 275

Notes on the Fecundation of the Egg of Sphærechinus granularis, and on the Maturation and Fertilisation of the Egg of Phallusia mammillata. By M. D. Hill, B.A.Oxon. (With Plate 17) 315

Further Remarks on the Cell-Theory, with a Reply to Mr. Bourne. By Adam Sedgwick, F.R.S. 331

CONTENTS OF No. 151, N.S., JANUARY, 1896.

MEMOIR: PAGE

The Development of Asterina gibbosa. By E. W. MacBride, Fellow of St. John's College; Demonstrator in Animal Morphology in the University of Cambridge. (With Plates 18—29) 339

CONTENTS OF No. 152, N.S., FEBRUARY, 1896.

MEMOIRS:

The Early Development of Amia. By Bashford Dean, Ph.D. (With Plates 30—32) 413

On Kynotus cingulatus, a New Species of Earthworm from Imerina in Madagascar. By W. Blaxland Benham, D.Sc. Lond., Hon.M.A.Oxon., Aldrichian Demonstrator in Comparative Anatomy in the University of Oxford. (With Plates 33 and 34) 445

Notes on the Ciliation of the Ectoderm of the Amphibian Embryo. By Richard Assheton, M.A. (With Plate 35) . . 465

Ontogenetic Differentiations of the Ectoderm in Necturus. Study II.—On the Development of the Peripheral Nervous System. By Julia B. Platt. (With Plates 36—38) . . . 485

On the Variation of Haliclystus octoradiatus.

By

Edward T. Browne, B.A.,
University College, London.

With Plate 1.

A NORMAL specimen of Haliclystus octoradiatus, Clark, has eight adradial groups of tentacles; eight adradial genital bands; eight colleto-cystophors, one midway between every two groups of tentacles; and four internal, interradial septa.

The variation in number, shape, and symmetry of these organs forms the subject of this paper. The specimens were collected by the officials of the Marine Biological Association at Plymouth during November, 1892, and the spring of 1893.

I examined 154 specimens, and found 120 specimens perfectly normal and 34 specimens abnormal. Some of the abnormal forms are beyond doubt good cases of congenital variation, and others are cases of an imperfect regeneration of organs damaged or completely destroyed by injury. Congenital variation is usually shown by an increase or decrease in the number of organs, which may either vary together or separately.

Only three specimens show a numerical variation in all the organs. One has six groups of tentacles, six colleto-cystophors, six genital bands, and three internal septa. Two specimens have twelve groups of tentacles, twelve colleto-cystophors, twelve genital bands, and six internal septa. In the last two specimens the increase in the number of organs is not followed by a corresponding increase in the amount of tissue. Each

organ is below the average in size, and the tentacles in each group are also below the normal number. I have noticed among the Ephyræ of Aurelia aurita, that when a great increase in the number of arms occurs, the arms are below the average in size.

Another specimen has seven groups of tentacles, seven colleto-cystophors, seven genital bands and five septa, an increase in the number of septa, but a decrease in the other organs.

A numerical variation of the septa only occurs in seven specimens (about $4\frac{1}{2}$ per cent.); four of these are given above, and the others, which have only three septa, will be described in another part of this paper. I think in all cases the numerical variation of the septa may be safely considered to be congenital. The septa run nearly the whole length of the body, and are not likely to be affected by an external injury. In the majority of abnormal specimens the septa have their normal number, and the groups of tentacles, colleto-cystophors, and genital bands show variation. Usually each set of organs shows an independent variation, either in number, shape, or position. One specimen, however, with the normal number of septa, has seven groups of tentacles, seven colleto-cystophors, and genital bands, all of which are symmetrically arranged. It is now probably the simplest plan to describe the variation in each set of organs separately, and to commence with the colleto-cystophors.

The Variation of the Colleto-cystophors.—In the five specimens already described the colleto-cystophors vary in number along with the groups of tentacles, and occupy their normal position on the margin of the umbrella. But many specimens show that the colleto-cystophors vary independently of the other organs.

Four specimens with eight groups of tentacles have nine colleto-cystophors. In two of these (figs. 2, 4, and 5) the increase is produced by the twinning of one of the colleto-cystophors. The other two specimens have the additional colleto-cystophor in an abnormal position. One is on the

margin of the umbrella, very near to a group of tentacles (fig. 1); the other is adradial and on the aboral side of a group of tentacles, a little way from the margin (fig. 3).

Five specimens have fewer colleto-cystophors than groups of tentacles; in each case one is missing. It is difficult to say whether the decrease is due to congenital variation or to the result of an injury.

One specimen (fig. 6) with seven groups of tentacles has eight colleto-cystophors. An examination of the specimen shows that two groups of tentacles are united into one group. The colleto-cystophor, which has been shut from its normal position by the union of the two groups, is situated close to, and on one side of, the double group.

Five other specimens show a similar union of groups of tentacles, but the colleto-cystophors correspond in number to the groups of tentacles.

Mr. Hornell (1) has examined many large specimens of Haliclystus octoradiatus taken at Jersey. He states that 33 per cent. show a variation either in the number of colleto-cystophors or in the groups of tentacles. More than half of these are cases in which a colleto-cystophor is absent from its proper position.

Only five of the Plymouth specimens have fewer colleto-cystophors than groups of tentacles (about 3 per cent.).

Mr. Hornell also examined 118 specimens taken at Jersey, and found 78 specimens (66 per cent.) with a capitate tentacle upon the apex of the colleto-cystophor. Some specimens have only a slight swelling at the apex with a few nematocysts, and others show various gradations up to a perfect capitate tentacle, just like an ordinary tentacle. The following table taken from Mr. Hornell's paper gives the number of colleto-cystophors with a capitate tentacle in each specimen.

14	specimens	with	1	colleto-cystophor	with a capitate tentacle.
15	,,		2	,,	,, ,,
15	,,		3	,,	,, ,,
9	,,		4	,,	,, ,,
8	,,		5	,,	,, ,,
5	,,		6	,,	,, ,,
4	,,		7	,,	,, ,,
8	,,		8	,,	,, ,,

I searched all the Plymouth specimens to see if a similar variation existed, and only found one doubtful case. This specimen (figs. 7 and 8) has seven groups of tentacles in the normal position and one group a little way inside the oral surface of the umbrella. The proper position of this group is occupied by a capitate tentacle with a swollen base, which may or may not be an abnormal colleto-cystophor. The specimen possesses the usual eight colleto-cystophors, normal in shape and position.

Two specimens have capitate tentacles on the margin of the umbrella in an abnormal position. One has three tentacles just above a double colleto-cystophor (figs. 4 and 5), and the other has three tentacles close to a colleto-cystophor (fig. 10).

I think the Jersey specimens give an excellent illustration of local variation of a species.

The Variation of the Genital Bands.—In a normal specimen there are eight adradial genital bands, separated into four distinct pairs by the interradial septa. Some specimens show a variation upon this arrangement. One specimen (fig. 9) has six adradial and two interradial genital bands. The change in position occurs through the union of two adjacent adradial groups of tentacles into one interradial group. This union has reduced the number of groups of tentacles and colleto-cystophors to seven, but the genital bands remain normal in number. The change in position of the two genital bands is also well shown by their being separated by an interradial septum occupying its normal position. I think this may be regarded as a good case of congenital variation.

Another specimen (fig. 6) has six adradial and two perradial genital bands. There are seven groups of tentacles. One

group is larger than the others, and has two genital bands running towards it. This large group is perradial, and represents the union of two adjacent adradial groups. Two other specimens show a similar variation. A slight alteration in this arrangement may take place by the union of two adjacent genital bands into one broad band (fig. 18). The genital bands usually start some distance down the body of the medusa and extend across the umbrella. Two specimens show an exception by having short bands commencing near the margin of the umbrella (figs. 19 and 20).

The Variation in the Position of the Groups of Tentacles.—In most cases the change in position of the groups of tentacles is due to a decrease in number, and usually affects the symmetry of the umbrella. The decrease is sometimes brought about by the union of two adjacent adradial groups of tentacles into an interradial (fig. 9) or a perradial group (figs. 6 and 18).

In a few cases the position occupied by a group is exceptionally abnormal. Two specimens have a group of tentacles on the oral side of the umbrella, some distance from the margin. One of these (figs. 7 and 8) has seven normal groups of tentacles, but the eighth group is a little way inside the margin, which projects beyond the group and has a tentacle-like colleto-cystophor in the position which is under normal conditions occupied by the eighth group of tentacles. The other specimen (figs. 12 and 15) has three septa, six colleto-cystophors, and five normal groups of tentacles. But there is also an abnormal group of tentacles upon a short stalk, which rises above the oral surface of the umbrella, and occupies a position about half-way between the centre and the margin of umbrella. Opposite this group of tentacles, upon the margin of the umbrella, there are two other sets of tentacles, close together, with the tentacles arranged in nearly a single row.

An unique case amongst the abnormal forms is that of a specimen (figs. 11 and 13) with eight groups of tentacles and colleto-cystophors in the normal position. One of these groups of tentacles is smaller than the others, and has, on its outer

side, a lateral outgrowth of the umbrella. This outgrowth contains two colleto-cystophors and two groups of tentacles, one behind the other. The specimen has altogether ten groups of tentacles and ten colleto-cystophors.

The Regeneration of Injured or Lost Organs.—It is evident from the mutilated condition of some specimens that a considerable amount of injury may happen to the umbrella without causing death to the medusa. The damaged or lost organs may be replaced by new ones, which may or may not resemble the old ones. A new symmetry may even arise through a decrease in the number of organs, which in some cases might be mistaken for congenital variation. The simplest case is the loss of one group of tentacles, well illustrated by a specimen (fig. 21) which has all its organs perfectly normal except that one group of tentacles is missing. The prolongation of the umbrella and the genital band suddenly terminates, as if the tentacles had been cut off with a knife. Another specimen (fig. 22) shows a similar abrupt termination of the genital band, but a few short tentacles are present which may be reasonably regarded as a new growth. The destruction and regrowth of tentacles are also well shown in a specimen (fig. 16) with five normal groups and with two groups having only a few short tentacles. A genital band and a short prolongation of the umbrella marks the position of the eighth group which is missing.

Two specimens show both congenital variation and an abnormality due to regeneration. One of these (fig. 14) has three septa, five genital bands (the sixth is absent, but its position is faintly marked), and six colleto-cystophors; but there are only four normal groups of tentacles present. The other two groups have evidently been destroyed and are again budding out afresh. The other specimen (fig. 17) has three septa, seven genital bands, six colleto-cystophors, and only four normal groups of tentacles. One half of the umbrella, containing these groups of tentacles, is normal in shape, but the other half has apparently been destroyed, and three new groups of tentacles are in the process of development.

Reference.

1. Hornell, J., 1893.—"Abnormalities in Haliclystus octoradiatus," 'Natural Science,' vol. iii, p. xxxiii.

DESCRIPTION OF PLATE 1,

Illustrating Mr. E. T. Browne's paper on "The Variation of Haliclystus octoradiatus."

PLATE 1.

Fig. 1.—A portion of the umbrella showing a colleto-cystophor in an abnormal position. Aboral side. × 10.

Fig. 2.—A portion of the umbrella showing the twinning of a colleto-cystophor. Aboral side. × 10.

Fig. 3.—Half of the umbrella showing a colleto-cystophor in an abnormal position. Aboral side. × 10.

Fig. 4.—Oral view of a specimen with a genital band in an abnormal position, and a double colleto-cystophor. × 9.

Fig. 5.—Double colleto-cystophor (Fig. 4) with tentacles on the margin of the umbrella. Aboral side. × 18.

Fig. 6.—Oral view of a specimen showing the union of two groups of tentacles. × 6.

Fig. 7.—Oral view of a specimen showing a group of tentacles inside the umbrella, and a tentacle-like colleto-cystophor.

Fig. 8.—Lateral view of the abnormal group of tentacles described in Fig. 7. × 10.

Fig. 9.—Oral view of a specimen showing the union of two groups of tentacles, and the double genital band separated by an interradial septum. × 6.

Fig. 10.—A portion of the umbrella with tentacles on the margin in an abnormal position. Oral side. × 10.

Fig. 11.—Oral view of a specimen with a lateral outgrowth of the umbrella. × 6.

Fig. 12.—Oral view of a specimen with a group of tentacles inside the umbrella. × 6.

Fig. 13.—Lateral view of the outgrowth of the umbrella (Fig. 11). × 8.

Fig. 14.—Oral view of a specimen showing congenital variation (three septa) and the new growth of tentacles. × 6.

Fig. 15.—Lateral view of the group of tentacles inside the umbrella (Fig. 12). × 6.

Fig. 16.—Oral view of a specimen showing the loss of tentacles by injury and the growth of new ones. × 6.

Fig. 17.—Oral view of a specimen showing the new growth of tentacles on the half of the umbrella which has been injured. × 6.

Fig. 18.—Oral view of a specimen showing the union of two groups of tentacles and two genital bands. × 6.

Figs. 19 and 20.—Oral view of specimens showing the commencement of genital bands near the margin. × 6.

Fig. 21.—Oral view of a specimen showing the loss of a group of tentacles by injury. × 5.

Fig. 22.—Oral view of a specimen showing the fresh growth of tentacles. × 6.

The Collar-cells of Heterocœla.

By

George Bidder.

With Plate 2.

SUMMARY.

THE collar-cells are in normal life short and barrel-shaped, with separated cylindrical collars, which are never united. In certain pathological conditions, probably connected with suffocation, they elongate very greatly, diminishing in the diameter of their upper part, or "collum;" and in some species, though not in Sycon compressum, the collars may then come into contact. In certain other pathological conditions the collar is lost, though apparently it can be regenerated. These metamorphoses appear unconnected with the ingestion of food, which also was not found to induce any migration of the collar-cells. On the other hand, migration seemed to occur under exceptionally unhealthy conditions.

The collar is made up of (in Sycon compressum) about thirty parallel rods united by a film of some other substance. The flagellum is intimately connected with the nuclear membrane. There is an interstitial substance between the bodies of the cells. The area inside the collar appears to be provided with a sphincter membrane.

Cells preserved and cut by the paraffin method show an average contraction of 5 : 4 linear in the best sections. In most preparations this contraction is uneven, producing Sollas's membrane and other fictitious appearances.

Prefatory Remarks.

The feeding experiments referred to in this paper were performed on Leucandra aspera and Sycon raphanus at the Naples Zoological Station,—some during an occupation of the Cambridge University table in 1887–8, some during later opportunities for work there, which I owe to the great kindness of Professor Dohrn. The observations on living cells were made chiefly on Sycon compressum at Plymouth; they were undertaken largely on the stimulus of the paper (19) by Vosmaer and Pekelharing. Some months during which Mr. Sedgwick has been good enough to allow me to work in his laboratory I have devoted to reviewing my permanent preparations of all species. Except where otherwise stated, the collar-cells of S. compressum are described below, this species having been preserved with the greatest care and success.

Sycon raphanus grows abundantly on the walls of the tanks of the Naples Zoological Station. It differs here from the varieties ordinarily met in the possession of a very long fur of fine linear spicules. It has the obvious advantage that physiological experiments can be made in surroundings natural to it; on the other hand, it is rather small and soft for free-hand living sections, and its collar-cells are comparatively small.

Leucandra aspera (var. gigantea, Vosm.) breeds in the port of Naples. It has the advantage of great size, large collar-cells, and a robust constitution habituated to the most poisonous surroundings; but its huge longitudinal spicules render free-hand sections practically impossible. It is very remarkable that in impure water it throws out a fur of fine spicules like that possessed by S. raphanus (var. aquariensis nova); it has occurred to me that this may be a filter against bacteria.

S. compressum grows abundantly on the tidal rocks within ten minutes' walk of the Plymouth Biological Station.

It appears to be annual, in common with S. ciliatum, Halichondria panicea (cf. Johnston, 1, p. 92), and Hymeniacidon sanguineum. The rocks were covered from December to March of 1894 with large specimens of these four species; in September of the same year there were in some localities crusts of Halichondria, but it was for the most part difficult to find any sponges, except that careful search revealed a large number of very minute Sycon. I am informed that a general absence of littoral sponges was noticed also in the autumn of 1893. Carter (No. 2) states that S. compressum breeds in May (larvæ at Plymouth July 13th, 1895).

This species is the best suited of all I know for examination under high powers during life. Its collar-cells are among the largest, if not as large as any known. Its strong radial spicules give a convenient consistency without impeding the razor; they also protect the section from being crushed on the slide. Such sections are necessarily of great thickness as compared with paraffin sections, but the chambers of the sponge are so wide and extensive that rows of collar-cells can always be found standing out freely either against the light or against quite transparent tissues. On the rocks above mentioned S. compressum is habitually left for an hour or two at every ebb-tide to live on the water contained in its canal-system; the conditions of life under the cover-slip are therefore only partially unnatural. In experience, unless the slide, razor, or finger holding the sponge have been dirty, the flagellar motion will continue two to two and a half hours after covering, though changes of form, detailed below, become apparent after about a quarter of an hour.

I have not yet used a gas-chamber, the sections having been merely placed in sea-water between an ordinary slide and cover-slip. Using a Leitz $\frac{1}{12}$ oil immersion with Zeiss oc. 3 (old system) the collars and moving flagella appear with diagrammatic distinctness. I employed an Abbé condenser and blue glass, with incandescent gauze light focussed exactly on the object. About fifty living sections were examined,

including two or three of S. ciliatum. Probably in all about 5000 living collar-cells were seen distinctly.

Since in the existing state of our knowledge it appears to be inconvenient to use names for the tissues of sponges which connote comparison with other groups of multicellular animals, I shall, where useful, employ the following terms:

Ectocyte. Any cell forming part of the external surface of a sponge, including the afferent system of canals.

Mesocyte. A parenchym cell.

Endocyte. Any cell forming part of the surface of the central cavity of a sponge, including the efferent system of canals and the flagellate chambers.

It also appears convenient to use the term gonocyte to designate a generative cell.

In this paper the "basal width" of a collar-cell is the length of a line passing through four or five cells side by side, divided by the number of cells. The "collar-width" is used shortly for the diameter of the collar at its origin from the cell. The "height" of the cell does not include the collar.

General Structure of the Living Collar-cells.

The collar-cells of S. compressum in normal life measure about 12 μ high by 6·6 μ extreme basal breadth (basal width); the width of the collar—the most constant dimension—being about 4·6 μ. A few measurements of S. raphanus in life give their height 7 μ, basal width 5 μ; judging from the permanent preparations of L. aspera, its cells are about the same size as those of S. compressum.[1]

[1] At Plymouth, in a sponge agreeing closely in spiculation with Carter's Acanthella stipitata (fide Ridley and Dendy), I have met with probably the smallest collar-cells yet recorded. The chambers (fig. 22) were about 6·7 μ to 8·3 μ, the apopyle about 3·3μ in diameter; the cells were greenish in life, about 1·7μ high and ·8 μ basal diameter, appearing as a mosaic in which the apopyle contrasted as a large round white hole. The smallest chambers measured by Ridley and Dendy are three times this diameter, but Ridley described the structure of Acanthella pulcherrima (fide Ridley and Dendy) as "a transparent, almost colourless mass, . . . containing

The protoplasm is in life greenish, and in normal condition of ground-glass appearance. Each cell contains from four or five to a dozen spherical granules, up to 1 μ, or rarely 2 μ in diameter, rather more refracting than the surrounding protoplasm. I have called such granules "basal spherules" (18, p. 476) from their strong tendency to segregation in the base of the cell.

The cells have nearly the form and relation to each other of full corn-sacks standing side by side in a granary (v. figs. 1, 3, 9a, 15, and 19); in the normal condition they are distinctly but not widely separated, appearing to be actually in contact only at their bases. The generally barrel-shaped lateral surface of the cell always shows a clear smooth line in optical section; the circle marking upon it the base of the collar is also a smooth and sharply defined line. On the other hand, the convex or irregular area inside the collar (intrachoanal area) has nearly always a fainter outline, as though it were less refractive; it is often irregular, often finely punctated, often strongly granular. This was observed also in S. raphanus.

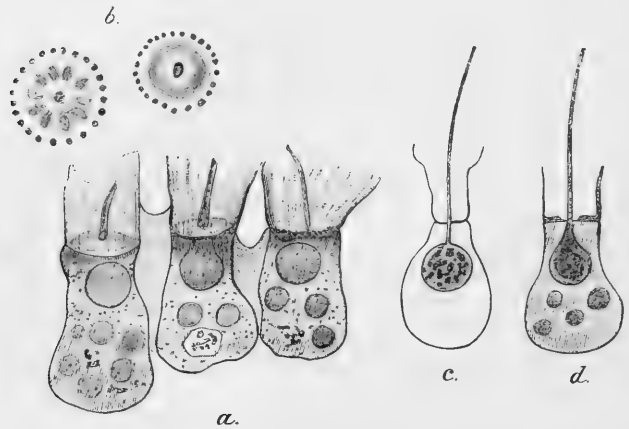
Collar.

The living healthy collar is from 2μ to 7μ in height, invariably an almost perfect cylinder, very little constricted at its base; ending sharply above without either rim or expansion (figs. 1, 2, 3, and 19). It has no vertical cleft, thereby differing from the spathiform collar of Choanoflagellata as described by Franze (19). From observations in life the thickness of collar or flagellum appeared to be $\frac{1}{5}\mu$ to $\frac{1}{10}\mu$.

Once in S. compressum, and once in S. raphanus, I observed in a fresh preparation the free edge of a collar, looked at from above, to present a "milling" or beaded appearance, as in fig. 7; in each case the cell had been some time under the cover-slip. Since the accompanying plate was engraved I have re-examined all my permanent preparations with a Zeiss

nucleoid bodies about ·007 to ·008 mm. in diameter." The identification of the chambers in my living specimen was unmistakable.

apochromatic 2·0 mm. objective of 1·40 aperture, ocular 8. With this power the beaded appearance from above is conspicuous in all cells of good sections strongly stained with hæmatoxylin. See subjoined woodcut, *b*.



a, *b*. Collar-cells over-stained in bulk (Series D), showing in *a* (profile) interstitial substance, and in *b* (from above) iris membrane. Same slide as fig. 15, *e*, Plate 2.

c. From Series A, stained borax carmine and hæmatoxylin, extracted with acid, focussed on flagellum to show connection with nucleus and perforation of nuclear membrane.

d. Series D, cleared with olive oil, stained on slide with hæmatoxylin, extracted with acid. Showing pear-shaped nucleus and perforation of iris.

From drawings made with Zeiss apochromatic immersion, 2 mm., ap. 1·40, oc. 8.

The "beads" remain sharply defined while focussing from top to bottom of the collar, each bead being about ·25 μ in diameter, and the less stained interspace between them about ·15 μ. The number seems to be fairly constant; though I never succeeded in counting exactly, it was in no collar estimated at less than twenty or more than six-and-thirty for S. compressum. The collar is, in fact, composed of a series of twenty or thirty parallel rods, or non-vibratile cilia, staining with hæmatoxylin, though less darkly than the flagellum, and united by a thinner film of non-staining substance. The

structure can be seen in profile, though generally less easily; but in a few cases the fibrils have become separated in the course of preservation, and stand out like the fringe of a tassel. I find that in Naples I once observed fresh preparations of Leucosolena primordialis in which the endocytes showed no collars or flagella, but appeared as if set with short cilia; the conditions were probably pathological.

As to Sollas's membrane, the statements of Vosmaer and Pehelharing[1] (19), which I originally went to Plymouth to confute, I can now only confirm.

The sponges were examined alive from rising tide, from ebbing tide, from deep tide-pools; after hours in a small vessel, after days and weeks in the aquarium. Many sections were watched on the slide until absolute death ensued. In no single instance was Sollas's membrane observed in a sea-water preparation.

As mere accident it would seem that often two neighbouring collars must be in contact, yet I only succeeded in observing with certainty two or three cases of this. There is never any membrane whatever in a plane at right angles to the axes of the collars. Neighbouring collars never pass into one another in a continuous curve. In every case that I have yet examined, where I had reason to believe that the sponge was thoroughly healthy, the collar-cells had the form shown in figs. 1, 2, and 19. In perfect health the collar is very little, if at all, expanded from the cylindrical; it is never trumpeted. After suffocation, as detailed below, the collars become conical, expanding distally; probably this is the explanation of my observations on S. raphanus (figs. 11*b* and 11*c*,—noted in 8, p. 630), especially as in this species F. E. Schulze (3) states that "unter Umständen kann eine solche Erweiterung des

[1] In their otherwise complete summary of the literature these authors have omitted Topsent's statement (9, p. 27) that in Cliona celata "les cellules sont unies entre elles par les collerettes. Collerettes et cils sont rétractiles comme les pseudopodes de cellules amiboïdes." Quite recently (24, p. 282) he writes, "Les choanocytes d'une même corbeille peuvent rester libres de toute adherence entre eux, ou bien ils se soudent, à l'occasion, par les bords de leurs collerettes."

aüsseren glatten Bautheiles vorkommen dass die benachbarten Collare sich fast beruhren." In S. compressum such contact does not occur, either in healthy life or in any of the morbid conditions I was able to investigate.

Sollas's membrane occurs, on the other hand, in paraffin sections of S. compressum (v. figs. 16—18, 20), preserved by any delicate method except the very best; careful examina- showing that it is always associated with great distortion of the cells, and that this is also the case in the drawings by other authors. Where there is no distortion (fig. 15) the membrane is not present.

Dendy was right in saying that cells showing the membrane may also possess flagella, though generally this is not the case. And the phrase "portions of flagella and collars irregularly sticking together" (19) is not descriptive of this very definite structure as it occurs in many sections. But these same sections have been prepared with great care (all being osmic acid preparations) from a sponge which I know in life had all its collars disunited and normally cylindrical; and in five cases the same individual was examined partly by a living section, partly by paraffin sections (cf. figs. 19 and 20). It is not disproved that union of the collars may occur in some living sponges—more probably in some dying sponges. But the evidence of ordinary paraffin sections for its existence must now be considered valueless, and, with exception of the observations quoted and explained above, there was no other evidence for its existence. There remains no reason to believe that it occurs in nature at all, and I must thank Dr. Vosmaer, my old friend and master, for yet another lesson in sponge lore.

Some measurements will be found in the note on distortion of cells at the end of this paper.

It is worth mentioning that in the living larva of S. raphanus (Naples, June) I found that the transparent ends of the flagellate cells, lettered by Barrois (5) as "collier," are solid and refractile, as faithfully figured by Schulze, × 5; the convex distal surfaces are correctly shown by both authors.

Flagellum.

In the living S. compressum, the flagellum may be 30 μ to 50 μ long. The movement is certainly asymmetrical, with a longer rest on one side than on the other.[1] In several cases it was also certain that the motion lay entirely in one plane. It is rare to see flagella moving more rapidly than about 10 beats to the second. I have guessed the greatest rapidity I observed to be 15 or 20 beats to the second. The thickness in life was estimated at $\frac{1}{5}\mu$ to $\frac{1}{10}\mu$; it appeared uniform, except sometimes for a thickening of the part inside the collar. In paraffin sections this thickening is also found, in perhaps a third of the cells; it does not extend for more than about 1 μ from the intra-choanal surface, the thickness above this point being uniform, and measured in different flagella from ·15 μ to ·3 μ. But in the paraffin sections the flagellum can be traced inside the substance of the cell to the nucleus (cf. cut, *c*), and in the osmic preparations stained in bulk it is not wider here than in its terminal portion. For greater definiteness I shall term the part of the flagellum below the general outline of the cell the radix of the flagellum.

Intra-choanal Area.

It has been stated above that the outline of the intra-choanal area is in life less definite than that of the sides of the cell. Vosmaer and Pekelharing mention carmine experiments which hint that here, as supposed by the early authors, food is taken in; and it will be seen below that careful examination of my own permanent preparations is far from contradicting this view.

In life, neither ingestion nor egestion were ever witnessed; but in a sponge which had been two hours in a basin of sea water, after two hours' exposure at low tide, almost every cell

[1] Minchin states this very definitely for Leucosolenia coriacea (16, p. 264). I have also a note that the same holds good for Leucandra aspera and another sponge (I think S. raphanus). These four are all sponges with tubular or thimble-shaped chambers; it is possible that the beat is symmetrical in short hemispherical chambers where the axis of the collar-cell is nearly parallel to that of the chamber.

possessed a globule containing angular dark particles,—sometimes, as in fig. 4, projecting on the surface between collar and flagellum. These globules were observed and drawn moving in the distal protoplasm of the cells; there were numerous bodies of similar appearance (cf. fig. 13 *a*) floating freely in the chamber. It is possible that they were some minute organism with whose appearance I am not acquainted; but the strong suggestion was that they were ejecta. I have often suspected, from paraffin sections, that the food vacuoles of sponges are filled with some gelatinous matter, coagulated in preservation.

The "vacuoles" in fig. 5 and fig. 9 were also moving in the protoplasm, but it does not seem impossible that they were nuclei (a view established since this was in type).

In paraffin sections stained with hæmatoxylin the free end of the cell very noticeably appears, with the ordinary immersion lens, as a dark band (figs. 15, 17, 18). Viewed from above, it is often seen that this stained area is really annular (cf. cut, *b*), the flagellum appearing as the dark centre of a white disc, which is generally about one third the radius of the intra-choanal area. And with the apochromatic immersion it can be seen in profile to be indeed the case that at the focus of the flagellum the terminal plate of stained matter is interrupted by an unstained interval, showing that the substance stained is arranged as a diaphragm, and not a complete disc (cf. cut, *d*). In the profile of cells treated with acid alcohol after staining, the hæmatoxylin is found to be confined to this diaphragm, the protoplasm beneath being comparatively unstained.

In many of the cells viewed from above the stained annulus shows a radial structure. Though marked in a few cases, in most cells it is impossible with the magnifying power employed to make certain whether this exists or not; but generally in the optical profile the dark line marking the section of the annulus is to some extent beaded, or broken, especially on focussing above or below the flagellum. Where the rays were recognised, their number was never more than ten or twelve (cf. cut, *b*); on the other hand, the root of the collar, focussed on

the surface with the cell in full profile, often seemed to show beads corresponding in number with the collar fibrils. On the whole, I believe that the radiation represents a condition existing in life. Vosmaer (19) figures a ring near the base of the collar ("at the base" in explanation of plate) in Spongilla, of which he promises a description; it is not beaded.

The substance which stains in this annular manner I shall call the iris, and the aperture in its centre the pupil. It is a natural suggestion that the iris is a contractile sphincter, and the pupil the ingestive and egestive aperture of the cell. Some sections support the view that the thickening at the base of many flagella is cell-protoplasm projecting in an amœboid cone through the pupil, the true flagellum running in the axis unthickened to the nucleus.

Nucleus.

There is nothing exact concerning the nucleus to be recorded from the observations of living cells. I have above referred to the "empty vacuoles" of figs. 5 and 9; one of similar position is shown in fig. 3. If the identification be correct, these indicate (1) that the nucleus is distal in life, (2) that it moves in the protoplasm. In a drawing made in life from the same preparation as fig. 13, of cells with very active flagella, there is a large clear sphere in each cell which can scarcely be other than a nucleus.

Preparations stained in bulk with borax-carmine show in the nuclei of collar-cells a well-defined chromatin reticulum surrounded by a stained nuclear membrane. In the wall of one chamber was a beautiful karyokinetic spindle; presumably the rather large cell in which it occurred was a collar-cell dividing in two.

In S. compressum hardened for one hour in 1 per cent. osmic acid, and stained carefully in bulk with hæmatoxylin, the nuclei are almost always spherical; the radix of the flagellum can be recognised as a refractile thread passing from the nucleus to the pupil of the iris. The same series of sections, stained also on the slide with hæmatoxylin and extracted with

acid alcohol, shows more often a fine, stained, tapering point, forming a distal prolongation to the nucleus, issuing through the pupil of the iris as the flagellum (cf. cut, *d*). In the nuclei of a preparation treated with $\frac{1}{2}$ per cent. osmic acid, stained in bulk with borax-carmine, and on the slide with hæmatoxylin, the two forms are also seen: where the nucleus is spherical the flagellar radix is seen as a faintly-stained thread piercing the dark nuclear membrane (cf. cut, *c*); where the nucleus is pointed, the point—that is, the radix of the flagellum—can often be seen to be a protrusion of the nuclear membrane. In either case the nuclear membrane is interrupted, so that in profile the outline shows a clear break opposite the flagellum.

In S. raphanus, treated with iodine followed by alcohol and borax-carmine, there is often a comparatively thick stained thread passing from the nucleus to the flagellum. In the same species, preserved in weak alcohol gradually strengthened, and stained in borax-carmine, very many of the nuclei appear pear-shaped, the distal half of the nucleus being a cone with its apex in the centre of the intrachoanal area.

In these last sections many of the cells have the nuclei filiform and ribbon-shaped, so that they probably do not give the living form; and in the cell shown in fig. 5, treated with weak alcohol under the microscope, showed the "vacuole" perfectly spherical, refracting, and absolutely distal. But the particular form of distortion described, assuming it distortion, points to a firm mechanical connection between flagellum and nucleus. It seems likely that the spherical nucleus, with a filiform radix issuing from it, represents an unaltered living structure;[1] we have then to consider whether the pear-shaped or bulb-shaped nucleus, which all additional reagents tend to develop represents the staining of other substances surrounding the radix, or a change in form of the nuclear membrane.

All that can be stated definitely is that the flagellum is firmly and intimately connected with the nuclear membrane, and that when this is spherical in outline the sphere shows a break at the point where the flagellum intersects it. The

[1] This was found to be true in the fresh tissue.—July, 1895.

appearances are consonant with the flagellum being a rod-like or tube-like process of the nuclear sheath.

Vosmaer (**19**, fig. 8), figures, without describing, such a connection in Halichondria; and Heider (**7**), in the larva of Oscarella, describes the root of the flagellum at the nucleus. With this structural disposition may be correlated the general (not invariable) distal position of the nucleus in collar-cells that are elongated, as shown for Heterocœla by myself (**18**, fig. 4) and Dendy (**20**, fig. 24). Leucosolenia is figured by Minchin (**17**, figs. 2 and 3) and myself (**18**, fig. 3) with a distal vacuole to each cell and a basal nucleus; Spongilla, according to Vosmaer's plate, differs in these respects from Halichondria precisely as Leucosolenia from the Heterocœla. A suggestion has been made to me that the nucleus serves the flagellum as a mechanical fulcrum in the semi-fluid protoplasm; and it is obvious that if the whole intra-choanal area be a cell-mouth the flagellum can have no permanent base except in the interior of the cell. If this view be correct, the same function would seem to be performed in certain lines of descent by the walls of a permanent vacuole, verifying for Leucosolenia an alternative suggestion of Minchin's (l. c.), who doubted "whether this space represents a 'Centralkörper,' or a kind of food-vacuole, or whether it is in some way connected with the movements of the flagellum and collar."[1]

Maas's embryological work on Silicea (**21**) seemed to point to the possibility that the relative size of nuclei might indicate

[1] I have no intention to discuss the classical literature on connections described in other groups between nuclei and flagella on cilia. But my friend Mr. J. J. Lister has kindly pointed out to me the description of Camptonema nutans (a Heliozoon-like organism) by Schaudinn (**25**) in which he describes the axis of each pseudopodium expanding to envelope a nucleus in a manner most suggestively recalling the condition drawn in my woodcut at *d*. Schaudinn puts forward tentatively the view "dass der Kern bei der Bewegung der Pseudopodien eine bedeutende Rolle, vielleicht als regulatorisches Centrum, spielt." I think we should first carefully test on Leucosolenia and Spongilla the hypothesis I have borrowed above before yielding to the ever-enticing temptation to appeal to the nucleus as cell-brain.

ontogenetic history, particularly as to whether in Sycon also the lining of the efferent system arises from the granular cells (with large nuclei) of the larva. Measuring thirty nuclei of each tissue, near the osculum of S. raphanus, gave the following average diameters :

Nuclei of collar-cells	2·15 μ.
„ of cloacal epithelium	2·8 μ.
„ of dermal epithelium	2·6 μ.

The largest cloacal nucleus is 4·7 μ, and two thirds were over 2·5 μ; the largest nucleus of a collar-cell is 2·6 μ, and there is no other over 2·5 μ. Between these two classes, therefore, the difference is very marked ; but on the other hand, three fourths of the collar-cell nuclei and dermal nuclei are mutually indistinguishable as regards size.

In a borax-carmine preparation of Leucandra aspera all cells but the gonocytes showed a nuclear reticulum, with the possible exception of two parenchym cells. Both in Sycon and Leucandra the gonocytes show the well-known large vesicular nucleus with nucleolus.

Interstitial Substance.

The interstitial jelly between the collar-cells, the existence of which I have never suspected from living preparations, proves in these permanent sections under the apochromatic lens to have considerable importance. It appears not only in the best sections of S. compressum, but also in sections made at Naples from S. raphanus. In permanent preparations of the normal condition it often reaches to the level of the base of the collar, as drawn by Dendy for Leucosolenia (**14**, pl. 8, fig. 3), sinking in a tension-curve between the two cells (cf. cut, *a*). In sections where Sollas's membrane has been produced, the membrane is seen uniting the tops of the collars, separated from the surface of the jelly by a vacant space, not being, as Lendenfeld suggested (**10**), a misinterpretation of this surface. I satisfied myself that in fig. 17 the line is not the outline of a jelly, but actual irregular fusion of collars, the effect being

that they have been forced into contact while of natural size, and then been subject to individual constriction.

I must admit that increased optical definition proves it was the surface of this substance, coinciding with the upper limit of the basal spherules and the constriction of the cells, which I mistook for an intracellular septum in the "column-and-plinth" cells (18). It will be shown that it is now probable that the form of these cells is not connected with nutrition, and that Dr. Dendy's surmise with regard to them was nearer the truth than my own.

Pathological Changes.

Two distinct series of changes in form, due to abnormal conditions, were noticeable from their constancy of character and sequence. They appear interesting not only for the light they throw on the histology recorded in preserved sponges, but also from the point of view of cell-physiology.

The first were observed in healthy living sections placed in a drop of sea water under the cover-slip on a glass slide; I shall call them "suffocation changes." They consist mainly of the formation and elongation of a transparent neck (collum of authors) to the normally barrel-shaped cell. Beginning with increased transparency of the upper (distal) part of the cell, the transparent region so distinguished soon becomes elongated and constricted, the spherules remaining in the wider and opaque base (figs. 5, 6, 8). Being narrower, the distal parts of the cells are obviously more separated than before. The collars become conical, expanding at the mouth—possibly in geometrical consequence of the constriction of the collum (figs. 8*c*, 9, 10, 11, 12). During these changes the flagella continue to move, so that the tissue must be considered living; they become very gradually slower, but after all motion has ceased it is long before the delicate flagellum and collar further change their outline. The extreme form drawn in fig. 12 was from a section that had been under observation one hour and three quarters; for another twenty minutes the cells were motionless, but unaltered.

The degree of change differs in different specimens; but usually after two hours every chamber presents an appearance it may be convenient to call "striated," the lumen being greatly reduced, the elongated thin cells forming a herring-bone pattern down the chamber, and appearing (if it be not, indeed, the fact) as if many of them became free.

Carter (2) draws two collar-cells from teased living preparations of S. compressum, of which his fig. 1 corresponds exactly to my fig. 10, and his fig. 2 to my fig. 11*a*. He describes changes on the slide to amœboid forms; but he is treating entirely of cells "scratched out from the body of the sponge," whereas I have confined my observations to cells in situ.

Fig. 11*a* is a sketch made from S. raphanus (Naples, Aug. 1892), with the note "a tendency to elongation of cells as the preparation dies;" while figs. 11*b* and 11*c*, made at the same time, bear the note "flagella" [in other parts] "still in motion, certainly none on these cells." I have already quoted Schulze's observation (3) as to occasional concrescence of collars in this species; the cells drawn by him as normal appear to have mostly entered on the phase of my figs. 6 and 8, that is, to have been twenty or thirty minutes under the cover-slip; his Taf. 14, fig. 4, is practically in the stage of my figs. 11 and 12.

The form of cell produced by this series of changes appears identical with that described by Dendy in his "x" chambers (12, 20), and is certainly so with the "column-and-plinth" cells described by me (18, p. 477). Similar cells in permanent preparations show the nucleus in most cases at the extreme distal end of the cell, the granules are in the base. The upper surface of this base coincides—at least in most instances—with the upper surface of the intercellular jelly, and the contours of the uppermost enclosed granules lie in the same plane. With the ordinary immersion objective the appearance of a septum is in many cases convincing, so that even now it is only with the apochromatic lens that I find it possible to resolve it into its component optical elements.

The second series of changes I will call "tide changes." S. compressum is a tidal sponge, and when removed from the water will live in a damp atmosphere for two or three days. The cells become rounded and transparent, they retain their flagella but lose their collars; after restoring the sponge to healthy conditions the collars reappear.

In some specimens gathered from bare rocks about four hours after the sea had left them, having been one and a half hours in drizzling rain, the cells were rather short, rather round, notably granular, and mostly without collars. The flagella were moving, in one sponge with greater violence than I have ever seen. In one cell, after ten minutes in fresh sea water, I thought I saw the collar reappear, but the observation was open to doubt.

In a sponge twenty-seven hours out of the water (in an empty corked bottle), the cells were very low, rounded, and transparent, with bright granules; the flagella were active, though not on all cells; collars were very rare. From the same sponge, after twelve hours in sea-water, another section showed the cells less transparent, and higher (fig. 13), with a few more collars; after another eighteen hours in sea-water there were in most parts of the sponge perfectly normal collared cells, in other parts the curious modification shown in fig. 14. Both forms of collar may be considered to have been regenerated, since two or three other "dry" sponges showed loss of collars from almost all cells, and it appears that few collars persist after a day's removal from the water.

While it is obviously impossible from these observations to point out with certainty the exact stimulus to which the changes are due, some of the facts available are worth reviewing. Increased salinity and retention of waste products in the chambers are common to the conditions producing both series, but the tidal changes also occurred when the salinity may be supposed to have been reduced. In all the suffocation changes the preparation had been brought to the warm temperature of the laboratory, but this was true for a much longer time of some sponges on which the tide phenomena were

observed. The latter were, however, always exposed to a considerable mass of air, and respiration may be supposed to have been still possible; under the cover-slip this was of course not the case. On the other hand, the radial chambers under the cover-slip each contained the excretory products of at the most two hours, for which time only they had been deprived of food; in the case of the tidal changes, nothing but gaseous matters could have been either received or eliminated for one or two days. It seems, therefore, plausible to suggest that the characteristic appearance results, in the suffocation changes, from want of oxygen or presence of carbonic acid; in the tidal changes, from starvation or the presence of non-gaseous excreta. The local suffocation transparency appears to be mere segregation, the tidal transparency may be due to starvation. It may possibly be important that the metamorphosis here attributed to lack of oxygen results in a maximum surface, that attributed to presence of poisonous products results in a minimum surface.

Tidal changes were never observed to originate under the cover-slip, nor on the other hand did cells so metamorphosed ever give rise to suffocation forms. The elongated suffocation-cells died extended, the hemispherical tidal cells died hemispherical, neither modification showing any signs of giving rise to the other. Only in one section (of a sponge twenty-seven hours out of the water) I found, after an hour on the slide, a chamber lined with the usual low, round, collarless cells (as in fig. 13), but with two collared-cells of the extreme suffocation form (as in fig. 12), 30 μ long, stretching almost across the chamber. The contrast was very striking, and seemed to hint that accompanying the loss of the collar is some change, perhaps of the lateral walls, which means the loss of power of extension. These two cells had escaped the tidal modification, and therefore were able to respond to the stimulus of suffocation. All appearances suggest that the extension under suffocation is due to constriction of the lateral wall—whether it be a contraction set up by these conditions, or a normal tone which the enfeebled cell-contents can no longer overcome.

Apparent migration of collar-cells into the parenchym

was observed in a sponge (S. compressum) which had been a month in the circulation of the aquarium, with other sponges, &c., allowed to decay in the dish containing it. The living section at first sight seemed to be full of embryos; these proved, however, to be the remnants of the flagellated chambers, some parts still exhibiting perfectly normal collared cells with active flagella and cylindrical separated collars; the space between the "Leucon"-like chambers being largely filled with parenchym. Paraffin sections showed many wide canals, resembling the normal afferent system. Only a few of the collar-cells are elongated, and the recognisable collar-cells in general are comparatively few in number; in some places they line only part of a chamber; in some places the chambers are shorter or narrower than in the normal sponge; in some places they form small closed chambers, or pseudo-blastulæ, consisting of as few as a dozen cells, lying in a plentiful gelatinous parenchym, into which appearances suggest that their fellows have migrated.

The condition appears identical with that recognised as common in winter for Spongilla (Lieberkühn, Metschnikoff, Weltner). It becomes a question whether we are not to ascribe the metamorphosis of Halisarca as described by Metschnikoff (6), and that of S. compressum described by Masterman (23), to conditions unfavourable to general vitality, rather than to the inception of nutritious sive innutritious particles.

Nutrition.

Vosmaer and Pekelharing (19) find carmine and milk, after one hour's feeding, in the choanocytes and in the lumen of the chamber, especially frequently in the collars themselves. After a longer time the particles are chiefly in the cell-bodies, rarely free or in the collars; after a still longer time they are found in the parenchyme.

Masterman (23) recently published an account of nutrition in S. compressum in which he describes an extraordinarily rapid cycle of events. It has been suggested above that he may have been deluded by pathological metamorphoses uncon-

nected with nutrition, as I was formerly (18) in my hypothesis as to changes of cell-form accompanying digestion.

Of my own experiments I printed shortly the main results in February, 1888. Omitting the passage (quoted in 19) on Sollas's membrane, I reprint the statement.[1]

"In Leuconia aspera I find that carmine granules are taken in freely by the collared cells, not appearing in the mesoderm, and only infinitesimally in the other epithelia. . . .

"I observed that during four hours a Leuconia plentifully supplied with carmine ejected none in its oscular stream, which was powerful and continuous. Its flagellate cells proved to be heavily charged with carmine grains. Such complete filtration would be uneconomical, if not impossible, were the carmine arrested merely by the ingestion of cells laterally bounding the current.

"I believe, from a consideration of the observations of others and the above facts, that the collared cells primitively both ingest and digest for the sponge; the function of digestion being in some sponges, but not in Leuconia, passed to cells situated in the mesoderm. I think that probably only under exceptional necessities of structure do other cells of a sponge ingest food in valuable quantity.

"My experiments were suggested by a recognition of the fact that in the current through a sponge the region of slowest motion, and therefore of greatest deposit and easiest arrest, is in the flagellate chambers, where the transverse area of the total channel for the water is greatest. This fact also explains

[1] Extracted from the 'Proceedings of the Cambridge Philosophical Society,' vol. vi, pt. iv, "Preliminary Note on the Physiology of Sponges." Fifty copies only were printed in full through a mistake owing to change of editorship by which an abstract of ten lines was substituted in the 'Proceedings' as issued; for this reason I print a "Preliminary Note" of work still, alas! unfinished. I hope soon to publish a discussion of the mechanical conditions here referred to. The lamellar forms of sponges are naturally independent of oscular velocity, since the stream of foul water is 180° from the stream of fresh water. It is the increase of this angle which leads to the number of stalked forms, from which are usually evolved the flabellar species and varieties.

the persistent union of nutritive with motor functions in the cells lining these chambers, since the flagella have their highest efficiency where the velocity is least. The healthy nutrition of a sponge (excepting lamellar forms) depends on the energy of the current from the osculum being high; the economy of its motor apparatus depends on the velocity of the water in its chambers being low. All transition from more to less primitive canal-systems exhibits an increase in the ratio between these quantities."

The mechanism of filtration we now know to have nothing to do with Sollas's membrane; the cardinal fact of filtration was very striking, and remains to be explained.[1]

As to the locality of ingestion and digestion, my permanent preparations available are in all from five specimens of S. raphanus, eight of Leucandra aspera, and one of Leucosolenia clathrus. The intervals between the first application of suspended particles (carmine, starch, &c., rubbed up in the sea-water), and that of the preserving fluid were respectively 5, 10, 10, 11, 14, 21, 27, 50, 60, 77 minutes, 4¼ hours, 18 hours, 22 hours, and 3 days. Most of the sponges were placed in clear sea water for various periods before killing; but the accumulations on the spicules, &c., render this of doubtful value.

Re-examining anew all these preparations very carefully with Zeiss's apochromatic immersion lens, I can support my old conclusions, and make some additions. Ingestion commences freely at once; on the whole, evidence is in favour of it taking place within the collar of the cell. After twenty minutes the foreign particles are often found enclosed in a vacuole, and they are more generally in the basal parts of the collar-cells.

Carmine is found here in S. raphanus which had been in pure sea water eighteen hours, after feeding for twenty minutes; only very fine particles are present, in the bases of

[1] I should warn anyone repeating the experiment that carmine is often soluble to a considerable extent in sea water. That which I used at Naples in 1887 was not soluble in the sea water of the aquarium.

the cells, and mostly in vacuoles. The cells containing foreign particles do not lose their collars, and the column-and-plinth appearance occurs independently of the amount of carmine contained. Nor do the collar-cells show any tendency to migration, even after being fed (L. aspera) for four and a half hours, when many are filled to their very outlines with carmine. In the sponge here referred to about 1 per cent. or fewer of the glandiform ectocytes contain a grain or two of carmine. This may be excretion, but there is no evidence against it being casual ingestion. In most recently fed preparations there are one or two canal ectocytes containing a grain of carmine.

Examination confirmed the statement (18) that there are a number of gonocytes connected by processes or pseudopodia with the basal surfaces of the collar-cells, and containing, in both body and process, spherules precisely resembling the basal spherules of these cells. I still believe, therefore, that the gonocytes nourish themselves on the basal spherules at the expense of the collar-cells; and in the hypothesis (which I think I owe to an oral suggestion of Miss Greenwood in 1888) that these spherules are stores of digested food. The preparations mainly examined are of the S. raphanus eighteen hours after feeding, where the carmine lies among the basal spherules. A large number of the gonocytes are in contact with collar-cells which contain plentiful carmine; in only two of them I found carmine-grains, and it is tempting to deduce that vacuoles and undigested food do not pass into the gonocyte.

In L. aspera and S. raphanus migration of the collar-cells into the parenchym certainly does not take place after satiation to any degree for any period with carmine; nor in L. aspera when a large proportion of the collar-cells contain completely ingested starch grains;[1] nor after fourteen minutes' feeding with carminate of alumina, freely ingested; nor after one hour's feeding with Indian ink, freely ingested. There is one clear

[1] The use of the polariscope for recognising starch grains is easily practicable with the highest powers. Without it vacuoles of the same size are often difficult to discriminate.

case (S. raphanus, eighteen hours after feeding) of carmine in the parenchym jelly among similar sized brownish particles, giving vividly the impression that they have been discharged from the collar-cell above. There is one apparently certain case (L. aspera) of a starch grain apparently enclosed between mesocytes in the parenchym near an afferent canal. I have seen no other instances, and there is nothing which leads me to suppose that as a rule undigested food ever passes into the parenchym, nor have I any observations which indicate the means of nutrition of the parenchym otherwise than as concerns the gonocytes. And it is worth stating that the few carmine-grains observed in ectocytes were never enclosed in vacuoles.

Though there are many cells containing carmine in S. raphanus after eighteen hours' feeding, the particles are fine and the mass small. L. aspera, twenty-one and a half hours after twenty-one minutes' feeding, shows no carmine.

As to the natural food and feeding of the sponge, S. compressum killed directly from the sea shows in the protoplasm of its collar-cells, besides and among the basal spherules, numerous minute irregular particles, often highly refractive; sometimes three or four in a vacuole-like structure (cf. cut, *a*). Many appear to be bacilli, being rod-like bodies 1 μ to 1·8 μ long by ·1 μ to ·2 μ broad. In another specimen there are lying freely in the chambers several specimens of what appears to be an alga, one a sphere of four cells, one probably of sixteen; also lying inside the collars of different collar-cells are several isolated spheres, of about the same size as the individual cells of the larger spheres, and similarly stained. In this preparation, and another of L. aspera, there are in the chambers several larger nucleate cells, possibly Protozoa, partly enveloped by the distended collars, sometimes more than one cell converging on them. I have not hitherto witnessed any similar phenomena in life, nor do I know of any such being recorded.

In several instances in the carmine preparations there are grains inside a collar, as Vosmaer and Pekelharing describe, and the evidence certainly so far points to ingestion by the

intra-choanal area, however difficult it may be to understand how the food is brought there. It is also obvious that where there is an interstitial substance the water cannot pass over the surface of the cell, as I formerly supposed. Therefore until direct evidence is obtained we must consider it probable that the pupil of the iris is the aperture both of ingestion and egestion. I have never witnessed in life anything suggesting pseudopodial action of the collar (except possibly change of length), but it is difficult otherwise to see how cells can ingest through the intra-choanal area starch-grains as wide as themselves.

It is commonly stated that sponges can be easily starved by filtering the water. Fig. 3 represents collared cells from S. raphanus which had been four days in water passed entirely through filter-paper; there was no difference apparent from sponges which had been detached on the same day and replaced in the water from which they had been gathered.

In L. aspera and S. raphanus the current is not stopped by the application of carmine,—which, as stated above, is ingested from the first. The current was stopped (L. aspera) after a few minutes by the carminate of alumina employed, which may have had with it some soluble poison producing this effect; but the sponge was preserved within fourteen minutes from first administration, and the collar-cells were found to have ingested the carminate freely. Far from the dermal pores closing for hours against suspended matter, powdered charcoal (L. aspera), and starch (S. raphanus), in sponges killed after seven minutes and five minutes respectively, were found solidly filling the afferent canals. With the starch the prosopyles were also filled, and widely open, and there was starch free in the flagellated chambers and even in the cloaca; the starch grains (and still more the particles of charcoal) were too large for easy ingestion, but they were adhering to and certainly occasionally ingested by the collar-cells.

Topsent (9) finds that with the parasitic Cliona "même d'y

mettre en suspension des granules de carmin, provoque l'occlusion relativement rapide des papilles." It is not clear from the words whether this is due to the presence of particles or only to stirring the water; but it is well known that these papillæ are exceedingly sensitive. For other sponges, and especially S. raphanus, Lendenfeld makes repeated statements (**11**, pp. 583, 592, 675, &c.) as to closure of pores against carmine (and not against milk). They are contradicted by the experiments of every other worker; and notwithstanding their picturesque elaboration, and the dramatic deductions for which these statements are responsible, the 149 experiments that he records include no evidence that the narrative is based on even erroneous observation.

Distortion of Cells in Preservation.

The following results may be of some interest to those who study histology on preserved material from other groups as well as sponges, though the measurements are too few to profess to be more than suggestive.

Measurements were made of the collar-cells in six series of sections, A, B, C, D, E, F, in order to compare their dimensions with those of life. The series were from five specimens (S. compressum), D and E being from one sponge; and in the case of all but A the collar-cells from a closely adjoining portion of the same individual were examined and measured during life.

All the sponges were preserved in osmic acid for one hour; followed by alcohol, benzol, and paraffin. In C, D, and F the change from water into absolute alcohol was effected by dialysis; in all but B the change from absolute alcohol into benzol was made in the same way; all were transferred by gradual changes of temperature and percentage through soft paraffin to hard paraffin of a temperature not exceeding 65° C., generally 62° C.

A was the only sponge preserved in $\frac{1}{2}$ per cent. instead of 1 per cent. osmic acid, it alone was decalcified (1 per cent. nitric acid in 90 per cent. alcohol), it alone was stained in

bulk with borax carmine, and alone was cut by the ribbon method, all the other sections being made with the oblique razor.

The distal expansion and fusion of the collars known as Sollas's membrane (fig. 18) appeared plentifully in the paraffin sections of A, B, C, and F; scarcely at all in D and E. It was not present in the living sections examined from any of the sponges; all alike showing the characters described in the previous paper.

It was found that the average cubical contraction of the cells is about to one half of their living dimensions:

Average volume of living collar-cell .	. 270 cubic μ.
„ „ of collar-cell in balsam	. 125 „

This was calculated from the linear measurements, which contract unequally in different directions:

Height[1] from 28 living cells .	. 12μ.
„ 86 balsam cells .	. $7{\cdot}5\mu$.
Basal width from 34 living cells	. $6{\cdot}4\mu$.
„ „ 203 balsam cells	. $5{\cdot}6\mu$.
Collar width from 50 living cells	. $4{\cdot}6\mu$.
„ „ 126 balsam cells	. $3{\cdot}4\mu$.

The best series of sections (D, drawn in fig. 15) and the worst series (A, drawn in figs. 17 and 18) show respectively the following ratios in their linear dimensions to those of life:

	in Series D.	in Series A.
Collar width . . .	·83	·5
Basal width . . .	·88	·7
Height	·8	·5
Height of collar . . .	1·0	1·0
Deduced ratio of volume of cell to that in life . . .	·55	·2
Deduced mean linear contraction ratio .	·82	·6

[1] "Collar-width" is measured at the origin of the collar from the cell; "basal width" is the length of a row of cells divided by the number of cells in the row; "height" is the distance between two parallel lines at right angles to the axis of the cell, and tangential to its apical and basal surfaces respectively. Contraction is here measured by the ratio of the final to the original magnitude, referred to briefly as the "contraction ratio."

The difference of the best two series of sections from all the others is in the uniformity of their contraction. It will be seen from the drawings that while the living form of the cell is barrel-shaped (figs. 1, 2, 3, 19), the tendency of preservation is to produce a sphere (figs. 17, 18, 20, 21; Dendy's figs. 24 and 25, plate 14, vol. xxxv, and fig. 38, plate 4, vol. xxxii, of this journal, &c.). This necessarily produces a highly disproportionate contraction at the base of the collar and it results that the measurement of the ratio of this dimension to the greatest width of the cell affords a fair index of the distortion which the preparation has suffered. Thus the artifact nature of Sollas's membrane is concisely demonstrated by the following figures, averaged from all the measurements:

Basal width in living cells	6·4 μ.
„ in balsam with separated collars	5·6 μ.
„ in balsam showing Sollas's membrane	5·6 μ.
Collar-width in living cells	4·6 μ.
„ in balsam with separated collars	4·2 μ.
„ in balsam showing Sollas's membrane	2·7 μ.

In life, as in the preparations where collars are separated, the collar-width—that is, the apical width of the cell—averages three fourths of the extreme width. Where Sollas's membrane is present the collar-width ranges from two thirds to one third of the extreme width of the cell.

The change can be best followed by comparing figs. 19 and 20 (series B), which are drawn from the same sponge to the same scale,—the one in life, the other from a paraffin section mounted in Canada balsam.

The nett result of the measurements may be seen in the averages of three series of paraffin sections, D, C, and A:

	cubic μ.		μ.
Cell-volume in life (cf. figs. 1, 19)	270	Collar-width in do.	4·6
„ in balsam, Series D (cf. fig. 15)	170	„ „	4·3
„ „ „ C	135	„ „	3·0
„ „ „ A (cf. figs. 17, 18)	865	„ „	2·2

There are, therefore, two principal phenomena due to the

transference of cells through osmic acid, alcohol, and benzol, into paraffin, and finally Canada balsam :

(1) There is a reduction in the total volume of the cell, which apparently cannot be avoided, corresponding to a mean linear contraction of about 5 : 4 in the best preparations, and 5 : 3 in the worst.

(2) Independently of the extent to which this takes place there is generally a change of form. It appears possible (cf. figs. 1, 15) almost entirely to avoid this, but by most methods the rectilinear and angular outlines of life (figs. 1, 2, 3, 19) are replaced by pyriform (figs. 20, 21), ovoid (fig. 17), spherical or even oblate (fig. 18) contours in the permanent preparations.

Thus, taking from the averages of the last table the consequent ratios of the linear dimensions to those of life, we obtain :

Mean linear contraction ratio in—		Contraction ratio of collar-width in same sections.
Series D (fig. 15)	·82	·83
Series C	·8	·68
Series A (figs. 17, 18)	·6	·46

It was experimentally shown that the extreme changes of cell-form were not produced in alcohol. Bringing part (E) of a sponge in four minutes through 30 per cent. and 50 per cent. into 70 per cent. alcohol, the cells were compared in paraffin sections with the part (D) of the same sponge treated uniformly by slow dialysis. The collar-width (4·0 μ) and the basal width (5·4 μ to 5·7 μ) in E retain their normal proportions to each other, and the collars are not united. It is true, however, that the mean contraction is greater (ratio ·74) than in D, and the height of the cells is disproportionately diminished (7·8 μ as against 9·5 μ in D and 12·0 μ in life).

It was also experimentally shown (fig. 16) that in some sections of the best series (D, cf. fig. 15) stained on the slide in the ordinary way through turpentine and four grades of alcohol into Grenacher's hæmatoxylin, the cells suffered considerable distortion, and in many cases developed Sollas's

membrane. Similar results were obtained on clearing the sections in benzol and in olive oil.

I am inclined to consider the chief engine of distortion to be the passage from alcohol into benzol, chloroform, or turpentine, and vice versâ. The cells of fig. 15 probably escaped, not only because the passage into benzol was effected by very gradual dialysis, but because they were first hardened in alcohol between 85 per cent. and absolute strength for some eighteen hours. It may be noted that the tendency of all the cells to assume a drop-like form proves that the force effecting their distortion is surface-tension.

It does not seem unlikely that the reduction in volume is due to the abstraction of water and soluble matters by the alcohol. It is not due to shrinkage of the paraffin block, for from the standard tables contraction through 45° C. would be in wax to ·96, and in paraffin not more than to ·99 of the original linear dimensions. I have no reason to suppose that there was any appreciable compression in cutting the sections; and since the nuclei remain spherical, and the collars are unaltered in length, this cannot be assumed. But it must be pointed out that the mean contraction-ratio is less certain than the amount of distortion, since it involves the measurement of the living cell-height, which can only be done accurately in fortunate instances.

The collar rarely contracts in length; this may either be due to its thinness, or to the nature of the rods which compose it. Sollas's membrane may be due to either a local constriction of the collar or the forcible contraction of its base throwing out the free lip; it should be noticed, however, that in such a section as is drawn in fig. 18, the chamber has so far contracted as a whole, that where the free ends of the collars remain of their living diameters, they must be pushed into contact.

By the definition of "basal width" employed, it will be seen that this measurement expresses the linear contraction of the wall of the chamber as a whole. There is generally least contraction in this plane, the tendency of the cell to become

spherical increasing the breadth in proportion to the height. In Series C the measurements give no evidence of contraction in this dimension; but the cells are spherical and even orange-shaped, showing that the absence of change in anatomical dimensions is no guarantee against the most profound cellular distortion.

Methods.

The main practical conclusion was that cell-form tends to be profoundly modified in the passage between alcohol and paraffin solvents, and that this may unfortunately be the case even in the process of staining on the slide. It seemed likely that the dangers of the embedding process are modified by very gradual dialysis from alcohol into benzol, and largely guarded against by super-hardening in 1 per cent. osmic acid and in absolute alcohol. For osmic acid even the sponge tissue requires to be cut in the smallest practicable pieces and repeatedly shaken, otherwise the inner chambers are not thoroughly hardened; the exposure used was one hour in the dark. Dialysis from water into absolute alcohol, or from alcohol into benzol, each took from six to twelve hours; they were left up to fifteen hours with good results. The best preparation (Series D) was stained in bulk with equal parts of Grenacher's hæmatoxylin and 70 per cent. alcohol, being brought into this solution from 40 per cent. alcohol by four equal changes of strength; no acid was used, and the result was a very valuable overstaining of the collars and iris membranes. The sections were fixed with water, the paraffin cleared in chloroform. It will be found convenient to have in a pipette a thin solution of balsam in chloroform, so that it can be squirted instantly on the sections after removal from the chloroform, to prevent drying before the thicker balsam has time to spread.

It will be seen that I am greatly indebted to the methods of Vosmaer and Pekelharing (19), which were closely followed up to the stage of embedding in paraffin; but I am convinced that staining on the slide is highly destructive of cell-form, unless the transference from benzol to alcohol be effected with

the tedious care used for the tissue in mass. I believe the form of the cells in Vosmaer's drawings has been influenced by this process, though the oval outlines of nuclei and vacuoles in the sections is probably attributable to the razor. Passage into glycerine is of course attended with the same necessity of preliminary passage into alcohol, but comparison with similar sections stained on the slide and mounted in Canada balsam show that the cells in glycerine are only equally distorted or contracted, and, as these authors state, the collars and flagella are more visible, and the preparation very brilliant.

References.

1. Johnston, G.—1842, 'A History of British Sponges and Lithophytes.'
2. Carter, H. J.—1875, 'Annals and Mag. Nat. Hist.,' vol. xvi, p. 1.
3. Schulze, F. E.—1875, 'Zeitschr. wiss. Zool.,' vol. xxv (suppl.), p. 247.
4. Barrois, C.—1876, 'Ann. Sc. Nat.,' ser. 6, vol. iii, art. 11.
5. Schulze, F. E.—1878, 'Zeitschr. wiss. Zool.,' vol. xxxi, p. 262.
6. Metschnikoff, E.—1879, ibid., vol. xxxii, p. 349.
7. Heider, K.—1886, 'Arb. Zool. Instit. Wien,' vol. vi, p. 175.
8. Bidder, G.—1888, 'Proc. Phil. Soc. Cambridge,' vol. vi, pt. 4.
9. Topsent, E.—1888, "Thèses presentées à la faculté des Sciences de Paris," 'Contributions données par la faculté,' Poitiers, Typographie Oudin.
10. Lendenfeld, R.—1889, 'Zool. Anz.,' xii, p. 361.
11. Lendenfeld, R.—1889, 'Zeitschr. wiss. Zool.,' vol. xlviii, p. 406.
12. Dendy, A.—1890, 'Quart. Journ. Micr. Sci.,' vol. xxxii, p. 1.
13. Chatin, J.—1890, 'Comptes Rendus,' p. 889.
14. Dendy, A.—1891, "A Monograph of the Victorian Sponges," part 1, Melbourne, 'Trans. Roy. Soc. Vict.,' vol. iii, p. 1.
15. Bidder, G.—1891, 'Quart. Journ. Micr. Sci.,' vol. xxxii, p. 625.
16. Minchin, E. A.—1892, ibid., vol. xxxiii, p. 251.
17. Minchin, E. A.—1892, 'Zool. Anz.,' xv, p. 180.
18. Bidder G.—1892, 'Proc. Roy. Soc.,' vol. li, p. 474.
19. —Vosmaer, G. C. J., and Pekelharing, C. A.—1893, 'Tijdschr. Nederl. Dierk. Ver.,' (ii), Deel. 4, p. 38.

20. Dendy, A.—1893, 'Quart. Journ. Micr. Sci.,' vol. xxxv, p. 159.
21. Maas, O.—1893, 'Z. Jahrb. Morph. Abth.,' Bd. vii, p. 331.
22. Franzé, R. H.—1893, 'Zool. Anz.,' xvi, p. 44.
23. Masterman, A. T.—1894, 'Annals and Mag. Nat. Hist.,' vol. xiii, p. 485
24. Topsent, E.—1894, 'Arch. Zool. Exp.,' vol. ii, p. 283.
25. Schaudinn, F.—1894, 'Sitz. Akad. Wiss.,' Berlin, lii, p. 1277.

EXPLANATION OF PLATE 2,

Illustrating Mr. G. Bidder's paper on "The Collar-cells of Heterocœla."

Figs. 1 to 14 and fig. 19 are from living cells. All the drawings except figs. 3 and 21 are multiplied about 1000 times linear; figs. 1, 2, 15, 16, 17, 18, 19, and 20 being drawn with the camera lucida,[1] figs. 9, 11 *c*, 12, and 22 drawn free-hand and scaled from micrometer measurements, the remaining figures are free-hand drawings approximately to the same scale. All drawings were made with Leitz $\frac{1}{12}$ oil-immersion, Zeiss oc. 3 old system, rarely oc. 4.

Figs. 3 and 11 are from Sycon raphanus, fig. 21 from Leucandra aspera, fig. 22 from Acanthella pulcherrima, the remainder from S. compressum.

Fig. 1.—Drawn from living S. compressum, forty minutes after it was taken from a tide-pool. These collar-cells were pressed against the cover-slip, hence they appear closer together and more in one place than in the other figures. Cf. fig. 15.

Fig. 2.—Another part of the same sponge, drawn immediately after the section was placed on the slide. The flagella were so active that only their bases could be drawn.

Fig. 3.—S. raphanus, living collar-cells (Naples, 1889), prob. × 2500. The shaded spherules were stained with Bismarck brown; the full number is not drawn in all the cells.

Fig. 4.—Two cells with distal globules (excreta ?), alive, flagella very active; from S. compressum two and a half hours exposed by the low tide, two

[1] The small numerals at the side of fig. 16 show the distortion found to exist in drawing with the Nachet camera when all adjustments are made with apparent accuracy.

hours in sea water after gathering. Very satisfactory preparation; all over it could be seen tall cylindrical cells, wide apart, with stiff cylindrical collars and flagella very active until two and a half hours after the preparation was made. These cells drawn in the first half-hour. Part of the same sponge placed when this preparation was made into osmic acid for an hour and a quarter, and dialysed through alcohol and benzol, showed in sections stained on the slide spherical or oblate collar-cells with a flat Sollas's membrane and few flagella (possibly due to imperfect dialysation in benzol).

Fig. 5.—From same sponge as fig. 19, six hours in a small saucer of sea-water; flagellar movement languid.

Fig. 6.—S. compressum, flagella moving.

Fig. 7.—S. compressum. Edge of collar showing beaded or milled-edge appearance, flagellum in optic section; same preparation as fig. 5.

Fig. 8.—S. compressum, living section; *a*, soon after the preparation was made; *b*, twenty minutes after, the flagella in very violent action; *c*, one hour forty minutes after (the two left-hand cells of *b*), the right flagellum was gone, the left still working; *d*, two hours twenty minutes after, the tops of the same cells, the bodies being hidden. Flagella were still moving in many of the chambers two hours thirty-five minutes from the time the preparation was made; many of the collar-cells were elongated to six or seven times their width.

Fig. 9.—Two successive drawings of a cell from the same sponge as fig. 4, but an hour and a half after the preparation was made. Part of the section was dead; the flagellum of this cell was moving well. Note the very long collar.

Figs. 10 and 12.—S. compressum gathered under a moist rock, placed for three hours in the circulation of the Biological Station. The first drawings from the living section present nearly the same appearance as fig. 1, the cells being short and more closely packed than usual. After three quarters of an hour the appearance is much as in fig. 8*a*, and the flagella are growing slack. Fig. 10 was drawn one hour and twenty minutes, and fig. 12 one hour and fifty minutes after preparation; the flagella were still moving in fig. 10, motionless in fig. 12. No further change was observed two and a quarter hours after preparation. Paraffin sections formed Series C of the text.

Fig. 11.—S. raphanus, some time under the cover-slip. There were flagella still moving in the preparation, though there were none visible on the cells drawn in *b* and *c*.

Fig. 12.—See fig. 10.

Fig. 13.—S. compressum, ten hours in sea water after twenty-seven hours absence from it; flagella moving actively. This is the typical form of cell, though there are a few with collars of the normal form. As noticed also

in other sponges there were in the chambers large masses containing hundreds of transparent globules (fig. 13 *a*) laden with small detritus. While their individual size and appearance strongly suggest ejecta from the cells (cf. figs. 4 and 10), their large aggregate mass makes this supposition difficult without stronger evidence.

Fig. 14.—From the same sponge after one day more in sea water. Most chambers showed perfectly normal collars and flagella; this (transitional?) form occurred in several places. Flagella active.

Fig. 15.—Series D of paraffin sections, preserved in osmic 1 per cent. at the time fig. 1 was drawn from the same sponge. The cells are very unvarying throughout the preparation, fusion of collars being rare and difficult to find; it occurs in a few cells. (See also woodcut *a*, *b*).

Fig. 16.—A typical set of cells from another slide of the same series of sections as fig. 15; fixed with water, cleared in turpentine, passed through absolute, 90 per cent., 70 per cent., 50 per cent., and 30 per cent. alcohol into Grenacher's hæmatoxylin; after two minutes back in the reverse order, half a minute in 30 per cent. and some minutes in each of the other alcohols, mounted through turpentine in Canada balsam and chloroform. Perhaps a quarter of the collars in this preparation are unaltered in form, most are either shortened or constricted, some of the cell-bodies are contracted.

Fig. 17.—S. compressum. A Sollas's membrane halfway up the collars, shown by careful focussing with the immersion lens to consist, as here drawn, of a series of bars and bands. With a dry lens it is seen as a strongly-stained line quite continuous round the chamber.

Figs. 17 and 18 are from Series A; in about half the chambers the collars are separated, in about half united. Preservation as in text, except that the passage into alcohol was by 10 per cent. changes every ten minutes, and the tissue was eighteen hours in paraffin at 63° C. before embedding. These two sections stained on the slide in Grenacher's hæmatoxylin and mounted in glycerine.

Fig. 18 (v. supra).—Typical Sollas's membrane, very frequent. The roughly shaded portion indicates the basal parts of cells above the focus, the under surface of the membrane being seen.

Fig. 19.—S. compressum, living cells, flagella in movement. See fig. 20.

Fig. 20.—Typical part of a section (Series B) made from the sponge from which fig. 19 was drawn; after preservation at the same time in osmic acid 1 per cent. eighty minutes, 10 per cent. changes of alcohol every eight minutes, 10 per cent. or 15 per cent. changes of benzol every quarter of an hour; stained on slide, Grenacher's hæmatoxylin.

Fig. 21.—Sollas's membrane from a paraffin section of L. aspera; preserved osmic acid 1 per cent., brought gradually through alcohols and decalcified with 1 per cent. formic acid in 90 per cent. alcohol, embedded through

chloroform. The outlines of cells in the adjoining chamber are shown; the dark spot and the black dots are carmine, with which the sponge had been fed for four hours and a quarter. The preparation is unstained; the light shading of the spherules is due to osmic acid.

Fig. 22.—Living flagellate chamber from Acanthella stipitata, Carter, drawn to the same scale as figs. 1, 2, 11 *c*, 12, &c. The shaded dots are the bases of collar-cells, the white space the apopyle.

The Metamorphosis of Echinoderms.

By

Henry Bury, M.A., F.L.S.,
Late Fellow of Trinity College, Cambridge.

With Plates 3—9.

Introduction.

Agassiz's view, put forward more than thirty years ago (1, p. 61), that the actinal and abactinal surfaces of Echinoderms (at least of Echinids, Asterids, and Ophiurids) are formed from the right and left enterocœl pouches ("water-tubes," as he calls them) respectively, has met with very general acceptance; but surprisingly little has been done to test it by modern methods.

Agassiz's observations were, of course, made on the whole larvæ, without any assistance from sections. Götte (9) in 1876 applied the section method to Antedon, and found Agassiz's view to hold good there; yet, on the other hand, Ludwig (15) in 1882 failed altogether to trace this symmetrical arrangement of the enterocœls in Asterina, while he showed conclusively that Agassiz was mistaken in supposing that the left enterocœl gives rise to nothing but the hydrocœl.

No one has ever attempted, so far as I know, to show how Holothurians can be included in Agassiz's scheme; and the conclusion of those who have studied their development has generally been (21, 31, 32) that in retaining the dorsal mesentery of the larva as a longitudinal mesentery in the adult (not a transverse one, as it is in Antedon, and should be on Agassiz's

hypothesis) they exhibit a primitive approach to a worm-like ancestor.

With a view to clearing up some of these differences of observation and opinion, I commenced in the spring of 1888 an examination of the metamorphosis of all available Echinoderm larvæ, paying special attention to the behaviour of the mesentery, in the belief that this would afford an important clue to the difficult question of the origin of Echinoderms and the relation of the radiate adult to the bilateral larva.

The solution of so wide a phylogenetic question cannot be hoped for, and should not be attempted as often as it is, from the narrow standpoint of the ontogeny of one or two forms; nor should too much reliance be placed upon the accounts of other observers, however careful they may be, whose attention has not specially been directed to the points at issue. As far as possible, therefore, I have worked out for myself the metamorphic changes of at least one form of larva in each of the five classes of Echinoderms.

I very soon found, however, that to understand these changes properly it was necessary to go back in almost every case to much earlier stages—in some cases right back to the earliest formation of the body-cavities from the archenteron. It would have been most undesirable to burden the pages of the present paper with the preliminary results thus obtained; and I therefore in 1888 (5) published an introductory paper on the subdivision of the enterocœl and the origin of the skeleton, which I intended to follow up as soon as possible with the present paper on the metamorphosis and phylogeny; but ill-health altogether stopped my work for a long period, and even now the difficulty of obtaining material has prevented me from carrying out my scheme as fully as I had hoped. It did not seem desirable, however, to withhold any longer the numerous facts bearing on the subject embodied in the subsequent pages.

Most of the material for these studies was obtained at the Zoological Station at Naples in the spring of 1888, but a second visit there in 1893 was necessary to complete my studies of Auricularia; while for most of my larvæ of Asterias rubens

I am indebted to the kindness of Professor McIntosh, who most generously placed at my disposal the resources of the Marine Laboratory at St. Andrews, where these larvæ are exceedingly abundant.

Of course it is not possible within the limits of this paper to give a detailed account of the development of every larva; and indeed so much is already known of most of them as to render this unnecessary; I have therefore, in most cases, given only such a brief outline of the facts as would render the metamorphosis intelligible and bring into prominence those features to which I attach theoretical importance. This method has indeed the disadvantage of making my work appear somewhat sketchy and superficial, but this seemed preferable to loading the pages with a number of facts having no connection with the theoretical views expressed in the second part of this paper, or with a mere recapitulation of observations originally made and recorded by other writers. In the case of Holothurians, however, it has been necessary to describe in some detail the metamorphosis of Synapta, as almost all the points which seem to bear on the origin of this class have been overlooked by previous observers.

To this alone is due the prominent position of the Holothurians in the ontogenetic portion of this paper—the order in which the various groups are taken being throughout purely a matter of convenience, and wholly independent of all phylogenetic considerations.

Part I.—Ontogeny.

A. Holothurians.

Metamorphosis of Synapta.

The earliest stage of Auricularia with which we need here concern ourselves is one in which the full size is already attained, and the eleven pouches of the hydrocœl (five large tentacles, five as yet small radial vessels, and one polian vesicle) distinctly visible. The right and left body-cavities are still small, and have hitherto been symmetrically arranged

and about equal in size; but now a small finger-like process is pushed out from the anterior end of the left cavity (fig. 1) which very soon meets the posterior end of the hydrocœl, and grows forward along its ventral surface. This process, which has no fellow on the right side, was recognised by Metschnikoff (21, pl. ii, fig. 11), but seems to have escaped Semon's notice. While it is forming, the two body-cavities begin to grow towards one another on the ventral side, a little behind the œsophagus, the growth both of this part and of the anterior process of the left cavity being marked by great pseudopodic activity of the cells forming the walls of the cavities (fig. 1).

About twenty-four hours after the appearance of this process of the left body-cavity, we reach the stage represented in fig. 2. The body-cavities have come close together ventrally, but not symmetrically, a portion of the left cavity overlapping the anterior edge of the right (fig. 3). It is further to be noticed that while the left cavity now extends anteriorly all along the ventral face of the hydrocœl ("oral cavity," fig. 3) it scarcely extends so far posteriorly as the right cavity. In sections (with the help of which I have confirmed all the observations embodied in the figures) the external wall of the left body-cavity exhibits a marked thickening in the region in which the two cavities approach one another; occasionally, though rarely, there seems to be already a fusion of the two cavities at this point.

The Auricularia has now reached its latest stage of development, and in a very few hours the whole aspect of the larva is changed, and the metamorphosis from the bilateral into the radial form has begun.

As several points in this change have been overlooked both by Metschnikoff (21) and by Semon (32), we must follow it in considerable detail. The onset of metamorphosis is marked externally by the breaking-up of the ciliated bands, the collapse of the stomach, and the growth of the hydrocœl round the œsophagus; and as far as possible these various groups of phenomena will be dealt with separately, though it is impossible to keep them wholly apart.

Ciliated Bands and Rings.[1]—Even before the collapse of the stomach the ciliated band of Auricularia shows signs of breaking up, at first only by a thinning of the band at certain points (fig. 2), and later by its complete separation into segments. Many of the changes which follow have already been correctly described (**21** and **32**), but as much has been misunderstood (especially by Semon) it will be better to describe the phenomena in full. To simplify this description as far as possible I shall begin at the posterior end of the larva, where the changes are least complicated; and with the help of figs. 3, 4, and 5, the formation of the two posterior (fourth and fifth) ciliated bands ought to be easily intelligible.

It is convenient to divide the ciliated band of Auricularia into three regions: (1) an anterior ventral, (2) a posterior ventral loop, and (3) a pair of longitudinal bands uniting these loops. These will at once be recognisable in any figure of Auricularia. In fig. 3 the whole band is outlined, but only those parts which persist and form the ciliated rings of the pupa are shaded—the dorsal parts (seen through the transparent tissue) being left lighter than the ventral. It must be understood that this arrangement is purely diagrammatic, and that the persistent parts of the band do not admit of accurate delimitation.

Fifth (posterior) Ciliated Ring.—This is usually formed from the two lateral (right and left) pieces of the ciliated band, which form the junction of the longitudinal bands with the posterior ventral loop (fig. 3, V); sometimes, however (for a short time time only), each piece is further divided into a dorsal and a ventral portion. The two halves (right and left) soon acquire a more transverse arrangement (figs. 4 and 5), and finally completely encircle the posterior end of the body, the anus having meanwhile assumed a terminal position.

Fourth Ciliated Ring.—This is invariably formed from four pieces (figs. 3 and 4, IV). The dorsal and ventral por-

[1] It is convenient to distinguish between the ciliated band of Auricularia and the ciliated rings of the pupa or "barrel" stage.

tions on each side unite first (fig. 5), then the two sides unite ventrally, and at a later period their union on the dorsal side completes the ring.

Mouth and Atrium.—Before describing the formation of the three anterior rings, we must turn for a moment to the behaviour of the mouth, and the formation of the atrial cavity.

Quite early in the metamorphosis four pieces of the ciliated band group themselves round the mouth and there form a ring (figs. 3 and 4; and **21**, pl. ii, figs. 14—16). The fate of the "nerve bands" (fig. 1) I have never been able to determine.

In fig. 4 it will be noticed that the mouth is pushed over to the left side of the larva, while the apex of the latter is turned considerably to the right. The latter change is partly visible in fig. 3, but varies considerably in different larvæ, being always most marked in spirit specimens in which some shrinkage has occurred.

In the next stage the mouth retreats into the interior, and an atrial cavity is formed, the external opening of which narrows rapidly and passes over to the left side (fig. 5); the apex of the larva has meantime nearly regained its original position, though it is still much to the right of a line passing through the longitudinal axis of the stomach.

The portions of the ciliated band which, as above described, encircle the mouth, now lie at the bottom of the atrial cavity, where they form, as Metschnikoff described, the epithelium of the tentacles. Though they form a ring, I have not included it among the ciliated rings of the pupa, of which there are five outside the atrium.

Figs. 4 and 5 represent such well-marked stages in the metamorphosis that it will be convenient to refer to them in future as marking, respectively, stage A (before the atrium is formed) and stage B) with the aperture of the atrium not yet terminal and the five ciliated rings still very incomplete). We will now return to these ciliated rings, to assist the study of which I have given in fig. 6 a diagrammatic view of the anterior pole of Auricularia, constructed on the same lines as fig. 3.

Third Ciliated Ring.—This is formed as simply as any, from one piece on each side (occasionally two on the left side, the ventral one being small, and soon joining the dorsal one). In stage B (fig. 5) they join on the ventral side, but remain apart much longer on the dorsal side, where they eventually unite in front of the water-pore.

First and Second Ciliated Rings.—Figs. 3 and 6 will show that we have now four pieces of the ciliated band with which to construct the two anterior rings. At first they form two calliper-like loops, one on each side (fig. 6), and this arrangement is still partly retained in stage A, of which fig. 7 is a polar view.

In stage B (figs. 5 and 8) the opening of the atrium has moved into the left loop, and it seems as if this loop were about to form the first ciliated ring. Fig. 9, however, shows that only the ventral portion of the loop is concerned in forming this ring, while three pieces (II *a*, *b*, and *c* in figs. 7, 8, and 9) form the second ring. The two pieces of the right side (II *b* and *c*) usually unite first (occasionally II *c* is absent), and a little later the right and left sides unite dorsally; lastly they unite on the ventral side, and the ring is complete.

The investigation of the phenomena just described requires a good deal of care and patience. The rapidity of the changes is one of the most serious difficulties, necessitating a rigorous search each day among one's specimens for signs of the impending change. At the time when most of my observations were made (Naples, March and April, 1888) the stage shown in figs. 2 and 3 was usually reached about ten o'clock on one morning, stage A late that evening, and the stage shown in fig. 9 about ten o'clock the next morning; after that the changes were slower. Development was much slowed down by keeping the larva in cold water, and in this way I was able to obtain all the intermediate stages without resorting to twenty-four hours' consecutive watching.

Another difficulty lies in the shape of the larva, which makes it hard to obtain prolonged views of any but the dorsal

and ventral surfaces ; and in their delicacy, which renders it almost an impossibility to preserve and embed them without losing much of the original form by shrinkage. I have succeeded fairly well by carefully embedding in celloidin, but the tissues are never so clear as in the living animal. Almost equally good results, with greater economy of material, may be obtained by balancing the living larva in a watch-glass with one hand, making a rough sketch with the other, and gradually correcting and adding details with the help of repeated observations. No doubt this requires practice, but it has the advantage that several stages can be followed on the same larva. All my polar diagrams (figs. 6—9) were made in this way, and though not drawn quite to the same scale, are accurate enough for my present purpose.

Semon has drawn attention to the marked diminution in size which accompanies metamorphosis, and has given some measurements (**32**, p. 29), but these can only be regarded as approximate, the variations in size being great.

The discrepancies between Metschnikoff's account and Semon's are so very large, that I naturally looked out carefully for any abnormalities which might help to reconcile them ; but though a good deal of variation was noticeable (some of it possibly due to specific differences), none of it threw any light on this point. Metschnikoff's description and figures are on the whole extremely accurate, and my account is rather an addition to his than a correction of it. It is curious, however, that he overlooked the asymmetrical position of the mouth and atrium, since two of his figures show it (**21**, pl. ii, figs. 16 and 18), one of them representing the water-pore and atrium on the same side of the body, which could not happen if they occupied the positions he assigns to them. His failure to understand the formation of the two anterior ciliated rings was probably due partly to the difficulty of obtaining polar views, which alone render the changes intelligible ; and partly to his having missed that stage, which, in my experience, usually occurs at night.

Semon's account I am wholly at a loss to understand, so

entirely does it differ from anything which has come under my notice. We can hardly have obtained different larvæ, since we both worked at the same place (Naples) and at about the same time of year (January to the end of May, 1888, in my case; November, 1885, to October, 1886, in his—but few larvæ found after March); and although during my subsequent visit to Naples in 1893 I met with many abnormal specimens (obviously pathological, as the condition of the whole ectoderm showed) yet none of them ever approached those figured by Semon as intermediate between Auricularia and the pupa (**32**, pl. i, figs. 5 and 6). Stranger still is the fact that while these figures are wholly irreconcilable with Metschnikoff's, he does not even allude, in this connection, to any differences between their accounts.

Hydrocœl, &c.—On a previous occasion (**5**, p. 11) I described the formation of a cavity which I regarded as the homologue of the left anterior body-cavity in the Echinoderms. As Ludwig (**17**, p. 609) seems to think this homology disproved by his observations on Cucumaria, it will be well to review briefly the grounds on which my suggestion was based.

When Ludwig first noticed this cavity (which he calls the "Madreporenblase") in Cucumaria it had the appearance of a simple swelling on the water-tube; and as he could not find any trace of it on the previous (fourth) day, he concludes that it is altogether secondary.

Whatever may be the case in Cucumaria, this conclusion is not justified in Auricularia. There, after the separation of the posterior body-cavities, the anterior portion of the cœlom forms a small, pear-shaped, thin-walled vesicle, from which a short tube with thicker walls ("pore-canal") runs to the exterior. Then the left wall of the vesicle thickens and presently produces the rudiments of the radial canals and tentacles; but the walls of the dorsal portion, into which the pore-canal opens, still remain thin. Shortly before metamorphosis this thin-walled portion becomes constricted and divided into two, the smaller of which remains in connection with

the thick-walled portion and forms the inner wall of the hydrocœl; while the other one (the dorsal), which is Ludwig's "Madreporenblase," is, in my opinion, the homologue of the anterior body-cavity in other Echinoderms (see figs. 1 and 2; and 5, figs. 22—25). The two cavities are rapidly pushed asunder by the formation of a second thick-walled tube, which almost immediately becomes continuous on one side of the "Madreporenblase" with the pore-canal. This new tube I regard as the true water-tube, though Ludwig, under the name of "Steinkanal," confuses it with the pore-canal (for the distinction see 5, p. 21).

No one from the study of Auricularia alone can say that one of these two cavities ("Madreporenblase" and hydrocœl) is more primitive than the other—they are parts of the same primary cœlomic pouch. Moreover, had Auricularia been opaque—had I been forced to rely on sections alone, it is very probable that I should have overlooked this division (as previous observers had done), so rapidly does it take place, and regarded the "Madreporenblase" as a later outgrowth of the water-tube. With all regard to Ludwig's admirable care in research, I cannot at present feel satisfied that he has not overlooked similar changes in Cucumaria.

To sum up, we find in Auricularia two cavities and a tube connecting them having precisely the structure and relations of the anterior body-cavity, hydrocœl, and water-tube in other Echinoderms; and to my mind it is far easier to believe that the "Madreporenblase" of Auricularia is the anterior body-cavity, than to admit that a cavity which is present in all other Echinoderm larvæ yet examined is totally absent in Holothurians.

In the fully-formed Auricularia (fig. 1) the hydrocœl, which is flattened dorso-ventrally, lies with its posterior end slightly nearer the ventral surface than its anterior end. Its inner face (that nearest to the œsophagus) is strongly concave, while its outer face, from which spring the radial vessels and tentacles, is convex.

The position of the water-tube, which enters on the inner

side, is certainly not easy to determine with relation to the radial vessels, which are on the outer side; but I have fully satisfied myself by means of sections of this and later stages that it was correctly given in my previous paper (**5**, p. 22), and I cannot admit the plea of variability with which Semon meets my criticism of his figures (**33**, p. 9).

The whole hydrocœl is pushed more and more towards the ventral surface of the larva by the elongation of the water-tube; but the obliquity which brings its posterior end even nearer this surface than its anterior becomes more marked, while the anterior end bends over at the same time more towards the middle line than the posterior (fig. 3).

The formation of the water-vascular ring round the œsophagus follows rapidly on the stage shown in figs. 2 and 3. The posterior end of the hydrocœl bends round on the ventral side of the œsophagus (close to its junction with the stomach) about as far as the middle line. The anterior end, on the other hand, crosses over on the dorsal side of the œsophagus to the right side of the larva, and then, bending posteriorly and ventrally, passes round to the ventral surface, where it eventually joins the other end of the hydrocœl, and completes the water-vascular ring.

It will be remembered that according to Metschnikoff the most posterior pouch of the hydrocœl Auricularia is the rudiment of the polian vesicle, while the most anterior becomes one of the radial canals. Semon expresses some doubt on this point; but a series of sections through the larva during stage A (before the closure of the water-vascular ring) sets this question at rest. The posterior pouch (which at the close of this stage may even be to the right of the middle line, though it seems to vary somewhat) turns slightly inwards, and pushes before it the wall of the left body-cavity (see figs. 10 and 13, in which, however, but little more than the peritoneal covering of the vesicle is visible). None of the other pouches ever project into the body-cavity in this way; and from this and later stages it is abundantly clear that Metschnikoff was right in identifying this posterior pouch with the polian vesicle. It

is clear also that the closure of the water-vascular ring is effected on the ventral side, just at the base of the polian vesicle. How near to the middle line this junction occurs is, however, difficult to determine. I have failed to observe the polian vesicle in external views, owing, no doubt, to its being turned inwards towards the body-cavity; and in sections so much distortion almost inevitably occurs that the exact middle line is hard to determine. Moreover it will be noticed (figs. 4 and 5) that, owing to the asymmetry of the larva in stages A and B, the middle line of the stomach does not correspond with that of the larva as a whole. The polian vesicle, however, is certainly to the right of the middle line in many larvæ at the close of stage A (fig. 13), though there is some reason to think that in later stages it moves back again somewhat to the left: at any rate, the closure of the water-vascular ring, whether to the right or left of this line, is not far removed from it.

In Cucumaria, Ludwig (17, p. 607) thinks that this closure occurs on the right side, not in the interradius of the polian vesicle; and in view of the remarkable discrepancies existing among Echinoderm larvæ on this point, it must be admitted that he is possibly right, but until every stage has been traced (Ludwig admits that he has missed the actual completion of the ring), it would be rash to assert that Cucumaria and Synapta differ in this respect.

With the commencement of stage B and the formation of the atrial cavity (to be further described later on) the hydrocœl ring, which is only completed at the commencement of this stage, assumes a new position. In stage A its oral surface was directed nearly towards the ventral surface of the larva, and only slightly towards the anterior end; now, however, it faces (approximately) the aperture of the atrium, and as this moves towards the anterior end, the water-vascular ring comes to lie more and more nearly at right angles to the longitudinal axis, a position which it finally assumes in the pupa or "barrel" stage. In stage B, however (fig. 5), the aperture of the atrium is not yet polar, and the oral surface of the

hydrocœl ring, facing it, is directed decidedly towards the left side.

This arrangement is of brief duration and easily overlooked, but, as will be shown in the second part of this paper, it affords an important clue to the probable phylogeny of the Holothurians.

In stage A the radial vessels and tentacles lie nearly in the same plane; but in stage B the latter turn up alongside the outer wall of the atrium, while the former bend back parallel to the long axis of the stomach, and grow rapidly towards the posterior pole of the larva. On the outer side of each runs a two-layered prolongation of the wall of the atrium; but this, and the ectodermic covering to the tentacles, are so well known from previous descriptions that they need not be dwelt on here.

Before describing the positions occupied by the parts of the hydrocœl in the fully-formed pupa, it will be well to come to some conclusion as to the nomenclature of the rays.

In adult Holothurians it is usual to speak of the rays as right and left dorsal, right and left ventral, and median ventral; the water-tube being in the dorsal interradius. Ludwig applies these terms to the young Cucumaria; but in the young Synapta their employment might lead to some confusion. Up to the end of stage B, and sometimes even in the pupa, the original right and left sides of the larva are distinguishable by means of the groups of calcareous discs at the posterior end, and this enables us to see that the water-pore remains, as in Auricularia, decidedly to the left of the middle line; and though its exact position in older stages (fig. 9 and later) is rather hard to determine (and perhaps variable), it is never truly median.

Again, the radial vessel which is nearest to the ventral median line in stage B (fig. 5) is certainly not the median vessel of the adult, but (as I believe) the left ventral. Since, then, the two planes of symmetry (larval and adult) do not correspond, it may be better to apply that nomenclature to the rays which has been adopted on morphological grounds in other

Echinoderms. The animal is looked at from the aboral pole, with the interradius of the water-pore directed away from the observer, this interradius being regarded as anterior. The ray immediately to the left of this is designated No. I, the next beyond it No. II, and so on, that immediately to the right of the anterior interradius being No. V. The interradii may be conveniently marked by the letters A, B, C, D, E—A being the anterior interradius, and the order of succession being the same as in the case of the rays. This system of numbering and lettering is adopted in fig. 14, in which the arrangement of the hydrocœl in the "pupa" is diagrammatically shown. As there seen, the water-tube is adradial, being nearer to radius V than to radius I, from which it is separated by one of the tentacles. The polian vesicle is also adradial, lying in interradius B, close to radius II.

Semon's figure (32, pl. ii, fig. 2) of these parts is extraordinarily inaccurate; not only, as I pointed out before (5, p. 22), is it out of harmony with his statement of the position of the water-tube in Auricularia, but this tube is represented on the wrong side of the polian vesicle; seen from the side, as he has drawn it, it should be on the right of this vesicle (compare fig. 14), but he has represented it on the left!

In none of my specimens can I detect that curious relation of the tentacles to the radial vessels which Ludwig describes in Cucumaria (16, p. 183); but if, as is probable, it obtains in Synapta also, in later stages than I have examined, it may help to explain one curious discrepancy between Synapta (and probably Holothurians as a whole) and other Echinoderms. In all Echinoderms, at any rate in young stages, the water-tube is adradial, but in most forms it is nearest to the left side of the interradius in which it lies; in the larva of Synapta, however, and of Cucumaria (16, pp. 187, 188; 17, p. 611) it lies, as shown in fig. 14, on the right of this interradius. Now if the tentacle which separates it from radius I belongs to this radius (as Ludwig says), it may be that the precocious development of this tentacle has pushed the water-tube (in appearance at least) somewhat out of its

true position; but even this does not wholly account for this peculiarity.

Alimentary Canal.—Although in stage A the mouth is much narrowed, yet it retains throughout this stage its ventral aspect. With the beginning of stage B, however, it follows the inward movement of the hydrocœl ring, and turning slightly inwards from the ventral surface, is bent upwards and towards the left side; in effecting this change it drags in not only the thick ectodermic ring derived from the ciliated band (**21**, pl. ii, fig. 16) but also some of the transparent tissue surrounding it, and an atrium is formed, lined by ectoderm.

The aperture of this atrium is at first fairly large, but it rapidly narrows, and passes up the left side of the larva towards the anterior pole; in doing so it gets further and further away from the mouth, and the atrium in consequence increasing in size, more of the transparent tissue becomes involved in its formation.

The stomach is sharply contracted at the onset of metamorphosis, diminishing to about half its original diameter; and its internal surface is consequently thrown for a time into strong folds. Otherwise it undergoes no important changes.

The intestine in Auricularia occupies a position which is nowhere else met with in Echinoderm larvæ, being directed downwards and backwards (figs. 1 and 2), instead of, as in all other cases, running forwards along the ventral surface of the stomach.

In stage A a small cæcal pouch usually (but not invariably) appears at the base of the intestine, running forward from the junction of the latter with the stomach. Early in stage B this part of the intestine widens rapidly, and its opening into the stomach is shifted to the left side. In this way the cæcum of the previous stage is transformed into a short transverse intestine (fig. 5; and **21**, pl. ii, fig. 16), from the right side of which the original, posteriorly directed intestine runs back to the anus, which is still median.

This transverse intestine rapidly increases in size, and becomes slightly bowed, its anterior margin being strongly

convex, and extending quickly forward along the ventral surface of the stomach nearly in the middle line. Although the concavity of its posterior margin is not so marked as this anterior convexity, yet it is for a short time sufficiently evident to enable us to distinguish an ascending portion running up on the left side from the junction with the stomach, and a descending portion, continuous with the remainder of the intestine, on the right. (It is curious that Semon in all his figures represents the ascending portion as being on the right side.)

The forward growth of the convex margin of the intestine reaches its limits in the young pupa, in which it extends nearly as far forward as the water-vascular ring, the polian vesicle being just internal to it (fig. 15). The combined width of the ascending and descending portions of the intestine, just where it bends over, very nearly equals that of the stomach.

After this the importance of this part rapidly declines; and before the ciliated bands of the pupa have wholly disappeared, the intestine is almost completely straightened out (see **21**, pl. iii, fig. 23).

Cœlom.—The question of the existence of an anterior enterocœl has already been discussed; and we have only here to consider the behaviour of the posterior body-cavities, which we left in Auricularia just meeting (but not uniting) on the ventral surface of the stomach, the left one having a tubular prolongation forward on the ventral side of the hydrocœl.

This is as far as the cavities can be traced in the living animal, the collapse of the stomach and increased opacity of the tissues rendering further observations, except by means of sections, almost impossible. Even in sections the changes are sufficiently hard to trace, owing to the rapidity with which they occur and to the excessive delicacy of the tissues. All through stage A there should not be a greater difference than three hours between the ages of the larvæ examined, while to avoid shrinkage the greatest care must be taken in every stage of preservation; embedding is best done first in celloidin

and then in paraffin—the latter alone, in my experience, gives no satisfactory results with any of the pelagic forms of Echinoderm larvæ.

The "oral cavity" (as I propose to call the anterior prolongation of the left body-cavity) closely follows the movements of the hydrocœl. Its anterior end is thus bent round the dorsal surface of the œsophagus, and down on the right side; while its posterior end, where it joins the left body-cavity, is brought down by the movements of the hydrocœl nearer to the ventral surface and to the middle line. This end of it, however, does not correspond with the extreme posterior end of the hydrocœl, but passes on to the surface of this organ at first between the polian vesicle and the second tentacle, and afterwards, when the polian vesicle has about reached the middle line, between the second tentacle and the first radial vessel. At this point, towards the end of stage A, it separates completely from the left body-cavity. Its two ends then come nearer and nearer together, and, as I believe, fuse together early in stage B, so as to form a complete ring on the oral surface of the water-vascular ring (figs. 11 and 15). It is just possible, indeed, that the two ends do not fuse, but that a thin mesentery remains separating them; but I have not been able to find anything of the kind.

The subsequent history of this cavity I have not traced. It may be identical with the cavity figured by Semon in the same position at a later stage (**32**, pl. iv, fig. 9), but none of my specimens are old enough to determine this.

Besides this oral cavity, we noticed in Auricularia another process of the left body-cavity overlapping the anterior end of the right body-cavity on the ventral side (fig. 3). This process (hardly recognisable in Auricularia) increases rapidly in size during stage A, and may be called the "ventral horn" of the left body-cavity. It grows rapidly round the right side of the œsophagus, on to the dorsal surface (figs. 10 and 11). The wall separating it from the right body-cavity very soon breaks down, starting from the ventral middle line; but up to the end of stage A a small portion of it remains (figs. 11—13), which

enables us to trace the true relations of this cavity, which without the greatest care in tracing its origin might be thought to be a diverticulum of the right, instead of the left, body-cavity. When it reaches the dorsal side it continues its growth past the middle line, till it reaches the water-tube, at the level of which it lies. Beyond this I have not been able to trace it with certainty; but since at this stage the most anterior portion of the right body-cavity is posterior to the water-tube, there is scarcely room for doubt that the "ventral horn" forms one side of the mesentery (very short in the pupa) which supports this tube—the other side being formed by the anterior part of the main mass of the left body-cavity, which gradually, during stage A, grows up the left side of the œsophagus (fig. 12).

By the time stage B is reached, all trace of separation between the right body-cavity and the ventral horn of the left cavity has disappeared; so that the mesentery of the water-tube (formed, as it seems, between two parts of the left cavity) is continuous with the main dorsal mesentery (formed between the right and left body-cavities), with which, indeed, Semon has confused it. The junction of the two is, however, still marked by a change in position—the water-tube and its mesentery being adradial (fig. 14), while the true dorsal mesentery is strictly interradial. It will be seen that the dorsal mesentery is even more on the left of the original middle line than the water-tube—not, as Semon considers it, in the median plane. Its posterior end is, however, nearer to this plane than its anterior.

This dorsal mesentery is first formed early in stage A by the meeting of the body-cavities about two-thirds of the way down the stomach, and from this point spreads rapidly forwards. Nearly at the posterior end of the stomach it passes sharply round to the left side, almost on to the ventral surface (fig. 16), thus forming a short transverse mesentery, which we shall see again in the pupa.

Very early in stage A the two body-cavities fuse ventrally along the ventral surface, at the point at which they so closely

approach one another in fig. 3. From this point the line of fusion runs obliquely forward, as we have seen, along the posterior edge of the "ventral horn" of the left body-cavity. Posteriorly the fusion usually (but perhaps not invariably) extends to the anterior margin of the intestine, apparently nearly in the middle line, but of this I cannot be quite sure.

Over the transverse portion of the intestine the left body-cavity (which at this level is much smaller than the right) pushes further across to the middle line (fig. 16) than the right body-cavity, and then passes even on to the right side, ending altogether just before the level of the posterior end of the stomach is reached. Its margin thus takes an oblique course, from left to right; but as the margin of the right body-cavity is still far removed from it at this stage, we cannot speak of a definite mesentery at this point. The right body-cavity extends somewhat further posteriorly than the left; but very little behind the posterior end of the stomach it also comes to an end.

Of stage B I have obtained but few examples, and none of them, I regret to say, show the mesenteries satisfactorily. In the fully-formed pupa the dorsal mesentery remains very much as we left it at the close of stage A, except that, as already mentioned, it is now continuous with the mesentery of the water-tube. Posteriorly, at about the level of the junction of the intestine and stomach (but in this stage a long way from the posterior end of the latter), it passes sharply round to the ventral side—in other words (since the original dorsal and ventral surfaces are no longer evident), it passes from the middle of interradius A into interradius B, thus forming the short transverse mesentery above mentioned. From this point it is continued into a mesentery which runs along the left edge of the ascending portion of the intestine, curves round the anterior margin of the latter, external to the polian vesicle (fig. 15, "mesentery"), and then descends for a short distance along the right edge of the descending portion of the intestine. How far this intestinal mesentery marks the division of the two body-cavities, and how far it is a new growth, the absence

of satisfactory sections of stage B prevents me from deciding; but the fact that the polian vesicle certainly hangs into the left cavity in stage A (figs. 10 and 13), as well as the appearance of certain specimens in which the fusion of the two cavities does not seem to take place, make it quite possible that this mesentery arises very nearly along the line of fusion, which, owing to the growth of the curved part of the intestine, has assumed rather a peculiar course.

This, however, is only conjecture; and I must admit that, except in the region of the œsophagus, I am quite unable to determine the exact limits of the body-cavities of the larva in the adult Synapta. It is, however, obvious that here, as in Cucumaria (**16** and **17**), it is by no means the simple matter which Semon represents it to be.

The descending part of the intestinal mesentery (lying in interradius D, close to radius III) is not continued, in the early pupa, on to that part of the intestine which lies posterior to the stomach—this part of the intestine being at this stage completely surrounded by the cœlom, though whether by the right and left cavities combined, or by the former alone (as certain early sections suggest) I cannot with accuracy determine. In later pupæ the intestine is tied to the body-wall by numerous threads of protoplasm; and only in the young Synaptæ which have completely lost the ciliated rings does the fully-developed mesentery, continuing the above-mentioned descending mesentery, appear.

B. Asterids.

Two very distinct types of development occur among Asterids, without, so far as I know, any connecting links between them. The most marked difference lies in the behaviour of the larval œsophagus, which survives in the adult in one form, but is replaced by an entirely new one in the other; there are, however, other important points of dissimilarity as well.

I propose to take first the type in which a new œsophagus is formed, since it has hitherto been but little investigated;

the other type, which includes the larva of Asterina gibbosa, is well known from Ludwig's researches (15), and has recently been further studied by MacBride (19).

1. Bipinnaria asterigera.

Under this head I propose to describe the more salient features in the development of a large Bipinnaria found in the Mediterranean, which closely resembles, if it is not identical with, "B. asterigera," described by Sars (29). My material was in part collected by myself at Naples in 1888 and 1893, and partly obtained through the kindness of Professor Kleinenberg from Messina. A fairly complete series has thus been secured, the only important gap being due to the entire absence of really early stages; but as it would be beyond the scope of the present paper to describe in detail all the stages observed, I must content myself for the present with calling attention to those features which have a direct bearing upon my theoretical conclusions.

I have been unable to discover from what adult form this larva springs, though there are some reasons for thinking that it may be from Luidia; all my specimens were obtained from the plankton, and the youngest of them had already acquired the long præoral lobe, terminating in a double fin-like expansion, which is so characteristic of this larva.

The arrangement of the body-cavities at this stage is as follows:—On the right the usual prolongation of the anterior body-cavity runs into the præoral lobe, and fuses with the left anterior cavity in front of the mouth; posteriorly it is continuous with the right posterior cavity, which lies beside the stomach and has a much greater dorsal and ventral extension than the anterior cavity. The left anterior body-cavity extends forwards like the right one, but its posterior end is not continued directly into the posterior body-cavity, but into the rudiment of the hydrocœl; this again opens into the posterior cavity, a deep constriction marking the point of junction. The arrangement is, in fact, almost exactly the

same as in Brachiolaria (belonging to the second type of Asterid development), as shown in fig. 17. Dorsally and ventrally the left posterior body-cavity curves forwards in two horns, which, as it were, embrace the hydrocœl, both horns being much longer than in fig. 17; the dorsal horn extends nearly to the water-pore, at the junction of stomach and œsophagus, while the ventral one runs alongside the intestine, and almost at the level of the anus curves over (between the stomach and intestine), and fuses with the right body-cavity. This fusion makes it impossible to determine the exact limits of the two cavities (right and left), but we shall probably not fall into any important error if we regard the point of junction as median.

Dorsally the right and left cavities already form a mesentery of no great thickness; but ventrally they are still widely separated over the intestine, except at its extreme posterior end.

The water-pore is, as usual, situated at the junction of œsophagus and stomach, rather to the left of the dorsal middle line. Slightly in front of the pore and in the median plane a cavity of irregular shape, absolutely unconnected with the body-cavities, lies over the base of the œsophagus. I mentioned it in a previous paper (5, p. 31) as probably of schizocœl origin, and this is rendered the more probable by the fact that the apparently homologous cavity in Asterias rubens and in Echinid Plutei arises in this way. In the present paper I propose to refer to it as the "dorsal sac."

The larva just described measured about 1·7 mm. in length. The next larva obtained measured nearly 4 mm. (exclusive of the arms), and is shown in ventral view in fig. 18. In it the posterior end of the hydrocœl is completely shut off from the body-cavity, and its anterior end, where it joins the anterior enterocœl, is further constricted. The dorsal horn of the left posterior cavity has fused with the anterior enterocœl from the pore to the commencement of the hydrocœl; and the right cavity extends across the anterior end of the stomach, on the dorsal side, rather to the left of the middle line, so that the

anterior end of the dorsal mesentery is left rather than median, though not so much so as the water-pore. In this way a portion of the right posterior body-cavity comes to lie close behind the dorsal sac, with which, owing to the thinness of the intervening wall, it is rather liable to be confused in sections. The two, however, are absolutely distinct.

The hydrocœl has thicker walls than before, especially in the five primary pouches, which now appear; and the rudiments of the water-tube and dorsal organ are also distinguishable, though I do not propose to deal with the details of their development in the present paper.

At this stage also the first rudiments of the skeleton appear —namely, five terminal plates, lying parallel to the mesentery, on its left side; two are dorsal, one is at the extreme posterior end, and two are ventral; the latter are shown in fig. 18. There are also a few plates, not shown in this figure, lying over the hydrocœl. The madreporite appears very soon after the terminals; but except for the fact that all the terminals lie over the left body-cavity, the skeleton has no special interest in our present inquiry.

The completion of the water-tube follows closely on the stage just described. It runs from the hydrocœl up to the immediate neighbourhood of the water-pore (where it remains permanently open to the anterior enterocœl) almost exactly along the line occupied at any earlier stage by the mesentery separating the anterior and posterior body-cavities. It is accompanied throughout its course by the dorsal organ, which now almost encircles the hydrocœl, and ends anteriorly underneath the dorsal sac, as we shall presently see is the case in other Asterid larvæ as well (see fig. 19). Its tubular nature is well marked at this point, as well as round the hydrocœl (fig. 20), but where it accompanies the water-tube it is usually very narrow, and its lumen, if present, is very hard to detect. With its further development we have no present concern.

Almost simultaneously with the completion of the water-tube, the separation of the hydrocœl from the anterior body-cavity is effected. Fig. 21 is a lateral view of a larva at this

stage, from which it will be seen that this separation occurs in the same interradius as the water-tube. Already the pouches of the hydrocœl have increased in number to twenty-five (they are much more conspicuous in sections than in external views), but there is no pause in this condition, fresh pouches being rapidly and continuously formed between the terminal one and the next adjoining it. In this way the hydrocœl spreads over the surface of the larva until its five main branches come in contact with and fuse with the five excrescences (see figs. 20 and 22) which contain the terminal plates; but it is to be noted that this is effected without that rotation of the two series of organs noticed by Ludwig in Asterina (**15**); the anterior dorsal pouch of the hydrocœl becomes enveloped by the anterior terminal plate, and the pouch nearest the anus by the corresponding terminal excrescence.

Fig. 21 will also serve to illustrate the relation of the anterior and posterior body-cavities on the left side in later stages. It will be seen that the "ventral horn" of the posterior cavity has there grown so far round that it forms a long thin mesentery with the ventral wall of the anterior cavity. The dorsal wall of the latter is marked, as we have seen, by the line of the water-tube; and along this line, at the close of larval life, a secondary separation of the two cavities is effected by the formation of a septum. It will at once suggest itself that in the remnant of the anterior cavity thus enclosed we have the equivalent of the "axial sinus" of the adult (see **5**, p. 37), and a study of its subsequent history shows that this is the case. It is also important to notice that this sinus is bounded on both sides by the left body-cavity. This is important for my theory, and though confirmed by MacBride (**19**, p. 433) is opposed to the statements of some other observers; Ludwig (**15**, p. 87) states that in Asterina the ventral wall of the sinus is part of the original mesentery separating the right and left cavities, while Semon (**32**, p. 37) regards the whole mesentery containing the water-tube as a part of the dorsal mesentery of Synapta.

No portion of the posterior body-cavity ever intervenes

between the outer body-wall and the hydrocœl, or between the centre of the latter and the stomach. Soon after the separation of the hydrocœl from the anterior enterocœl a small circular depression appears in the outer wall of the former, as shown in fig. 20. As it is not invariably present even at this stage, and seems to be generally absent in the next, I am inclined to regard it as due to shrinkage after death; but even so it marks a special weakness of the wall at this place, which is not unimportant as the first indication of the position of the adult mouth. In the next stage an outgrowth of the stomach presses against the inner wall of the hydrocœl, and a very little later pierces right through this organ and fuses with the body-wall beyond; probably it forms a part of the permanent œsophagus, but most of the latter seems to be derived, at a much later stage, from the ectoderm (fig. 23). By this piercing of the hydrocœl the water-vascular ring is formed, as Metschnikoff observed. This, at least, is the conclusion I draw from my sections; but this method of formation is unknown in other Echinoderms, and it is just possible that an invagination of the side of the hydrocœl (such as occurs in Echinids) may take place; it must, however, be formed and obliterated with remarkable rapidity to have entirely escaped notice, and I am not disposed to believe in its existence. After all, this unusual mode of formation of the water-vascular ring is no more remarkable than the extreme variation in the point of closure of this ring exhibited by other Echinoderms (see **5**, fig. 28).

With the help of fig. 22, which is a dorsal view at about the same stage as figs. 20 and 21, the general course of the longitudinal dorsal mesentery can be followed; it starts rather to the left of the middle line, under the posterior margin of the madreporic plate, and runs somewhat obliquely backwards on the right side of, and parallel to, the line of the terminals. This obliquity increases in later stages; but as at the same time the centre of the hydrocœl pushes itself further and further back, the plane of this organ is always (as in fig. 22) parallel to the plane of the mesentery; while the latter, as will

be readily seen, is at right angles to the plane of the water-tube.

This obliquity of the mesentery causes the right body-cavity to appear, in sections through the extreme posterior end of the larva, somewhat smaller than the left; but this is not the case in sections through more anterior regions (fig. 20), and indeed the total bulk of the right posterior body-cavity (deducting the anterior body-cavity, which may be said to end near the anterior margin of the stomach) is, at the time of the formation of the water-vascular ring, decidedly greater than that of the left posterior cavity.

The later stages of development do not call for any detailed description in this paper. After the junction of the radial tubes of the hydrocœl with the excrescences of the body-wall which contain the terminals, the two grow out together and form the arms, into which, in the larva, only the left body-cavity extends. By this means, as well as by increased growth within the disc, the left body-cavity gradually exceeds the right in size, though even in the oldest larva I have obtained (with fifteen pairs of tube-feet to each arm, and a total diameter of 3·9 mm.) there is still, within the disc alone, not very much difference between them (fig. 23).

In this latest stage the former protuberance of the stomach in the centre of the water-vascular ring has been pushed back by the formation of an ectodermic stomodæum; but the adult œsophagus is not yet complete. The stomach is bound to the body-wall not only by the mesentery, but also by five interradial septa, one of which, as we saw, forms one wall of the axial sinus. Radially the stomach has five pairs of pouches, as seen in fig. 24, and the relation of these to the mesentery is shown in section in fig. 25; as will be seen, the right body-cavity is dorsal to the stomach, no portion of it being visible in fig. 24. The anus is, as shown, still in the same interradius as the water-tube. As I have never succeeded in rearing a post-larval specimen from this Bipinnaria, I am quite unable to trace the migration of the anus into the position which it occupies in the adult; but it is probable that the larval anus is

entirely obliterated, since in a young Luidia in which the disc measures about 5 mm. in diameter, I find no trace of any anus at all.

This Luidia presents certain other features which merit a brief description. The dorsal sac is very conspicuous on the aboral side of the water-pore, and a considerable portion of the dorsal organ projects into it; it is quite distinct from the ampulla, as well as from the rest of the cœlom, and is evidently identical with the space which Ludwig found (**13**, p. 159) containing the aboral termination of the dorsal organ. Immediately below this sac, at the level of the original dorsal mesentery (persisting at this stage transverse to the axis of the œsophagus and stomach), the genital cord starts from close by the dorsal organ (though I cannot from my own observations assert the connection of the two structures); but it is still very short, and cannot be followed far round the disc. For the most part the original mesentery is fragmentary (at any rate in my sections), but enough of it remains to indicate that it follows the growth of the hepatic cæca into the arms—these cæca being derived from the ten stomachic pouches seen in fig. 24 (compare figs. 25 and 26). This extension, however, of the right body-cavity into the arms is probably to be regarded as secondary, as I shall explain later. There is much, in my opinion, to point to the conclusion that the more primitive line of division of the two cavities is marked in the adult by the ring of the genital cord; but the proof of this is indirect, and cannot be entered upon here.

2. Brachiolaria.

I have selected Brachiolaria as the representative of the second type of Asterid development because it is the form which I have had most opportunity of studying; but the same general plan of development is found in many true Bipinnariæ, as well as in the larva of Asterina, which is, as it seems, only a modified Brachiolaria (**1**, p. 62; **15**, p. 154).

The general external features of Brachiolaria have been admirably figured by Agassiz (**1**); while the internal anatomy

of the larva of Asterina is not only the subject of Ludwig's well-known paper, but has recently received further attention from MacBride (19), whose full account may be expected shortly. It is unnecessary, therefore, for me to spend time on a detailed description, and I may confine my account entirely to those points which bear most on my theoretical views, and those which the absence of the intestine in the larval Asterina tends to obscure in that form.

As already mentioned, most of my material was obtained at St. Andrews; but the earlier stages have been more especially studied in larvæ, probably of Asterias glacialis, obtained at Naples.

There is no need to describe here the earliest stages of all, as the few points in which my observations are at variance with Field's (8) have no special connection with the present paper.

The hydrocœl seems to be always marked out before the left posterior body-cavity is separated off, but the exact form of the cœlom at this stage is subject to much variation; fig. 17, however, may be taken as a fairly typical example.

In the next stage, in all cases examined by me, the separation of the posterior enterocœl from the combined anterior enterocœl and hydrocœl is complete. The posterior cavity then pushes a "dorsal horn" forwards on the dorsal side, close up to the water-pore, while a "ventral horn" grows rapidly forwards alongside the intestine, and then, at the point at which the latter (closely pressed against the intestine throughout most of its course) curves outwards towards the anus, this "ventral horn" of the left body-cavity crosses over to the right side. Meeting with no opposition from the right body-cavity, which at this stage only reaches the ventral surface at its posterior end, the "ventral horn" widens out, and passes down the right side of the intestine, near the posterior end of which it meets the right body-cavity, and forms with it an oblique mesentery, shown in fig. 27. All the stages of this movement can, in perfectly healthy larvæ, be easily followed in the living animal, from which fig. 27 is drawn; but I have fully confirmed the

facts shown in this figure by means of sections, one of which is shown in fig. 28.

The anterior end of this mesentery soon breaks down, but before it does so the rudiments of the terminal plates appear (fig. 27) lying over the left body-cavity, though one of them, as the figure shows, is very much to the right of the intestine. This constitutes one of the most striking distinctions between the two types of Asterid larvæ (compare fig. 18); the other principal differences may be briefly summed up as follows:

1. The larval œsophagus persists in Brachiolaria, and is not replaced by a new one, though it loses its functional activity for some time after the metamorphosis.

2. Up to the moment the metamorphosis begins, the hydrocœl is still open to the anterior body-cavity, but this opening is not (for the most part, at least) in the same interradius as the water-tube and water-pore—one of the pouches of the hydrocœl lying (see **9**, pl. xxvi, fig. 22) anterior to the pore.

The closing of the hydrocœl ring I have not satisfactorily followed in Brachiolaria, but it is certainly in the interradius indicated by Ludwig (**15**, p. 169, and pl. vii, fig. 95), and probably in every way the same as in Asterina.

3. As in Asterina, the most anterior hydrocœl pouch of the larva does not unite with the most anterior of the dorsal arm-rudiments, but with the one just behind the anus—the whole hydrocœl ring being rotated (viewing the animal from the oral surface of the adult—left side of the larva) in a direction opposite to that of the hands of a watch; or in other words, the hydrocœl is, so to speak, unscrewed slightly from the rest of the body.

4. In the later stages of Bipinnaria asterigera the future oral surface is turned rather towards the posterior end of the larva; in Brachiolaria, however, it is the future aboral surface which is directed backwards (compare **24**, pl. ii, figs. 1 and 5).

The growth of the left body-cavity round the hydrocœl, and the formation of an axial sinus between the two horns of this cavity, takes place as in Bipinnaria asterigera.

The "dorsal sac" appears at a very early stage as a space

of schizocœl origin, lying over the posterior end of the œsophagus. I have cut numerous sections to satisfy myself that it has no connection with the cœlom, from which, indeed, in the earlier stages it is somewhat widely separated. In later stages the right body-cavity pushes its way up close to it, but is at no time connected with it. Field (**8**, p. 118) found the same cavity in Asterias vulgaris, and gives figures of its schizocœl origin; he seems, however, unaware that I had already drawn attention to it (**5**, p. 31). MacBride (**19**) does not mention it in Asterina, but describes a cavity, derived from the anterior enterocœl, in apparently very nearly the same situation; this he regards as the homologue of the right collar-cavity in Balanoglossus; but until a similar cavity has been found in other Echinoderms—and especially in one which conforms more nearly to the usual plan of development—this conclusion appears to be somewhat rash.

In very old Brachiolariæ, just before metamorphosis, I have in a few cases observed a faint pulsation in this region—apparently in the floor of this cavity; but the opacity and activity of the larva make it very hard to study, and it is moreover much slower and less regular than in Echinid Plutei.

Immediately under the "dorsal sac" in older Brachiolariæ lies the termination of the "dorsal organ," as seen in fig. 19; but this, and the ultimate fate of the dorsal sac, have been already described in connection with Bipinnaria asterigera.

Metamorphosis is ushered in, in the larva of Asterias rubens, by the fixation of the larva by the knobbed arms of the Brachiolaria, and the ciliated pit which they surround; and this fixation, at first voluntary, very soon becomes as complete as in Asterina (**19**, p. 433). Then the præoral lobe shrivels up, and all that remains of it is a small stalk lying in the interradius of the adult anus (interradius E). The larval intestine and anus soon disappear, but the latter, when last seen by me, still lay nearly in the interradius of the water-pore (judging the interradii by the arm-rudiments, not by the hydrocœl pouches), or perhaps rather more nearly opposite radius V.

c. Echinids.

My studies of the internal anatomy of Echinids (both larvæ and adults) have been chiefly made on Echinus microtuberculatus; but so far as other forms have been examined all Regular Urchins follow much the same plan of development.

It is unnecessary to repeat here the description which I gave in a previous paper (5) of the origin and general arrangement of the parts of the cœlom in this group; but attention may be called to the migration of the hydrocœl along the left side of the stomach. Originating at the junction of œsophagus and stomach, it pushes its way backwards over the surface of the latter till it comes to lie about in the middle of the left side, where it forms a ring through which (at a later stage) the œsophagus of the adult grows—the larval œsophagus not being permanent. Round this hydrocœl ring the left posterior body-cavity grows in the form of a crescent, the dorsal and ventral horns of which eventually unite and fuse together anteriorly. I believe that this fusion occurs along the line of the water-tube, which, as in Bipinnaria, lies close against the wall of the stomach; but I have not yet been able to obtain decisive proof of this.

The limits of the body-cavities in older larvæ are extremely difficult to determine, but they appear to be separated by a mesentery starting just behind the water-pore, and running back along the middle dorsal line to the extreme posterior end, where it turns and runs forward along the ventral surface of the intestine. I have not been able to trace any communication between the cavities between the anus and stomach, such as occurs in Asterids, though there are some indications that it occurs just before metamorphosis.

The general arrangement of the left anterior enterocœl and the organs adjacent to it was described and figured by me in a previous paper (5, p. 18, fig. 9); but some further details may be added here.

The "pulsating vesicle" arises at a fairly early period from

a group of cells situated over the middle line of the œsophagus, to the right and rather in front of the water-pore. A schizocœl space soon appears in this mass, and rapidly increases in size (fig. 29). It is certainly distinct from the cœlom in its origin, and, to the best of my belief, throughout larval and adult life. The similarity of its origin and position, as well as its relation to the dorsal organ (to be described later) lead me to regard it as homologous with the "dorsal sac" of Asterids, and by this name I shall in future refer to it.

In late Plutei its floor projects far into its cavity (fig. 30), but the extent to which this is seen in sections varies considerably in different specimens, apparently depending on the methods used in preservation. As far as I can judge from a careful study of the living animal under high powers of the microscope, it is this part, and not the cavity as a whole, which pulsates. This pulsation, I may mention, is certainly continued in the earliest post-larval stages, though whether it occurs in the adult I am unable to say.

The left anterior enterocœl forms at a fairly early stage a large ampulla where the pore-canal and water-tube open into it (fig. 31). In later stages it extends backwards for some distance alongside the water-tube—further than in fig. 30, but exactly how far I am still doubtful.

The dorsal organ is rather difficult to trace with accuracy, but subsequent stages make it almost certain that a mass of cells (usually filled with yellow granules), projecting into the dorsal sac on the one hand (fig. 30) and into the ampulla on the other (fig. 31), is the rudiment of this organ. Besides this, a cord of cells is frequently noticeable (fig. 30) lying alongside the water-tube (on the ventral side of this structure in the larva), which may be a part of the dorsal organ; but I am unable to distinguish it clearly in transverse section, or to trace it into connection with the above-mentioned mass of cells with yellow granules. The aspect of all these parts varies much with the methods employed, and great care should be taken to kill the animal in an extended condition. Where this is not done the great mass of transparent tissue projecting

into the dorsal sac (fig. 30) often shrivels up and forms, with the adjacent mass of yellow cells, a compact knot of tissue seemingly composed mainly of nuclei; even this, however, is instructive, since it presents a close resemblance to the dorsal organ as it projects into the dorsal sac and axial canal in post-larval stages.

The water-tube lies throughout its course close against the stomach, and finally enters the water-vascular ring adradially—being, when the animal is viewed from the right (future aboral) side, on the left side of its interradius.

When the time for metamorphosis is reached, the hydrocœl seems to have moved even beyond the middle of the left side, and the ambulacral surface is directed somewhat backwards, as in Bipinnaria asterigera; but at the same time the original apex is pushed over to the right, and the mesentery separating the right and left body-cavities becoming somewhat oblique, its plane still continues to be about parallel to that of the ambulacral surface, and at right angles to the axis of the adult œsophagus.

The larva at this stage creeps about on the bottom by means of its five primary tentacles, the larval arms elevated, and the thin membranous "amnion" spread out like an umbrella, supported on the spines of the young Echinid.

The actual metamorphosis is accomplished very rapidly; the "amnion" contracts and is absorbed, while the spines which were embedded in it become erect; then the larval œsophagus is absorbed, and the spicules of the larval arms are (usually) broken off by the force of the accompanying contractions of the ectoderm—so that in less than an hour a perfect Pluteus is transformed into a small rounded Echinid, in which radial symmetry entirely replaces the bilateral symmetry of the larva. This young Echinid is usually rendered extremely opaque by a species of histolysis, which begins in the Pluteus with the proliferation of cells into the cavity of the stomach, and afterwards extends to other tissues, rendering the examination of the internal organs extremely difficult Exactly the same thing usually occurs in the larva of Antedon,

as well as, in a less degree, in Ophiurid Plutei and Brachiolaria; but the fact that one or two of my Echinid Plutei hardly showed it at all, and that Seeliger was not troubled with it at all in Antedon, indicates that, as that author suggests (30), it is probably pathological. In all larvæ kept under satisfactory conditions it soon clears off, and the tissues return to their normal condition.

Although this histolysis has prevented me from following the details of the anatomy of the youngest Echinid to my satisfaction, yet we may state positively that the essential relations of the organs are not much altered during metamorphosis. If any mesentery exists at this stage (which is doubtful) it must still occupy the same position as before—parallel to the ambulacral surface, at the level of the water-pore; but since the total bulk of hydrocœl and left body-cavity combined is far greater than that of the right body-cavity alone, it follows that the divisional line between the two cavities lies far on the aboral side of the equator of the young Echinid. Its approximate position is shown by a dotted line in the diagram, fig. 35, which will be presently described in connection with the skeleton.

In a previous paper (5) I described the position of the basal plates in the Pluteus; though they do not form such a regular longitudinal series as in Asterids, yet all (except perhaps the madreporic plate) are formed over the right body-cavity. Most of them possess spines, which when first formed usually seem to end in three points, but when fully formed are seen to be quadrangular and to terminate in four points, not always of equal length; two of them are shown in fig. 32.

Besides these plates a number of others are formed on the left side, round the base of the "amnion," into which their spines project. They are most difficult to study in the Pluteus, and I have not yet determined, in spite of much time spent on them, their order of development; apparently a large number of them are formed within a few hours of one another. In a young post-larval stage their positions are more easily

determined, and I have therefore confined my figures to this stage.

Their spines are of two different kinds, and are of great assistance in identifying the different series of plates, the general arrangement of which can be made out from figs. 34 and 35, and from the ventral view given by Lovèn (11, pl. xvii, fig. 149). On the aboral side of each primary tentacle lies a plate with two quadrangular spines, such as we have seen on the basals. In my latest stage (fig. 34) they curve round as if to embrace the tentacle. Alternating with these, and usually rather nearer the oral surface, are five interradial plates each bearing one spine of quite a different pattern, with six longitudinal rods instead of four (fig. 33). Théel (35, fig. 99) has given figures of the development of these plates in Echinocyamus, which agree with what I have observed in Echinus, though rather too diagrammatically regular. Several of the other plates, the position of which need not be described, bear spines of the same pattern (see figs. 34 and 35)—indeed those over the primary tentacles are the only plates developed on the left side which have spines of the quadrilateral pattern.

All these plates and spines have been carefully studied, both in whole specimens and by means of maceration and dissection. In my oldest larva, however, an accident prevented the use of the latter method, and it is therefore possible that I have exaggerated in fig. 34 the amount of curvature existing in the plates overlying the tentacles; I do not think, however, that this is the case. This (fig. 34) is the latest stage to which I have been able to rear, from the egg, the young Echinus microtuberculatus. The next stage (still of the same species) which I have been able to obtain is very much older, and a considerable gap is left between the two. Being unable to see the plates clearly in the whole animal, I cut off the aboral portion and examined it as a transparent object, the result of my observations being shown in fig. 36: many of the spines are broken, and the plates at the margin have been much injured in the process of section, but still the figure shows much that is interesting. The basal plates have already (5) been traced from the plates

of the right side of the larva; but I may add here that in figs. 11 and 12 of that paper the water-pore was drawn much too large (a superficial hollow in the plate being mistaken for it), and that the presence of two pedicellariæ on the second basal plate is much more common in the Pluteus than I then supposed. Outside the ring of basals, and alternating with them, we see the five oculars; each of them bears a pair of quadrilateral spines, which are not found on any other plates except the basals. When we compare these plates with those just described in fig. 34, and take into consideration the rarity of these quadrilateral spines, the various facts known about the disappearance of the primary tentacles, and the termination of the radial water-vessel of the adult in the eye-spot, on the oral side of the ocular plate, we can scarcely, I think, doubt that these oculars are identical with the five plates which lie above the primary tentacles in fig. 34. But these plates are developed on the left side, as are the terminals of Asterids and Ophiurids, which also in the adult embrace the terminations of the radial water-vessels; so that we have, as it seems to me, new and important grounds for accepting the homology, often suggested but never proved, of the oculars of Echinids with the terminals of Ophiurids and Asterids.

Another consideration follows from this identification of the plates in figs. 34 and 36. In the younger stage the line of original division between the right and left body-cavities (the mesentery having probably disappeared already) lies somewhere between the basal and ocular plates—probably in the position of the dotted line in fig. 35, and nearly at the level of the water-pore. In adults these two rows of plates fit into one another, but the genital rachis encircles the intestine at the level of the basals (through which the genital ducts pass) and of the water-pore; so that we have here somewhat better grounds than in Asterids for believing that the genital rachis marks the line of division between the right and left body-cavities.

The arrangement of the water-tube and the neighbouring organs is almost exactly the same in Echinus microtuber-

culatus as in Dorocidaris (27), except that in the oldest specimen I have cut (diameter 3·5 mm.) the "espace sous-madréporique" of Prouho does not extend down the side of the water-tube.

In spite of the unsatisfactory histological condition of my youngest radial specimens, and the gap which still exists between them and the next stage obtained, the relations of the parts in the Pluteus to those in the adult can be fairly easily followed.

The pore or pores of the madreporic plate and the upper end of the water-tube open in all stages into an ampulla (figs. 30 and 39), which is continued down into a canal lying alongside the water-tube, and enclosing the axial organ. This is the "canal aquifere annexe" of Prouho, and being derived from the anterior enterocœl is apparently homologous with the axial sinus of Asterids. (Fig. 39, "axial canal.")

Lying under the madreporic plate, on the aboral side of the water-pore, is a closed vesicle into the floor of which projects a portion of the dorsal organ, which passes round the water-tube into the axial sinus at a lower level than the section drawn in fig. 39. This closed vesicle is Pronho's "espace sous-madréporique," and the portion of the dorsal organ projecting into it is his "processus glandularis." Its position is exactly that of the "pulsating vesicle" of the Pluteus (for what is on the right of the pore in the larva is on its aboral side in the adult), and its obvious similarity to the dorsal sac of Asterids justifies the conclusion already arrived at that this sac is homologous with the "pulsating vesicle" of the Pluteus.

Fig. 39 very well illustrates the general relation of these parts to one another and to the intestine and ovary, though the junction of ampulla and axial canal takes place at a higher level, and the connection of the latter with the dorsal organ (as already mentioned) at a lower level. The water-pores are clearly adradial, being on the left of the ovary, which is interradial. The water-tube in this figure appears to be on the left side of this interradius, but at its oral end it opens into

the water-vascular ring in the same adradius as the water-pores.

Bütschli (6) has already called attention to the fact that in Lovèn's figure of a young Echinid (11, pl. xxi, fig. 170) the flattened side of the dorso-central plate is directed towards interradius E (compare fig. 36), and he infers from this that the anus was originally in this position. I had observed the same point in 18 88, before Bütschli's paper appeared, and am able to give proofs of that which he could only conjecture. As the anus does not break through till the young Echinus microtuberculatus has attained a diameter of from 5 to 7 mm., it is no difficult matter to obtain the necessary material; and by adding a few drops of chloral hydrate to the water containing the animals, they can be stupefied in an extended condition and easily examined.

Fig. 37 shows the position of the anus in one of the youngest specimens in which it could be detected with absolute certainty; it will be seen that it lies well within interradius E. As the small plates of the perianal area (which sometimes show a marked bilateral symmetry) increase in number, the anus works gradually across into radius IV (fig. 38), and thence into its adult position; but it is quite unnecessary to illustrate the whole series of changes.

I have not succeeded in tracing the steps by which the long and tortuous alimentary canal of the adult is evolved out of the globular stomach and short intestine of the Pluteus. The anus closes at the moment metamorphosis begins, and in my youngest radial specimens I cannot even trace the intestine with certainty. It is, however, interesting to note that in the adult the œsophagus lies in interradius A, and from this point the alimentary canal makes a complete circuit of the disc till it again reaches this interradius. Then it turns back (compare 14, pl. xiii, fig. 7) as far as interradius B, from which it runs sharply back again to the anus in interradius D. I am almost certain that in the youngest specimen of which I have sections (diameter 1 mm.) this last loop (from interradius D to interradius E and back again) does not exist,

and it seems to me not improbable that most of this coiling of the intestine is secondary, and that the original condition was that found in Crinoids—a simple curve round the disc, beginning and ending in interradius A.

Lovèn's figure of Gonocidaris canaliculata (**12**, pl. iii, fig. 10) is suggestive of this; but of course the matter can only be settled by careful study of the development, for which I have not been able to obtain the necessary material.

D. Ophiurids.

For a general account of the external changes which an Ophiurid Pluteus undergoes before and during metamorphosis, I must refer to my former paper (**5**, p. 26); here I shall only call attention to those features which will be referred to in the second half of this paper, in connection with the phylogeny of the group.

Though not perhaps a very primitive group in themselves, their Plutei offer us, in their external features at least, what seems to me to be a much more accurate epitome of phylogenetic history than is met with in other Echinoderm larvæ. Unfortunately, however, the difficulty of obtaining satisfactory sections is so great that few of the internal changes can at present be followed in detail. I am full of confidence that better methods will give all the facts we require, as all my material was used up before I adopted the celloidin-in-paraffin method, which has given me such satisfactory results elsewhere; but meantime I must content myself with inferences from studies of the exterior and from our knowledge of other Echinoderm larvæ, which, though attaining to great probability, are never so satisfactory as direct observation.

The first stage in development which we need pause to consider is that shown in **5**, fig. 4. In it the hydrocœl forms a nearly complete ring round the œsophagus, while the posterior part of the body still retains its primitive bilateral symmetry; the plane of the hydrocœl is in fact about at right angles to that of the mesentery.

I have already described and figured the changes of position

which the calcareous plates of the Pluteus undergo at the time of metamorphosis, showing that the plates developed over the right body-cavity (radials and dorso-central) pass up on to the dorsal surface of the larva, while those over the left body-cavity (terminals) pass on to the ventral side. At the same time the madreporic plate is pushed forwards to the anterior end (finally resting to the right of the middle line, see fig. 43), while the mouth assumes a more decidedly ventral position, turning as it does so towards the left (fig. 43).

All these changes in the skeletal plates are very easily followed on the living Pluteus; but a question of much more importance to us now is, how far do the body-cavities follow this movement—how far does the longitudinal mesentery remain parallel to the lines of the radial and terminal plates?

It is on this point that my sections have most conspicuously failed; but fortunately enough evidence remains to place the matter (bearing in mind the analogy of Asterids and Echinids) beyond any reasonable doubt. When the radial plates of the dorsal side first begin to move across to the left, the edge of the right body-cavity is still plainly visible in external views, and certainly follows their movement as long as it can be traced —that is, nearly to the stage shown in 5, fig. 5. Similarly, on the ventral side the edge of the left body-cavity can be seen passing, still parallel to the line of the terminal plates, over to the right side (fig. 42). Between this and the first truly pentamerous form, I have no observations worth recording; but in this stage (5, fig. 6) I find in sections traces of a mesentery running round the edge of the stomach, still parallel to the lines of the skeletal plates, though now at right angles to the axis of the œsophagus (fig. 41). I cannot trace it all round the body—indeed it appears to be in a very fragmentary condition at this stage—but enough remains to make it almost certain that we have here a remnant of the larval longitudinal mesentery, which therefore in Ophiurids as in other Echinoderms assumes in the pentamerous form a transverse position.

If this is admitted, there cannot, I think, remain much doubt that the left body-cavity, in the course of its movement, encircles the œsophagus; though whether it ever forms a mesentery in the interradius of the water-tube, must be left for future investigation to decide.

Another point requiring further evidence is the behaviour of the intestine. If the movement of the mesentery be admitted, it is scarcely possible to doubt that the stomach moves with it; and it would be at least a plausible conjecture that the intestine, if it survived, would retain its primitive relation to the stomach and body-cavities, and so assume a position transverse to the axis of the adult œsophagus. As a matter of fact I believe in most larvæ the intestine and anus disappear almost the moment metamorphosis sets in; but in a few larvæ I have thought that I could trace the intestine, at the stage shown in fig. 42, bending over to the right, exactly as we should expect it to do. I have not, however, succeeded in obtaining sections which prove this; and until I have done so, I do not like to assert that it really occurs, though analogy renders it extremely probable.

Up to the stage immediately preceding metamorphosis (fig. 40) the two body-cavities are practically equal in size; and it would seem from fig. 41 that no great difference between them exists even when the radial symmetry is fully acquired.

At this stage (**5**, fig. 6) the water-pore is nearly at the edge of the disc; and from close by it starts (much later) the genital rachis, which grows as a ring round the body (**18**, p. 138). Subsequently, when the arms grow out, the water-pore moves on to the ventral surface, while the radial portions of the genital rachis remain dorsal to the arms, so that the whole rachis assumes the form of an undulating cord, described by Ludwig as an aboral vascular ring. Now I have no evidence to offer except analogy; but I would suggest as possible, and worthy of investigation, that this genital rachis may mark, as it does in Crinoids, and I believe in Asterids and Echinids also, the position of the larval mesentery—the original line of separation between the two body-cavities. In this connection

it is worth while to remember, though no great stress can be laid upon it, that the cavities of the arms, both in Crinoids and Asterids, are primarily parts of the left body-cavity only, though in both these forms (but not apparently in Ophiurids) the right cavity secondarily extends into them.

Erratum.—I take this opportunity of pointing out that the calcareous plate marked over the "anterior enterocœl" in fig. 4 of my former paper (**5**) is entirely due to a lithographer's error. A similar error has caused the omission of the madreporite in Bipinnaria (**5**, fig. 14).

e. Crinoids.

The development of Antedon rosacea, which alone among Crinoids has been studied, is too well known to need any description here; and though Seeliger's (**30**) careful examination, while undoubtedly correcting some of my errors (**4**), contains much debatable matter, yet fortunately few of the points to which I wish here to call attention are open to dispute.

(1) The plane of the hydrocœl is not parallel to that of the mesentery in the larva, but forms an angle with it. I may have exaggerated this in my figure (**4**, fig. 59), but it is unquestionably very marked (**30**, pl. xvi, fig. 67), and may be compared with the somewhat similar, though by no means identical arrangement in Asterina (**15**, woodcut iii, p. 156).

(2) The stalk, though connected with the præoral lobe of the larva, does not include in the adult the whole of this lobe; while it does contain in the larva other parts which do not seem primarily to belong to this region. Thus the anterior body-cavity, which extends into this region in all larvæ which possess a præoral lobe, is far removed from the stalk in the Cystid stage of Antedon; while several skeletal plates, and part of the right body-cavity, are present in this region of the larva. Now whatever may be said about the identification of the terminal plate of the stalk with the dorso-central of other Echinoderms, we have strong reasons for thinking that the ancestral Crinoid (like many Cystidea) was sessile; and there-

fore it seems to me that the presence of stem-joints in the larval Antedon indicates that this region is not a pure præoral lobe, but a mixture of this with a structure (the stalk) belonging to a much later (phylogenetic) date. The forward prolongation of the right body-cavity is due to the same precocious development of the stalk; for it is impossible to compare this, which arises so late, with the right anterior enterocœl of other Echinoderm larvæ, which is one of the first parts of the cœlom to appear.

(3) The left body-cavity grows round the œsophagus at the time of metamorphosis, forming with the right body-cavity a mesentery parallel to the plane of the hydrocœl ring. The two horns of the left body-cavity certainly both reach the interradius of the water-tube, and appear to me to form a mesentery supporting the latter; but this, in the face of Seeliger's researches (**30**, p. 292) must be admitted to be doubtful.

(4) In the free-swimming larva the right and left body-cavities are approximately equal in size (Seeliger rightly points out that in my diagrams I represented the right cavity too small). In the Cystid, however, the left cavity is much smaller than the right, and though, when the arms begin to grow out, its relative size increases for a time, yet the subsequent extension of the right cavity into the arms apparently neutralises this. The important point for us, however, is the fact that the actual metamorphosis cannot in any way be said to be due to, or even accompanied by, "predominance of the left posterior body-cavity" (**19**, p. 434).

(5) The order of development of the first-formed pairs of tube-feet in other Echinoderms is a little doubtful, but apparently they are from the first (as the later pairs certainly are) in centrifugal succession. In Antedon, however, the second pair is undoubtedly formed later than the first pair of each ray; while the development of the later tentacles in triplets (**26**, p. 177) is utterly unlike anything observable elsewhere, and goes far to establish an absence of homology between the arms of Crinoids and those of other brachiate Echinoderms.

(6) Perrier's description (26) seems to leave little room for doubt that the genital cords start at the level of the transverse mesentery. They grow out, according to him (26, p. 202), from the oral end of the dorsal organ, and pass along to the arms in the septum which separates the oral and aboral arm-cavities—a continuation (26, pl. ix, figs. 62 and 62) of the transverse mesentery.

(7) In my former figures (4) I represented the stalk of the larva as lying in radius V (compare fig. 14 of this paper). It is extremely difficult, in the absence of radial plates, to determine its exact position, but I am strongly disposed now to believe that its true position is adradial—close to radius V, but actually in interradius A. There is some indication that it undergoes a change of position during metamorphosis; but further investigation of this point is required.

Part II.—Phylogeny.

Interesting as are the problems involved in the history of the probable bilateral ancestor of Echinoderms, they have very little to do with the subject of the present paper. The relation of this ancestor to the Enteropneusta will indeed be briefly discussed later on; but for the present we may confine our attention to the first appearance of radial symmetry, and the changes represented in ontogeny by the metamorphosis.

On this point many opinions have been expressed in the last few years, some of them of a highly speculative character; but few attempts have been made to trace these changes in detail from an embryological standpoint. The more general sketches of phylogenetic possibilities, and expressions of opinion founded on observation of post-larval and adult examples only, can, where they call for any special remark, be more conveniently dealt with in connection with those details of my views with which they are immediately connected; but those accounts which endeavour to give a fuller explanation of the meta-

morphic changes must be briefly reviewed before my own opinions are set forth.

Semon (**32**) assumes a bilaterally symmetrical ancestor, which he calls "Pentactæa." In it the hydrocœl, which formed a ring round the mouth, had five tentacles, and was connected by the water-tube with the water-pore in the dorsal interradius. A dorsal mesentery, embracing the water-tube, ran back from near the mouth to the posterior end in the middle line; the mouth and anus were ventral, and the animal was fixed at a point somewhere on the dorsal surface. The representative of this ancestor he professes to find in the young stages of all Echinoderms ("Pentactula" stage)—regarding the mesentery of the water-tube as identical with the longitudinal mesentery of the larva. He admits subsequent changes of position of mouth and anus, but only in Crinoids does he recognise the parallelism of the mesentery (or a part of it only, as he thinks) to the plane of the hydrocœl, and accounts for it briefly as due to a secondary "Drehung des Darmes."

How utterly he has misunderstood the position of the larval mesentery in Asterids and Echinids will be evident without further comment to any one who will compare his figures (**32**, pl. vi, figs. 4 and 5) with the descriptions given in the foregoing pages of this paper; while Ludwig's observations on Cucumaria (**16** and **17**) and my own re-examination of Synapta (on which Semon's views are founded) suggest that the symmetry of adult Holothurians has not been quite so simply derived from that of the larva as Semon would have us believe. In fact, without entering further upon the details of his theory, I think we may say that his fundamental assumption of the retention of bilateral symmetry by the complete radiate form is opposed to embryological evidence. To the further assumption of the fixation of the common ancestor I shall return later.

Bütschli's theory (**6**) is extremely ingenious and very carefully reasoned; but, while resting on no personal observation, is a little too prone to ignore, or set aside on purely theoretical grounds, those statements of other observers which do not conveniently fit into it. It is impossible here to review

his paper as fully as the obvious care bestowed upon it deserves, but the following is a brief summary of its main points:

The ancestor has a hydrocœl with eight tentacles surrounding the mouth (which is ventral) and two symmetrically disposed body-cavities. It then becomes fixed by the tentacles of the right side, three of which are thereby suppressed; and after this the point of fixation shifts to the centre of the right side, while the mouth (surrounded by the five remaining tentacles) moves into the left side.

In this way he arrives at an arrangement of the body-cavities and mesentery with relation to the alimentary canal which I believe to be very nearly correct; but the steps by which this position is reached are open to grave objections. In the first place, there is no sort of evidence that more than five tentacles ever existed. Bütschli begins with ten, but afterwards reduces them to eight, with suppression of three, for the sole purpose of explaining the remarkable bilateral symmetry observed by Lovèn in Echinids. For the details of this explanation reference must be made to the original paper; but as we have no sort of evidence that this symmetry extends to other groups, I venture to think that the assumptions made to explain it are wholly unwarranted. Of fixation by the right side there is no more embryological evidence than of the fixation by the dorsal side assumed by Semon; but the whole assumption of universal fixation is founded mainly on palæontological evidence (6, p. 137), to which I shall return later.

In addition to these more important assumptions, there are many other points unsupported by evidence (e. g. the pulling by the œsophagus of a part of the right body-cavity into the oral side—see p. 146; and the derivation of one of the five permanent tentacles from the right side—see p. 157, note), as well as others in which serious distortion of evidence occurs. Thus he accepts Ludwig's determination of the position of the anus in Crinoids, in preference to mine (since proved by Seeliger to be correct), because it agrees with his theory (6, p. 156), regardless of the evidence I produced in my last paper to show that in other Echinoderms also the anus was

probably primarily in the same interradius as the water-pore. Again, on purely theoretical grounds he assumes that the closure of the water-vascular ring in ontogeny ought to take place where we find it in Asterina; and he therefore boldly denies (p. 157) the accuracy of the observations tabulated in my last paper (**5**, fig. 28), except in the case of Ophiurids, in which he assumes that the hydrocœl has been turned completely round in ontogeny—its anterior end in the larva being the original posterior end! I leave the facts in these cases to speak for themselves; but I cannot help expressing a regret that Bütschli should have allowed his theories to carry him so far without taking the smallest pains to find out for himself where the truth lay.

MacBride's hypothesis (**19**) is in many respects more nearly in accordance with the facts of embryology than its predecessors, and contains much that is suggestive; but though it is perhaps unfair to criticise it while only an abstract of it is before us, yet it gives one the impression of being based too completely upon the ontogeny of Asterina, the only form, apparently, in which MacBride has personally followed the metamorphosis.

He assumes that the bilateral ancestor possessed two hydrocœls ("collar-cavities"), of which one at least (the left) had five tentacles. Whether there were five more on the right side I cannot certainly gather, but at least they are not represented. This bilateral form became fixed by the præoral lobe, and then the left hydrocœl and left posterior enterocœl grew round the œsophagus, the former embracing the base of the præoral lobe in Echinozoa, but not in Crinoids. This encircling of the œsophagus is regarded as brought about by "the curious, and as yet unexplained, peculiarity of Echinoderms, the predominance of the left side (left hydrocœl and left posterior body-cavity"). On these views I offer the following comments:

(1) Whatever may be thought of the existence at an early period of a second (right) hydrocœl (and to this question we shall return later), it is improbable that it could have retained enough importance to bear tentacles at the stage under consideration, without leaving more trace of them in ontogeny.

But if, on the other hand, as MacBride's figure (fig. 2) indicates, only the left hydrocœl of the bilateral ancestor had tentacles, then these tentacles, running down the left side, without any special relation to the mouth, occupied an unparalleled and, to my mind, most improbable position. Moreover, the position assigned to them in this figure, though suiting fairly well the larva of Asterina, is not at all in accordance with what we find in Ophiurid Plutei, in which the hydrocœl surrounds the œsophagus at right angles to the dorsal mesentery, before bilateral symmetry is lost. I shall endeavour to show later that Ophiurids are more likely to be primitive in this respect than Asterina.

(2) MacBride's idea of the fixation of the ancestor, followed by different changes in Echinozoa and Pelmatozoa respectively (**19**, p. 434), will not suit palæontologists, but is for all that more likely, in my opinion, to be right than those theories (such as Semon's, Bütschli's, &c.) designed to satisfy the supposed teachings of palæontology, to which we shall return. It must, however, be borne in mind that it rests solely on the fact that Asterina, as well as Antedon, becomes fixed by the præoral lobe; no trace of such fixation has yet been found in any other Echinozoan larva.

(3) We have seen that in post-larval stages of all Echinoderms, except Holothurians, the mesentery of the water-tube (formed, as MacBride rightly recognises, by the left body-cavity only) is at right angles to the original longitudinal mesentery of the bilateral stage, while the water-pore is almost at the level of this longitudinal (now transverse) mesentery. I am at a loss to understand how this condition can be brought about by any amount of "predominance of the left side." No doubt the greater this predominance the more nearly will this condition be approached, but it can never be actually reached, and the hypertrophy must be enormous before anything like it is attained. But what is the evidence of this hypertrophy? No doubt it occurs early in Brachiolaria and Asterina, and perhaps too in Echinid Plutei; but, on the other hand, metamorphosis seems to be accomplished in Ophiurids without any

marked change in the proportions of the two body-cavities; while in Crinoids it actually ends in the left cavity being smaller than the right. On Holothurians I offer no opinion, but I fail to see in them any support of MacBride's views; while in Bipinnaria asterigera, lastly, in which the adult relation of hydrocœl and body-cavities is assumed long before metamorphosis, I have shown that the preponderance of the left cavity only arises when the arms are formed, and apparently as a consequence of this formation—certainly not as a consequence of the growth of the hydrocœl and left body-cavity round the œsophagus. Here, again, as it seems to me, MacBride relies too much on the larva immediately under his notice.

Those who have approached the question of the origin of Echinoderms from a palæontological standpoint, have almost without exception derived all existing forms from the Cystidea. Various genera are pressed into service as ancestral, but at some period or other all the Echinozoa are supposed to have passed through a stage in which they are fixed by the aboral pole. Of this there is not the slightest embryological evidence, for even if we follow MacBride in regarding the fixation of Asterina as an ancestral feature, that fixation is by the oral, and not by the aboral surface, so that it does not in the least satisfy the requirements of the palæontologists. Nevertheless, almost all embryologists, apparently out of deference to palæontological conclusions, have thought it necessary to assume that ontogeny is misleading, and that a period of fixation really did take place, of which all traces have since disappeared.

Now this involves us in a question of fundamental importance. If palæontologists have really proved beyond any reasonable doubt that the Echinozoa are derived from fixed ancestors, then ontogeny is misleading; but if it is misleading to such an extent as to obliterate all traces of a process of such immense importance, I for my part do not see how we can trust it in other particulars, and those who rely upon it for indications of phylogenetic history had better re-consider their position. (The fixation of Brachiolaria and Asterina obviously

does not help us: if, with MacBride, we regard it as primitive, we directly oppose the palæontological position; if it is secondary, Asterids are in the same position as other Echinozoa —they retain no trace of aboral fixation.) But after all, have palæontologists so completely established their position as to compel us to accept it? From the nature of the case no details can be known of the internal anatomy of the Cystidea, and consequently in connecting them with modern forms we are obliged to rely solely on the general arrangement of the skeleton, and on the position of the few apertures (mouth, anus, &c.) which we can distinguish. The latter seldom help us far, while the untrustworthiness of the former is proved by the fact, so clearly emphasised by Neumayr (**25**, p. 497), that in order to derive the Echinozoa directly from the Cystids at present known, we are compelled to regard the resemblances of the skeleton in Crinoids and Echinozoa as due to homoplasy, not to homology. Neumayr thinks that the origin of Echinids can be very clearly traced through the Cystid Cystocidaris (**25**, p. 400); yet his description of this form does not give a single really convincing item of homological resemblance, while there is much that it is quite as easy to lay down to homoplasy as the far more striking similarity of the basal plates in Crinoids on the one hand, and Asterids and Echinids on the other.

It is beyond the scope of the present paper to pursue this subject further, but I submit that until palæontologists have produced some far more striking intermediate forms between fixed Cystids and free Echinozoa than are at present forthcoming, embryologists may be forgiven if they do not follow them.

But even if we deny, on embryological grounds, that the Echinozoa ever had a stalk or disc of fixation on the aboral surface, there remains the further question whether they may not have been fixed, as MacBride supposes, by the præoral lobe, which afterwards shifted—in them to the oral side, in Pelmatozoa to the aboral pole. Since this actually occurs in some Asterids, it cannot be said that this view is so violently opposed to embryology as the one we have just been discussing;

but here again the negative evidence ought, it seems to me, to weigh very heavily with those who rely, as much as MacBride does, upon ontogeny as a repetition of phylogeny. At present the positive evidence is exceedingly weak—only in Asterids, and not even in all of them, has this fixation been found, in spite of most diligent search for it; and so weak is the atavistic tendency to recover this supposed phylogenetic character that Amphiura squamata, though fixed to the body of the mother in its young stages, is fixed by the posterior, not by the anterior end. Here the fixation is clearly secondary, and until further evidence is brought forward I am strongly disposed to regard the fixation of Asterina as also secondary, and quite independent of the fixation of Antedon. Fixation by the præoral lobe is no uncommon thing, so that it may easily arise over and over again; and when we consider the apparent difficulties of the transition from the bilateral to the pentamerous stage in other larvæ (as evidenced by the rapidity of the metamorphosis and the frequent obliteration of the œsophagus), we can easily see the advantages of such fixation, especially to a shallow-water form exposed to wave action. This, however, is pure speculation; what is really important is, I repeat, the strength of the negative evidence, which to my mind is so great as to make it unwise to assume the fixation of the ancestor so long as any other explanation is possible. That the phenomena can be accounted for without this assumption I shall endeavour to show in the following pages. I am aware that the proof of my views is still far from complete, and for that reason I shall not attempt to follow out the details so far as some of my predecessors have done; but the large number of speculative suggestions as to the origin of Echinoderms which have appeared in the last few years, almost all assuming original fixation in some form or other, seemed to make it advisable that I should attempt to show that such an assumption is neither embryologically sound nor necessary as a basis for phylogenetic speculation.

The Bilateral Ancestor.

Into the most primitive stages of the bilateral ancestor I do not propose to enter here; some few remarks on the subject will be offered at the end of this paper, when I come to discuss the relation of the Echinodermata with the Enteropneusta, but for present purposes it will be enough to start with a stage —of the existence of which I believe there is sufficient evidence— in which the hydrocœl already formed a ring round the œsophagus, and five tentacles already laid the foundation of the future pentamerous symmetry, while behind the œsophagus the alimentary canal and body-cavities still retained the primitive bilateral symmetry. Such a form is shown in figs. 44 and 45, and may be described as follows:—The mouth was bent down on to the ventral surface, and probably opened into an atrial cavity, though this is uncertain; the stomach was globular or slightly elongated antero-posteriorly, and from its posterior end the intestine ran forward, opening on the ventral surface not far from the level of the anterior margin of the stomach. Round the œsophagus was the water-vascular ring, with five tentacles or tube-feet, suitable to progression, but capable of retraction within the atrial cavity (if that existed at this stage). One tentacle was median and posterior, while in the dorsal interradius, on the left side of it, was the water-tube running back to the water-pore, which was situated over the anterior end of the stomach, rather to the left of the middle line. The left anterior enterocœl was probably already reduced to a simple ampulla at the junction of water-tube and pore-canal, such as we find in adult Echinoderms, though it probably ran a little forward (see fig. 45) alongside and on the dorsal side of the water-tube. The right anterior enterocœl had probably already disappeared. The two large body-cavities were symmetrically disposed on the right and left sides of the stomach, and there was certainly between them a longitudinal dorsal mesentery, though how far this extended round to the ventral surface is uncertain. In the middle line, just to the right of the water-pore, I believe there was a "dorsal sac,"

and under it a "dorsal organ" extending probably forwards towards the water-vascular ring; but though I have introduced these into fig. 45, I shall, for the sake of clearness, omit them almost entirely from my subsequent description, returning to them when I deal with the relation to Enteropneusta.

The ancestral form just described is closely similar to Semon's "Pentactæa," from which it differs in not being fixed, and in the body-cavities not extending forwards far enough to form a mesentery enclosing the water-tube. Still more closely does it resemble an Ophiurid Pluteus, just before metamorphosis, deprived of its arms and its pelagic habits; indeed, almost the only important points of difference lie in the facts that the Pluteus has a pentamerously-arranged skeleton (which I shall deal with immediately), but has not a very definite atrial cavity, which, however, is not essential to the ancestor.

One of the first questions which meets us in trying to reconstruct the history of Echinoderms is—what organ, or group of organs, originated the pentamerous arrangement? The Sarasins (**28**, p. 147) have given precedence on this point to the longitudinal nerves and muscles; but I think most embryologists will be inclined to follow more closely the teachings of ontogeny, which seem to point to either the hydrocœl or the skeleton as the first to exhibit this symmetry. Now in spite of the markedly metameric arrangement of the skeleton in many larvæ, there are very serious difficulties in the way of making it the starting-point of the pentamerous symmetry; indeed, all homologies of the skeleton between Pelmatozoa and Echinozoa have been of late strenuously denied. But without entering upon this very difficult question at present, let us examine the primitive nature of the five pouches of the hydrocœl, and see to what results it will lead us; for, after all, such assumptions must be judged more by the results deducible from them than by the direct evidence in their favour—provided always that ontogeny establishes a fair primâ facie case, which is not, I think, true of the Sarasins' supposition.

It is not my purpose in this paper to discuss how or why the hydrocœl came to encircle the œsophagus; our present evidence does not seem to me to be sufficient to allow of even a plausible guess; but that it did assume this position before the general bilateral symmetry was lost, there is a good deal of evidence to show. In the first place, it actually does assume this position in Ophiurids; and secondly, in many larvæ its plane forms a marked angle with that of the longitudinal mesentery—an arrangement easily derivable from that of Ophiurids. This is seen in Asterina (15, p. 156, fig. 3, taking the "antiambulacralen Armanlagen" as parallel with the mesentery), and in Crinoids (4, fig. 59). As we shall see in the next stage, this condition may be considered as a derivative from that seen in Ophiurids, approximating to a later arrangement; while it is not easy to understand, if this or the later arrangement (with the planes of hydrocœl and mesentery parallel) is primitive, how the Ophiurid position was ever arrived at. In Holothurians, on which Semon's Pentactæa is founded, this point is not so clearly defined as he supposed, as my description of Synapta will show; but as there is certainly nothing in this form opposed to the view that the Ophiurid arrangement is primitive, this need not detain us now; while the fact that no trace of this arrangement is seen in Bipinnaria asterigera or in Echinid Plutei is easily explained, when we consider that in these larvæ the hydrocœl ring is arranged in relation to the secondary, not the primary, œsophagus. But all this will be clearer when we have considered the next stage in ancestral history. The same is true of several details in the positions assigned to the water-tube, &c., but the situation of the water-pore is clearly in accordance with what we find in most larvæ, while the adradial position of the opening of the water-tube into the water-vascular ring has been frequently commented on in the foregoing pages. It is this position of the water-tube, together with the probability of a symmetrical arrangement, which has led me to assign a dorsal position to one interradius; for though it is tempting to assume a mechanical function for the posterior unpaired tentacle, and perhaps for the number (five) of the

primary tentacles, it would be very unsafe to attach any importance to such a speculation. That an atrial cavity was present in the common ancestor at some stage or other is rendered almost certain by its presence in such widely separated groups as Crinoids, Echinids, and Holothurians; and whether it arose at the stage now under consideration or at a later one is a question of no importance.

The only other point requiring justification is the limit placed on the forward extension of the posterior body-cavities. It has apparently escaped the notice of Semon and Bütschli that these cavities rarely extend beyond the anterior margin of the stomach; even in Ophiurid Plutei (5, fig. 4) where they extend further than elsewhere, they still lie posterior to the water-pore. Asterid larvæ must, of course, be judged by those early stages in which the anterior and posterior cavities are separated on the left side, and they will then be found to agree with other larvæ in this respect.

It will be noticed that I have given this ancestor neither præoral lobe nor point of fixation; a rudiment of the former (which probably existed at an earlier epoch) may have been present, and there may have been on it a sucker for temporary fixation (though I refuse to admit that complete fixation can have occurred without leaving stronger evidence behind); but such assumptions, though leaving the main outline of my hypothesis untouched, are wholly unnecessary, and to my mind not justified by the evidence at present before us.

Transition to Radial Symmetry.

In all recent adult Echinoderms (with the exception of Holothurians, to which we shall return later) the plane of the water-vascular ring is at right angles to the axis of the œsophagus and parallel to the longitudinal mesentery of the larva. To arrive at this condition from that of our hypothetical ancestor, it is only necessary to assume a movement of the œsophagus, with the water-vascular ring, into the left side of the animal. Of course I do not mean to assert dogmatically that an actual migration, and that alone, of the œsophagus

occurred, for it is quite possible that the result was produced by a complicated system of hypertrophies and atrophies; but the predication of an actual migration gives us the simplest process for descriptive purposes, and at the same time emphasises my objection to MacBride's assumption that hypertrophy of the left side has been the cause of the change of symmetry.

Though the water-vascular ring almost necessarily moved with the œsophagus, which it surrounds, the water-pore, lying over the anterior end of the stomach, was not involved in this movement. The moment the œsophagus began to move it came in contact with the margin of the left body-cavity, which seems to have become invaginated to receive it, and by the time the œsophagus reached the centre of the left side of the stomach, to have surrounded it completely (see figs. 47 and 48), possibly forming a mesentery to support the water-tube—but to that we shall return. Figs. 46 and 47 will render the transitional stages sufficiently intelligible, while figs. 48 and 49 show the arrangement of the principal organs when the œsophagus has reached its resting-place in the centre of what was the left side of the stomach. The water-tube is elongated so as to run over the surface of the stomach from the water-vascular ring to the water-pore, and parallel to it as before runs the anterior enterocœl (ampulla). Almost at the same level as the water-pore the originally longitudinal mesentery forms a sort of equatorial band round the stomach, and in this plane lies the intestine, which has not changed its position relative to the stomach and mesentery.

It will be noticed that in these diagrams (figs. 48 and 49) I have assigned positions to the tentacles which do not quite accord with the supposition of a simple movement of the hydrocœl into the left side. In the bilateral form the tentacles were arranged symmetrically about a dorso-ventral plane, one on the ventral side being median. If the movement into the left side were as simple as I have so far represented it, this plane of symmetry would now lie approximately along the line X–Y in fig. 48 (its exact position depends on how we construct fig. 44). But this, if we assume the water-tube still to open

into the water-vascular ring in the interradius through which this plane passes, would give (as the construction of a simple diagram will easily show) a very oblique course to the water-tube between the water-vascular ring and the pore; and in order to obtain the straight course for this tube, which it invariably possesses, we must assume either that the tube has shifted the position of its opening into the water-vascular ring, or that the latter has been rotated. That the latter is the more probable solution we shall see when we have examined the justification of the main features of my hypothesis afforded by ontogeny.

If we may judge by external appearances, Ophiurid Plutei repeat with most completeness the features of the supposed ancestral migration of the œsophagus and water-vascular ring. On the other hand, in Bipinnaria asterigera the hydrocœl lies from the first nearly in the middle of the left side of the stomach, and a new œsophagus is formed in the centre of it, so that there is no migration of either organ. Between these two extremes we have several very instructive intermediate steps.

In Crinoids there is no œsophagus, but the atrial cavity and water-vascular ring both migrate, so as to place the plane of the latter more and more parallel to the cœlomic mesentery; but the nature of the process is much obscured by the early change of position of the body-cavities and by the migration being directed towards what appears to be the posterior pole. In Echinids there is no migration of the œsophagus, but the atrial cavity and hydrocœl move over the left side of the stomach, arriving fairly early at their final position. The formation of a new œsophagus is obviously secondary, but otherwise the ancestral process seems to be fairly closely followed.

Brachiolaria (including Asterina) is still more interesting. Here, as in Bipinnaria, the hydrocœl is from the first nearly in the centre of the left side (its position there being in my opinion secondary), and it consequently undergoes no migration. But the larval œsophagus is retained in the adult, and at the time of metamorphosis is bent sharply into the middle of the hydrocœl ring; at the same time it undergoes a complete change of position

with relation to the longitudinal mesentery and water-tube, which justifies us in asserting that here, too, there is an actual migration over the left side of the stomach.

All these differences between the various larvæ are easily intelligible as shortenings in ontogeny of the phylogenetic migration—the shortest process of all being found in Bipinnaria asterigera, in which both hydrocœl ring and permanent œsophagus are produced in the positions which they will ultimately occupy.

In close connection with this lies the point already alluded to in connection with the bilateral ancestor—the angle which the plane of the water-vascular ring forms with that of the mesentery. If the facts above given are referred to, it will be found that here too we have represented the steps by which the ontogenetic process has been shortened—the two planes being at right angles to one another in Ophiurids; inclined at a lesser angle in Brachiolaria and Crinoids; and parallel from the first in the Bipinnaria.

The facts just quoted with regard to the migration of the œsophagus and water-vascular ring greatly strengthen the conclusion that the Ophiurid position of the latter—at right angles to the mesentery—is the primitive one.

The encircling of the œsophagus by the left body-cavity has been sufficiently emphasised in the early part of this paper; but the frequent occurrence of a communication between the right and left cavities makes it often difficult to say how far this process is carried, and how far we are justified in asserting that the mesentery of the water-tube ("oral mesentery," let us call it for brevity's sake) is really bounded on both sides by the left body-cavity. The following summary of the facts will help us to a conclusion.

In Echinids there seems to be no fusion of the body-cavities before metamorphosis; but the dorsal and ventral horns of the left cavity fuse completely, the oral mesentery being formed later. Further investigation of these larvæ is, however, desirable.

In Brachiolaria it is fairly certain that the left body-cavity bounds the oral mesentery (containing the axial sinus) on the

ventral side of the larva; for though the right and left cavities are united, the point of union takes place so far to the right of the intestine as to make it almost inconceivable that the right cavity should have anything to do with this region. The dorsal horn of the left body-cavity fuses with the axial sinus.

In Bipinnaria the early fusion of the two body-cavities makes accurate determination of their limits impossible. The dorsal horn of the left cavity behaves as in Brachiolaria.

In Crinoids, again, the ventral horn of the left cavity cannot be traced with certainty up to the oral mesentery; but the opposite side of this mesentery is unquestionably bounded by the dorsal horn.

These facts, taken together, seem to me to establish almost beyond doubt that the growth of the left cavity round the œsophagus is complete (except for the possible intervention of a mesentery between the two horns), and that the right cavity has no connection with this region. Whether the oral mesentery is primary or (as Echinids seem to indicate) secondary is a question which need not detain us now.

That the water-pore did not move with the water-vascular ring, but retained its original relation to the longitudinal (now transverse) mesentery, the facts of ontogeny abundantly testify. That it has since moved slightly either to oral or aboral side of this position need not surprise us; but in all larvæ, even after the establishment of pentamerous symmetry, it will be found practically at the level of the mesentery, and I do not think that even in adults it has ever migrated to any large extent.

The course of the intestine along the equatorial line marked by the mesentery, and the opening of the anus in the interradius of the water-pore, as shown in fig. 48, are best attested by the Crinoids; but the former is well enough seen in Echinid Plutei also, while in the same group, after metamorphosis, the intestine still coils right round the disc as far as the above-mentioned interradius, though the anus does not open there. Fig. 23 shows the intestine for the most part on the right (aboral) side of the stomach in Bipinnaria, but the mesentery is attached to its edge; and the anus in this larva

is in the same interradius as the water-pore (see fig. 24) before metamorphosis. A further indication that this was the primitive position of the anus is given by its relation to the calcareous plates in Ophiurid Plutei. No doubt these plates (which mark the radii) are precociously developed, but their situation, when we compare this larva with Bipinnaria or Brachiolaria, points pretty clearly to the conclusion just arrived at. On the position of the anus in adult Asterids and Echinids, much nearer the aboral pole than the water-pore, I shall have some further remarks to offer later on.

Of course this question of the situation of the anus, though interesting, has no vital bearing upon my hypothesis; it is important, indeed, that the general course of the intestine should be parallel to the plane of the water-vascular ring, and that in early stages of ontogeny it should lie at the level of the equatorial zone (mesentery); but the anus may be placed in any interradius that ontogeny demands—that is simply a question of the length of the intestine. In my diagrams I have made it long, so as to bring it into the interradius of the water-pore;[1] but it would have been quite as easy to shorten it into the interradius which it occupies in Asterids, or still further into that in which it is found in adult Echinids.

We saw that a straight course for the water-tube from water-pore to water-vascular ring, which is demanded by the anatomy of all known Echinoderms, was inconsistent with the very simple movement of this ring into the left side which I had postulated; and we must now examine how the observed position may have been brought about.

Seeliger (39, p. 261) takes up the somewhat remarkable position that the water-pore is not in the same interradius in all Echinoderms, giving as evidence the fact that it is in the middle dorsal line in Holothurians (which is not strictly true), but far removed from it (on the left side) in the larva of Antedon. In making this statement, however, he seems to have forgotten the fact that, before the pore appears, the body-cavi-

[1] A slight alteration of my drawing has brought it into a radial position in fig. 48.

ties have already shifted their position in Antedon, so that the relation of the pore to the "Medianebene" of the larva as a whole no longer has any morphological importance.

If we compare Antedon in the "Cystid" stage with an Echinid, we shall see that in both the water-pore may be stated to lie in the same interradius as the œsophagus, alongside which runs the mesentery holding the water-tube. From this point in both cases the alimentary canal runs round the disc till it again reaches this same interradius. It is true that only in Antedon does the intestine end here, but remembering that evidence which Echinids afford of the variability of the anal interradius, this is of small importance; and it is a bold thing to deny that the interradius of the water-pore is not homologous in these two forms, yet Echinid Plutei have the water-pore even more nearly in the "Medianebene" than the Holothurians on which Seeliger relies. A further argument might be derived from the skeletal plates, but it is unnecessary to pursue it; it is enough for my purpose to show, not the impossibility of Seeliger's assumption, but the extreme weakness of the evidence. In the larvæ of all Echinozoa the water-pore lies at the anterior end of the dorsal mesentery, though usually somewhat to the left. In Bipinnaria the mesentery becomes oblique (fig. 22), and the pore is consequently pushed to the left of the "Medianebene"; in Antedon this mesentery is still more oblique at the time the pore appears, and consequently the latter is situated further still over on the left side—even, indeed, on the ventral surface. This, at least, appears to me to be a far easier explanation of the phenomena than Seeliger's supposition that the pore has changed its interradius.

A change of position of the union of the water-tube with the water-vascular ring would be a good deal more difficult to detect; but in the entire absence of any evidence that it has taken place, we are not justified in assuming it as the cause of the straight course of the former, so long as any other explanation is possible. That another cause is not only conceivable, but actually supported by ontogeny, I shall now endeavour to show.

Turning to fig. 48, we see that a gradual rotation of the water-vascular ring, as it moved into the left side, would suffice to maintain the straight course of the water-tube, without any change of interradius of either of its extremities —the total angle of rotation being about 70°. Now compare Ludwig's diagram (15, p. 157, fig. v) of the changes by which the "ambulacralen Armanlagen" (hydrocœl pouches) are brought into connection with the "antiambulacralen Armanlagen" (formed over the left body-cavity, parallel with the mesentery), and it will at once be evident that we have here a twisting of the hydrocœl through a considerable angle (though not as much as 70°) in exactly the same direction (remembering that Ludwig gives a dorsal view and I a ventral) as I have postulated; the movement, in fact, may in both cases be described as tending to unscrew the water-vascular ring from the stomach.

Here, then, we have a possible explanation of a very remarkable phenomenon. Of course so long as we are ignorant of the meaning of the extraordinary variability in the point of closure of the water-vascular ring noticeable in Echinoderm larvæ, we cannot hope to explain all the peculiarities of development presented by Brachiolaria; but the meaning just suggested for one of the most striking of them fits in so completely with the needs of my hypothesis that I cannot help attaching a good deal of importance to it. In considering why this change is more strikingly presented by Brachiolaria than by any other larva, we must remember that this form is peculiar in that the hydrocœl early arrives at its position alongside the stomach, while the œsophagus only joins it there at the time of metamorphosis. In Bipinnaria and in Echinids the hydrocœl very early assumes its final position, and a new œsophagus is formed; so that the rotation of the hydrocœl (if it is not entirely omitted in ontogeny) probably takes place before the development of the tentacles enables us to recognise it. In Ophiurids the rotation ought to take place exactly as in the ancestor; and I think when we come to consider the matter carefully we are bound to admit that it does so, though the

details are a good deal masked by what appear to be secondary and purely ontogenetic processes.

Hitherto, for simplicity of description, I have regarded the stomach as fixed and the œsophagus as undergoing movement; but seeing that the mouth is always on the ground, to which the animal adheres by its tentacles, it might have been more accurate to regard this as the fixed point, and speak of a movement of the stomach across the base of the œsophagus. One of the first results of this movement is to throw the water-pore and front end of the mesentery across to the right side, as seen in fig. 46, and this finds its counterpart in ontogeny in the position of the water-pore in a late Ophiurid Pluteus (fig. 43). But a close comparison of these two figures will show us that in the Pluteus some other changes have occurred as well. The mouth was from the first ventral, and in order that the left body-cavity may surround it, there must be a movement of this cavity towards the ventral side. There are in fact two movements, one tending to make the left side of the stomach anterior, and the right side posterior (as in fig. 46); and the other pushing the left side on to the ventral, and the right side on to the dorsal surface. The former would lead to great asymmetry, which the latter would to some extent counteract; and it is just possible that we have here the reason of this second movement, though as we are as far as ever from the reason of the first, this suggestion is not of much value.

Now if we consider carefully what the nature of this second movement is, we shall see that it involves exactly that "unscrewing" of the stomach from the œsophagus which we have already seen in Brachiolaria, and which we now see must also take place in Ophiurid Plutei. An examination of the hydrocœl pouches in this larva points in the same direction. At first they are arranged as a longitudinal series along the left side of the œsophagus (fig. 40); then one by one they pass across the dorsal surface of this organ, and encircle it; and finally, at the time of metamorphosis the water-pore also moves forwards and to the right (by a process already explained), and we reach the rather curious result that that tentacular pouch, which was

originally posterior, is now the most anterior of all. It would be difficult and tedious to describe in full the changes of position which the other pouches undergo, but in fig. 40 I have placed numbers against them so that a comparison with 5, figs. 2—6, will show with which arm-rudiment each unites. Much of this complicated process no doubt is due to ontogenetic causes, and is unconnected with phylogeny, but the movement by which the primarily anterior tentacle (see 5, figs. 2 and 4) passes all round the œsophagus and finally is embraced by the second terminal plate is so peculiar, and so exactly corresponds in direction with the movement of the hydrocœl in Asterina, that it can hardly be without significance. In both larvæ it will be noticed that the pouch which lies immediately anterior to the water-tube subsequently unites with that terminal plate which is the most posterior of the longitudinal series in the larvæ (5, "Terminal 5" in fig. 2).

The two movements spoken of above are combined in such a manner in Ophiurid Plutei that at the end of metamorphosis the original antero-posterior axis, still traceable with the help of the arms of the Pluteus, passes distinctly to the left of the water-pore (compare 5, figs. 5 and 6); this is due, as already explained, to the first movement of the above description, while the second movement is responsible for the fact that the former left side of the stomach is at this stage (if we may trust the external evidence of the calcareous plates) entirely on the original ventral surface. In Crinoids, on the other hand, it would seem that the first movement has little effect, while the second carries even the pore far over to the left of the original "Medianebene." An intermediate condition between these two is seen, as already indicated, in Bipinnaria (fig. 22).

As previously mentioned, the position ascribed to the dorsal sac and organ in the bilateral ancestor will be discussed especially in connection with the relation to the Enteropneusta; but it is worth while here to show that, if that position be admitted, the movements we have just been considering will bring these organs exactly into the relation to surrounding structures which is observable in adult Echinoderms. The dorsal sac has so far

only been found in the larvæ of two groups—Asterids (both forms of larvæ) and Echinids, but I shall assume for the present that it has a phylogenetic value. Situated, as it is in these larvæ and in my hypothetical ancestor, just to the right of the water-pore, it seems, like the latter, to have escaped the movement into the left side, or perhaps I ought to say, has moved with the pore and the anterior end of the stomach into an equatorial position. A glance at my diagrams will show that what was formerly on the right side of the water-pore must now be on its aboral side; and this is precisely the position of this sac in adult Asterids and Echinids.

The axial organ was assumed to run forward along the dorsal surface of the œsophagus, and it seems to have accompanied the latter in its change of position relative to the stomach and mesentery. As it lay to the right of the water-tube in the bilateral stage, so now it still occupies the same relative position when the animal is viewed from the aboral side with the interradius of the water-pore directed away from the observer. One end of the dorsal organ, however, was assumed to lie under the dorsal sac; if it retained this position it must, in the pentamerous stage, pass round at the aboral end of the water-tube from the right side of the latter to the aboral side of the water-pore, and this is precisely the course which, as Ludwig has shown (13, p. 159) it follows in adult Asterids. It seems to me that, apart from any possible homologies with Enteropneusta, it is not an unimportant feature of my hypothesis that it enables us to derive this very peculiar and asymmetrical course from an originally symmetrical one; and I believe that, if ever the axial organ is discovered in the bilateral stage of an Ophiurid Pluteus, it will be found to occupy exactly the position ascribed to it in my bilateral ancestor.

In fig. 45 I gave a forward extension to the anterior body-cavity, in order that it, with the water-tube and dorsal organ, might be brought down into the left side, and there by enveloping these two structures form the "axial sinus." That it might be made to do so is sufficiently obvious; but until

more is known of the development of this sinus not much importance can be attached to the suggestion. The fusion of left anterior and posterior cavities in Asterids makes it rather doubtful whether I was justified in asserting (5, p. 37) that the axial sinus was a part of the former, though I see that MacBride (19, p. 433) accepts my position. In Ophiurids, on the other hand, the same authority states (18, p. 135) that the axial sinus arises from the posterior cavity, quite distinct from the ampulla (anterior cavity). In Echinids I confess I am not sufficiently confident in my observations to assert a definite origin for this sinus. Further investigation must be left to settle this point, which fortunately is not very important to our present inquiry.

The nomenclature of all these parts is in a great state of confusion; but as long as we use the various synonyms merely as names, and not as descriptions of position, not much harm will accrue. Seeliger objects to the term "dorsal organ" because this structure is apparently more ventral than dorsal in Antedon; but what is to be said of the application of the epithet "axial," which he prefers, to Ophiurids and Asterids? My hypothesis indicates that this organ may have been at one time dorsal, but it certainly is not so in true Echinoderms, and this being the case, both terms (axial and dorsal) seem to me equally objectionable if held to be descriptive, and equally unobjectionable if used merely as convenient names.

Further Development of Radial Symmetry.

The ancestral form at which we have now arrived would probably, if found in a fossil state, be included in that very heterogeneous group, the Cystidea, from the simpler forms of which it differs chiefly in possessing only five tentacles. If, however, I am right in supposing that it was not fixed, and that these tentacles were used for locomotion, it must have been of such extremely small size as to render its discovery as a fossil very improbable; for though, for the sake of clearness, I have in my diagrams (figs. 45 and 46) made the tentacles more slender than is probable, yet it is impossible to imagine an animal of any large size supporting itself upon only five of such

organs. It was probably increase of size which led to the further development of the ambulacral system, and this in its turn led to the completion of that radial symmetry which is among the most striking possessions of the Echinodermata.

The first addition to the ambulacral system probably took the form of the production of five pairs of tentacles at the bases of the original five, so that the total was raised to fifteen. The next stage is not so easy to follow. In Crinoids five more pairs are added between the previous pairs and the mouth, that is to say, in centripetal order; and then, with a total of twenty-five, there is a long pause. Many Cystidea never get beyond this total, so that we may regard it as primitive, at any rate for the Pelmatozoa.

As regards the Echinozoa, there is something of a pause when the number twenty-five is reached in Ophiurids, but not, I think, in other groups; and even here there is no evidence to show in what order the pairs arise. In all later-formed tube-feet the succession in Echinozoa is invariably centrifugal (acropetal), and this seems to be the case even from the first in Holothurians. In Crinoids, however, a totally distinct order is observable. We are then driven to the conclusion that the Pelmatozoa branched off at least as early as the stage with twenty-five tentacles, and there is some evidence that the common ancestor did not get beyond a total of fifteen.

At this point we may leave the Pelmatozoa, the origin of which will be discussed later, and for the present direct our attention chiefly to the Echinozoa, in which, so far as we can see, the increase in number of tube-feet (tentacles) went on pretty steadily, in acropetal order.

It is obvious that no great number of tentacles could start from the water-vascular ring itself, nor would their concentration there allow of any great increase in bulk in the body as a whole, and this seems to have led, from a very early stage, to the development of radial vessels, which may be described as elongations of the bases of the five primary tentacles, from which the paired tentacles sprang; and since these vessels would have had no strength if they had grown out, like

tentacles, free from the rest of the body, they were forced to spread over the oral surface, which thus became divided up into radial and interradial areas. This spreading of the ambulacral area, which to a large extent seems to have occurred independently in Echinozoa and Pelmatozoa, has apparently been the cause of that "radial segmentation" of various organs which I shall now attempt, very briefly, to follow.

It is probable—though we have no direct evidence on the subject—that the concentration of a portion of the nervous system into a ring round the mouth took place at an early period, and that this ring supplied the tentacles. When the water-vascular system spread over the surface of the body, the nerve-bands would naturally accompany it, and thus we should get a definitely radial arrangement of the nervous system. A special development of the muscular system along the same lines might be expected, and so it too would exhibit radial symmetry.

The Generative Organs appear to have followed the new (radial) symmetry at a fairly early period. What form they assumed in the bilateral ancestor we are not in a position to assert; they may very possibly have consisted, as they do now in Elasipoda, of a pair of glands opening by a median aperture on the dorsal side, just behind the water-pore; then, when radial symmetry was established, other pairs of glands (one pair for each interradius) may have been developed, connected together by the genital rachis; but whether this segmentation of the gonads took place in the common ancestor, and was afterwards lost in Holothurians, or whether the latter branched off from the parent stock before this was accomplished (and therefore before the separation of Echinozoa and Pelmatozoa), I am quite unable to determine. In any case the assumption of a primary pore just behind the water-pore (or rather, being median, behind the dorsal sac), enables us to understand why it is, as I have endeavoured to show, that the genital rachis marks the original line of division of the body-cavities—its growth simply following the line of the mesentery, in which

the primary genital pore lay. It also agrees with the direction of the growth of the rachis round the disc in ontogeny.

Whether the Skeleton was one of the first structures to assume radial symmetry, or whether this symmetry has been independently acquired by Echinozoa and Pelmatozoa respectively, is a question which is still too much sub judice for me to attempt to decide it. It may freely be admitted that when once the new symmetry was thoroughly impressed upon the body, any set of organs might acquire it in independent groups (we shall see directly that this has been the case); so that there is no reason why homoplasy should not be responsible for all the supposed fundamental homologies that P. H. Carpenter and others tried to establish. But between this and asserting that all the apparent resemblances are homoplastic, there is a very great difference. I admit that we cannot rely upon the skeleton alone to settle this question; but until we have arrived at far more certainty than at present as to the relations of the various classes of Echinoderms, I do not think we are justified in absolutely denying the possibility of homology between, for example, the dorso-central and basal plates of Crinoids, and the similarly-named plates in Echinozoa. We ought rather to return an open verdict and wait for fresh evidence.

The utter unimportance of this question to my hypothesis led me, as far as convenient, to omit all mention of the skeletal plates in the first part of this paper; one or two bits of fresh evidence, however, there introduced are worthy of consideration. In the first place there is not the smallest embryological ground for Neumayr's statement (25, p. 498) that the primitive arrangement of the plates in Echinids is in rows of ten; indeed, the invariable development of all plates in Echinoderm larvæ not directly connected with the ambulacral system in groups of five, is a very striking coincidence if the symmetrical arrangement has arisen out of primitive chaos, at various times and in ancestors of various sizes, independently; while it is easily intelligible if the number five was established, at any rate in a few plates, at that early stage when the

ancestor was still extremely small and the tentacles few in number.

Next I would point out that the correspondence of the series of plates with the body-cavities indicates that their symmetrical arrangement arose at a period when the distinction between these cavities was still very strongly impressed upon the animal. In recent adult Echinoderms this relic of bilateral symmetry is almost entirely lost; but in ontogeny, as we have seen, it is often retained (with approximate equality of the body-cavities) for some time after metamorphosis.

Lastly, my observations go far to prove that, whatever may be true of other skeletal plates, we have in Echinids, Asterids, and Ophiurids at least one set—the terminals—which are homologous. They are developed over the left body-cavity, and in all cases embrace the unpaired tentacles at the ends of the radial canals, which may be the reason that in these groups, though not apparently in Holothurians, the ambulacral system has failed to extend itself over the region of the right body-cavity. It must be admitted, however, that this is also true of Crinoids, in which the terminal plates do not exist.

The organs hitherto considered may (though this is uncertain) have assumed a radial arrangement before the separation from the parent stem of any of the recognised classes; but there are a few cases of radial symmetry which must have been arrived at independently by the groups in which they occur. This is the case in the water-pores (five in number) of Rhizocrinus, which seem to form a primitive feature in Crinoids (since Antedon passes through such a stage), but are not found in any other Echinoderms. Whether four of them were developed (simultaneously (one of course being primitive), or in succession as in Antedon), is not quite evident.

A similar case of the acquisition of radial symmetry by individual organs is seen in the form of the stomach in Asterids and Ophiurids. In Echinids, Holothurians, and Crinoids the alimentary canal is a thin tube winding round the disc without any trace of radial arrangement.

We are strongly reminded by the above phenomena of the varying degrees in which metameric segmentation has been acquired in other animals. Indeed Bateson (3, p. 432) regards the rays of Echinoderms as a successive, not a truly radial series. This is not invariably true in ontogeny; and if the explanation given above of the origin of radial symmetry in Echinoderms is correct (though I am far from asserting that it is), it will be seen that this symmetry was acquired by all organs except the water-vascular system in the radial stage, and that therefore the existence of pentamerism in the skeleton of bilateral larvæ is precocious.

We have seen that, in the remote ancestors we have been considering, there was probably but little difference in size between the two body-cavities; indeed, if the position of the anus may be taken as an indication of the level of the mesentery, the right body-cavity was, in many of the simpler Cystids, much larger than the left, as in fact it is in the "Cystid" stage of Antedon. In Echinids and Asterids, however, the growth of the ambulacral area (connected, as we have seen, with the left cavity only) has led to an enormous preponderance of the left over the right cavity. Hence the bulk of the alimentary canal came to lie in the region of the left cavity, and it need not surprise us if we find the mesentery forsaking its old position and following where it is most needed. This seems to have happened in Echinids, in which the intestinal mesentery undulates up and down in the region of the left cavity; while the old line of separation of the cavities is still marked by the genital rachis, and, to a less extent, by the skeletal plates.

In Asterids the mesentery probably retained its original position for a longer period, the stomach being supported by the septa; but with the outgrowth of the hepatic cæca into the arms, it too forsook its former position, which the genital rachis now alone marks.

The curious distribution of the two cavities in Ophiurids (as indicated by the genital rachis) may perhaps be due to an early

connection of the water-pore with one of the plates of the left body-cavity (the so-called "orals"). This pore was originally situated between the two series of skeletal plates, as ontogeny shows, and its connection with one or other of the plates adjoining it has apparently occurred independently in the different classes; but in no case, to the best of my belief, has it ever wandered far away from the original line of separation of the two body-cavities.

The freedom of the intestinal mesentery to move where it is required has led in some cases to a portion of it traversing part of the right body-cavity. In my hypothetical ancestor the anus is placed in interradius A, at the level of the division of the body-cavities, as we find it in the larva of Antedon. In Asterids and young Echinids, however, we see that it has shifted into interradius E, and into the region of the right body-cavity—being aboral to the basal plates. This, however, may be due not to a shortening but to a lengthening of the intestine, which, in Echinids at any rate (and in the larvæ of Asterids) reaches interradius A, and then (in Echinids) turns back to the anus in interradius E.

It is just possible that this position of the anus may be primitive; that at the very time that the mouth was working its way into the left side, the anus may have been moving into the right, but being (conceivably) prevented from reaching the pole by the dorso-central plate, it turned a little to the side into interradius E. In this case the position observed in Antedon must be regarded as secondary, due to a shortening of the intestine consequent upon the occupation of the aboral pole by the disc of fixation; and the "aboral longitudinal mesentery," found nowhere but in the larva of Antedon, and running from the aboral pole in interradius E to the level of the transverse mesentery in interradius A, very possibly marks the original course of the terminal portion of the intestine. I put this suggestion forward for what it is worth, knowing full well the weakness of the evidence. The ontogeny of other Crinoids may show us its true value, but the absence of the intestine in the larva of Antedon deprives it of all importance in this connection.

It would be altogether beyond the scope of the present paper to try and follow further the separation of the different classes of Echinoderms. The special features of the Pelmatozoa and Holothurians will be briefly considered in the following pages: but the exact period of separation of these and the remaining classes seems to me to require much further evidence.

I will only add here, as following closely upon the point we have just been discussing, a protest against Cuénot's (7) attempt to attach classificatory and phylogenetic value to the persistence or closure of the anus in ontogeny; it may or may not possess this value, but in the present state of our evidence it would seem almost as reasonable to attach importance to the persistence of the larval œsophagus in Ophiurids, some Asterids, and some Holothurians, or to the entire absence of the intestine in the young stages of Antedon and Asterina, as opposed to its invariable presence in all pelagic larvæ.

Origin of Pelmatozoa.

I have already given my reasons for believing that the Echinozoa were never fixed by the aboral pole, and that the utmost that embryological evidence will allow of is a fixation of the bilateral ancestor by the præoral lobe; and even this fixation, if it had been complete, and not a mere voluntary process effected by means of a sucker, would, in my opinion, have left more traces behind it than the study of embryology has yet afforded us. The existence of such a sucker is certainly possible, though the evidence for it is weak, and I do not think the assumption of it necessary. But before pursuing the subject further it is worth while to point out that whether the idea of primitive fixation is accepted or rejected, it remains certain that the changes of symmetry which we have been considering in the foregoing pages are common both to Echinozoa and Pelmatozoa; and since the acceptance of this idea, in whatever form, does not provide us with any explanation of how these changes came about, my hypothesis—which, indeed, only deals with the nature, not the cause, of these changes—is obviously independent of it.

Accepting for the present the supposition of a præoral sucker in the bilateral ancestor, it would seem that it ought, in the pentamerous form, to lie in the same interradius as other organs (water-pore, water-tube, dorsal organ, &c.) belonging to the same region; and here, I think, we meet with the one serious objection to this view, which involves the homology of the stalk by which Asterina is fixed with that of Antedon. The latter, as we have seen, is either in radius V, or, as I think, in interradius A, while the former, externally at least, is in the next interradius (E). It is true that it has its roots, so to speak, in interradius A, since it is there that we find the remnant of the anterior body-cavity, which before metamorphosis extended far into the præoral lobe; and the whole stalk has undoubtedly a very oblique aspect in Asterias rubens, though I have not satisfactorily traced its internal relations. It is to be hoped that MacBride, in his detailed account of Asterina, will give us some explanation of this point, which at present seems to me opposed to the homology he supports.

Thus the evidence of the existence of even a sucker in the common ancestor is extremely weak, and it is worth while to see whether the supposition that the Pelmatozoa became fixed after the change of symmetry, instead of before it, is not at least as probable.

Let us imagine that a sucker arose in the interradius of the water-pore, somewhere between that pore and the mouth; how or why it arose I cannot attempt to determine (explanations of this kind are, indeed, very seldom possible), but at least I see no a priori objection to the suggestion. The selection of this particular radius may have been purely a matter of chance, but the eccentric position of the mouth in the Cystid larva of Antedon, as well as certain peculiarities in the ambulacral fields—"hydrospires palmées" of Barrande (2)—of some Cystids suggest that perhaps this radius may have been rather different from the rest in early stages of pentamerism—possibly, even, it may have been (as it still is morphologically) the anterior interradius, and loco-

motion may have been, as in the bilateral stage, in its direction. However this may be, if fixation, beginning probably with a mere sucker, afterwards assumed a more permanent character, we have plenty of parallel cases to show the possibility (or even, perhaps, probability) that the mouth would move away from this point, and eventually reach the opposite pole. Here again no certain cause can be assigned; we may suggest that this movement was necessary to place the mouth in an advantageous position for obtaining food; but if the earlier position was disadvantageous, why did fixation ever take place? All we can say is that, if we may trust embryology, such a movement is a very common sequel to fixation by a point near the mouth.

The exact position of this sucker in the ancestor of the Pelmatozoa I cannot determine with any certainty. It may have been anywhere on the oral surface, but we do not even know what the extent of this surface was. In the later Cystid stages of Antedon (**26**, pl. x, fig. 90) the mesentery is oblique and the oral surface is fairly flat right up to the level of the water-pore, and this is true also of young Ophiurids; while in some Cystids, though this surface is not so flat, an equatorial line dividing the animal into equal oral and aboral halves would lie far on the aboral side of the anus and water-pore, if the identification of these apertures can be trusted. But without going to such extreme cases as this, it is very easy to understand that the hypothetical sucker may have occupied almost any position between the transverse mesentery and the mouth, though probably not within the atrial cavity. As its subsequent migration to the aboral pole does not seem to have affected the water-pore, it is probable that it lay nearly over the centre of this interradius, and therefore to the right of the pore, which, as we have seen, is adradial. But, while thus avoiding both water-pore and water-tube, it may possibly have involved some portion of the dorsal organ, which, as the diagrams show, lay further to the right than either of these structures; or if it was very broad, and was situated at the level of the mesentery, it might (though this is less probable) involve in its movements the dorsal sac.

In one of these two ways, it seems to me, we may find a possible explanation of the very curious course taken by the dorsal organ in Crinoids. So striking is the difference of position of this organ in Echinozoa and Pelmatozoa, that Cuénot denies the usually accepted homology. In combating this view MacBride (18, p. 147) suggests that the dorsal organ of Crinoids may be homologous with that portion of the "ovoid gland" in Asterids and Ophiurids which lies on the aboral side of the genital rachis. But this seems to me to imply misconception of the relations of the parts involved. The remarkable point about Crinoids is that the dorsal organ traverses the right body-cavity, no part of it, so far as we know at present, lying in the region of the left cavity. In Echinozoa, on the other hand, almost its entire course is in the region of the left cavity, the only portion which extends beyond this region being the small aboral portion which ends in the dorsal sac in Asterids and Echinids, and lies under "sinus b" in Amphiura (18, fig. 2, *e*). There is not the smallest evidence that the dorsal organ in any of the Echinozoa extends beyond the mesentery into the region of the right body-cavity. This, so far as it goes, furnishes an argument in Cuénot's favour; but the other objections to his view are so many and important, that most embryologists will be loth to accept it, so long as any other explanation is possible.

Now if, as I have supposed, the ancestor of the Pelmatozoa was fixed by a point lying over the dorsal organ, and this point afterwards migrated to the aboral pole, might not the subjacent organ share in this migration, and afterwards, taking the shortest course from this pole to the mesentery, pass, as it does in Antedon, through the right body-cavity, in the concavity of the alimentary canal?

Of course this is only put forward as a suggestion, and would require a great deal more evidence to prove it; but at least it seems to me to be more satisfactory than Cuénot's denial of homology, which no amount of fresh evidence seems likely to make wholly satisfactory, placing, as it does, a wider gulf between the Echinozoa and Pelmatozoa than the many

similarities between the two groups would justify us in recognising. With this exception the supposed migration of the disc of fixation to the aboral pole would be for the most part superficial in its effects, and the general arrangement of the internal organs would not be altered by it.

If a dorso-central plate existed at the aboral pole of the ancestor, I see no difficulty in supposing that the disc of fixation might come to lie external to it, the animal up to this stage remaining sessile; and that when the stalk was formed this plate was borne out on the end of it. But whether we accept this skeletal homology or not, I do not see sufficient grounds for adopting MacBride's conclusion (19, p. 436) "that the abactinal poles of Asterina and Comatula are not comparable with each other, and that all conclusions based on the supposed homology of the dorso-central of Echinids and Asterids, and that in Crinoids, are incorrect."

The exact stage at which this fixation may be supposed to have occurred is not, perhaps, a matter of great consequence. The frequent occurrence in Cystids (some of which may almost certainly be regarded as the earliest Pelmatozoa) of twenty-five tentacles (five to each radius) suggests that this may have been the number reached by the common ancestor of all the Echinoderms; but, as already mentioned, the absence of any pause at this stage in most Echinozoa, as well as the apparently anomalous order of development of these twenty-five in Antedon, render this extremely doubtful, and make it perhaps more probable that the separation of these two main divisions of the Echinodermata took place at a still earlier period, though whether at a stage with fifteen tentacles or with only five there is no evidence to prove.

It remains for me to show that the hypothesis put forward above is not inconsistent with the apparent teaching of embryology that fixation took place by the præoral lobe. It seems to me that we are apt to speak of this lobe as if it were a definite organ, instead of a region of the body possessing a great number of parts; and that though Brachiolaria and the larva of Antedon are both fixed, apparently, by the præoral

lobe, we are not at present in possession of any facts to show that the same part of the lobe is involved in both these cases; so that even the proof of the homology of their position is yet imperfect, while the proof that they had a common origin, and are therefore completely homologous, is further off still.

We have seen that the organs connected with the præoral lobe of Echinoderm larvæ are situated in the radial stage, in what I have called the anterior interradius; and it is evident that a disc of fixation arising in this interradius might very easily appear, in ontogeny, to be a part of the præoral lobe itself. That some such confusion of two phylogenetically distinct structures does occur in the larva of Antedon is very strongly suggested by the fact, to which I have already drawn attention, that the so-called præoral lobe of this larva contains, at the time of fixation, certain structures (skeleton and continuation of the right body-cavity) which cannot well be regarded as primarily belonging to this lobe, but rather to the stalk, which is itself apparently a secondary structure—the earliest Pelmatozoa having been, as palæontology teaches, sessile.

The position of the water-pore in the larva of Antedon also accords very well with the view here advanced, though it is not inexplicable on other hypotheses. It is difficult to illustrate this point without undue multiplication of diagrams, but perhaps fig. 50 may be of some service. I have assumed that the disc of fixation arose in the interradius of the anus and water-pore in such a stage as is shown in fig. 48, and then moved round to the aboral pole. If, before this movement had proceeded far, the stalk and the extension of the right body-cavity into it were, in ontogeny, precociously developed, something very like fig. 50 would be reached; compare this with the larva of Antedon at the moment of fixation, and we shall see a possible reason why the water-pore in this larva is so far removed from that dorsal position which it assumes in other Echinoderm larvæ.

Of course I am well aware that the above suggestions as to

the origin of the Pelmatozoa are of an extremely speculative character, and will require a great deal of evidence to support them; but a too rigid adherence to the apparent teachings of the larva of Antedon is really open to equally strong objections. In opposition to those who, like Seeliger, regard the ontogeny of this larva as a safe guide to phylogeny, it cannot be too strongly urged that at present we only know the development of one Pelmatozoan larva, and that we have no reason for regarding this as specially primitive; on the contrary, the very early loss of bilateral symmetry in the arrangement of the body-cavities, as well as the entire absence, before fixation, of either œsophagus or intestine, point most conclusively to its being a much altered form.

Origin of Holothurians.

Until phenomena similar to those which I have described in Synapta have been observed in other Holothurians, it would be rash to attempt more than a cautious suggestion as to the origin of this class. Even in Synapta my investigations are unfortunately far from complete, but so far as they go they appear to teach a fairly definite lesson.

In the light of our knowledge of other Echinoderms, we are justified in regarding the asymmetrical movement of the atrial aperture, and the formation of the mesentery of the water-tube by the left body-cavity (a portion of which grows round the œsophagus for this purpose), as indications that in the ancestor of Holothurians also the movement of the œsophagus into the left side has taken place. But, on the other hand, although we cannot determine the exact limits of the larval body-cavities in adult Holothurians, we can certainly assert that the left cavity is not symmetrically disposed about the œsophagus, and that the mesentery of the water-pore is not, as in other Echinoderms, at right angles to the mesentery of the stomach and intestine (dorsal mesentery of the larva), but nearly in a straight line with it. These facts, coupled with the observed migration of the atrial aperture towards the anterior pole,

suggest that the mouth, after first moving into the left side, has undergone a secondary change of position, accompanied of course by the water-vascular ring.

To some extent this is parallelled by the Spatangidea, and (possibly) by Actinometra; but the case of the Holothurians presents certain marked pecularities. In the first place the secondary migration of the mouth has apparently been such as exactly to retrace the line of the original movement—that is to say, it has occurred in the direction of interradius A (compare figs. 47 and 48); though in this it does not differ very widely from the Spatangidea, in which the secondary movement has been towards radius I (see **14**, pl. xiii, fig. 8). Secondly, it has not been a simple movement; for that, while it would reduce the length of the mesentery of the water-tube, would still leave the same angle between this and the mesentery of the stomach and intestine which we have found in other Echinoderms. But in Holothurians these two mesenteries are nearly in the same straight line; and this can, I think, only be accounted for by supposing that the torsion of the water-vascular ring which we traced in the common ancestor has been reversed and undone during the secondary movement, so that this ring has returned very nearly to the position assumed for it in the bilateral ancestor (fig. 45), in which the dorsal mesentery may be said to lie in interradius A (the interradius of the water-tube). One great difference, however, exists between this secondary Holothurian ancestor and the ancestor shown in fig. 45. In the latter the body-cavities do not run forward on to the œsophagus, but in the former, during the time when, in common with other Echinoderms, its mouth was on the left side, the left body-cavity learnt, so to speak, to surround the œsophagus and form a mesentery for the water-tube; and this peculiarity, once acquired, was not lost during the secondary changes, but is still traceable in the larva of Synapta; and it accords very well with this view that, whereas in other forms (compare fig. 17) a dorsal as well as a ventral horn of the left body-cavity is observable, in Synapta the ventral horn alone is conspicuous; this, however, is a point

which cannot well be illustrated by figures in two dimensions, but will be found by those who take the trouble to construct models, to offer interesting evidence in favour of the view here advanced. In fact, the only obstacle to this view with which I am acquainted lies in the unusual position of the water-pore on the right, instead of as usual on the left, of interradius A (see fig. 14); but this is equally puzzling on any other hypothesis, except Semon's untenable one that the five tentacles of Auricularia are radial; and indeed, as already suggested, the difficulty may be more apparent than real, being perhaps due to the precocious development of the tentacles of radius I, while one of those belonging to radius V only appears later (see **16**, p. 183).

The further question of the exact stage at which the Holothurians branched off from the parent stock, is not one on which I care to express any very decided opinion. I would point out, however, that it is difficult to conceive of any torsion of the water-vascular ring occurring after the radial canals had spread far over the disc and become united with parts of the body-wall; and consequently I am inclined to the belief that the separation of this class occurred very early, perhaps even before that of the Pelmatozoa. The possibly primitive character of the genital organs in the Elasipoda fits in very well with this supposition; while the fact that the ambulacral fields are limited to the region of the left body-cavity in other Echinoderms, but run to the extreme posterior end regardless of the body-cavities in Holothurians, is, so far as it goes, opposed to the derivation of the latter from any of the other groups of the Echinozoa.

Relation of Echinodermata to Enteropneusta.

Of the various features which have from time to time been supposed to show affinities between the Echinodermata and Enteropneusta, probably the least important, though one of the first to attract attention, is the outward resemblance of certain Echinoderm larvæ to Tornaria. No one who has seen the latter and Auricularia alive can fail to be struck with their general

similarity; but it is difficult to regard Auricularia as a primitive larval form, and even if we could, the details of the likeness are not sufficiently strong to prove a common origin.

Spengel ignores this resemblance, and suggests that Morgan's mention of Auricularia is a lapsus calami for Bipinnaria,[1] though he does not himself attach any phylogenetic importance to the resemblance of this larva to Tornaria. But though these larvæ resemble one another in having the ciliated band divided into two at the anterior pole, yet it is impossible to regard this as a primitive feature in Bipinnaria, seeing that no other Echinoderm larva normally possesses it; while the ease with which it may be independently acquired is attested by the fact that I observed precisely the same division of the band into two, in one instance, in Auricularia.

Of far more importance is the presence in Echinoderms as well as in Enteropneusta of at least one anterior body-cavity, opening by a pore at its posterior end on the left side of the body. My identification of this cavity in Echinoderm larvæ (see 4 and 5) has met with a good deal of opposition, it being by many regarded as a mere appendage to the hydrocœl; but the facts (1) that it always arises as early as, often earlier than, the posterior cavities—generally earlier than the hydrocœl; (2) that it is constant in position, arising and remaining anterior to the stomach, with the pore at its posterior end; (3) that it always has thin walls, while the hydrocœl after the first moment of its appearance has thick walls, seem to me to go far towards refuting this view, and establishing the primitive nature of the cavity in question. The homology of it with the proboscis cavity of Balanoglossus may not be so well established, but at least has too much plausibility to be lightly set aside.

The existence of a second anterior cavity in many Echinoderm larvæ is of less importance, since it seems to carry us back to a far earlier period than the separation from a common stock of Echinodermata and Enteropneusta. There is no clear

[1] Morgan (22) has certainly made several statements about Auricularia which are true, I believe, only of Bipinnaria.

evidence that the latter ever had a second anterior cavity, though there are some grounds for believing it; but if they had, its importance must have been subordinate before the divergence of the two lines of descent, for we can hardly suppose that the predominance of the left cavity has been independently acquired.

The same applies to the second pore discovered by Field, and present in some Enteropneusta; if it is really an ancestral feature (which is not yet fully proved) it must have been lost before the separation of the two groups, or we should not be likely to find only one (the left) in the adult form of both.

Spengel's suggestion, that the left collar-cavity of Tornaria may be comparable with the hydrocœl of Echinoderms, is robbed of much of its value by his curious error in supposing that Field and I have described in the latter a second (right) hydrocœl. There is, however, some plausibility in the adoption of this comparision in the form which it assumes in MacBride's hands—a comparison of the true hydrocœl (as distinguished from the anterior enterocœl) with the collar-cavity of Enteropneusta. The situation of the hydrocœl in young larvæ is certainly strongly suggestive of this; and the obvious objection that the hydrocœl is unpaired in most larvæ is met by MacBride's supposed discovery of a second hydrocœl in Asterina; and this writer even goes so far as to suggest that in a pore leading directly from the hydrocœl to the exterior which he has found in one larva of Asterina, we have the homologue of the collar-pore (20). As I have utterly failed to find any trace of this second hydrocœl in any of the larvæ I have examined, I may perhaps be forgiven if I refuse, at present, to accept the evidence of such an obviously secondary form as the larva of Asterina; and I would moreover point out that one of MacBride's own figures (19, fig. 4) is out of accordance with his hypothesis, since the "collar-cavity" should be posterior to the anterior body-cavity, and should not embrace its posterior end, though I must confess that I am at a loss to understand why he ever drew his figure in this form, as it is unnecessary for his hypothesis, and unsup-

ported by evidence. Spengel's idea evidently is that the whole hydrocœl ring is comparable to the two collar-cavities combined; and if there were any evidence of its two-fold origin, we might readily adopt this view. It is just conceivable that the variation in the point of closure of the ring is in some way connected with this, and that the hydrocœl really does contain in itself a right and a left element—its present development on the left side alone being almost exactly paralleled by the development of the right body-cavity in Auricularia from the asymmetrical (left) hydro-enterocœl rudiment. But on this point I refrain from offering an opinion.

As to MacBride's "collar-pore" in Asterina, I would point out the a priori improbability that the ancestral Echinoderm ever possessed such a pore; for if it did, why has the hydrocœl lost it own pore, and entered into a secondary connection with that of the anterior enterocœl? Until further examples of its occurrence have demonstrated a constant position for this pore, it seems to me far more probable that we have in it simply a case of multiplication of water-pores, such as has led in Rhizocrinus to the presence of one in each interradius.

To the resemblances above mentioned, and some urged by Morgan (22 and 23), I would suggest the addition of others, which, if supported by further investigations, would go far to bind the two groups together. The suggestions are not wholly new, various scattered hints at them being found in the pages of other writers; but the present paper offers, I think, far more evidence than has hitherto been attempted.

At the base of the proboscis of Balanoglossus lies a closed vesicle ("pericardium," "Herzblase," "sac of proboscis gland"), which is dorsal to the alimentary canal (notochord), and, being in the middle line, has the water-pore on its left. Underneath this "pericardium," and deriving its muscular coat from it, lies the pulsating organ known as the "heart;" it is simply a lacunar space, as are the blood-vessels with which it communicates, and may be regarded as a remnant of the embryonic segmentation-cavity.

In intimate connection with these structures is the "pro-

boscis gland." It consists, according to Spengel, of a number of folds of the epithelium of the proboscis cavity (anterior body-cavity), and contains a number of cells with yellow granules—apparently excretory; it is also supplied with blood by a number of lacunæ.

Now I believe that we can recognise all these structures in Echinoderms, though the metamorphic changes which the larvæ undergo greatly obscures their true position. In Asterid and Echinid larvæ we find on the dorsal side, just over the junction of stomach and œsophagus, and to the right of the water-pore, a closed vesicle of schizocœl origin. The floor of this "dorsal sac," as I have called it, is raised up in Echinid Plutei, and in these larvæ (and occasionally in Asterids) a pulsation may be observed—not of the vesicle as a whole, but apparently of its floor only.

Besides this, we have in all Echinoderms (except Holothurians) a glandular organ known as the "dorsal organ," "axial organ," "ovoid gland," &c. The origin of this is somewhat obscure. In Crinoids and apparently in Ophiurids (18) it is at first a solid mass of cells; but in Asterids (Asterina) MacBride describes it as "an ingrowth of the left posterior cœlom into the septum separating the posterior cœlomic cavities from the axial sinus" (19, p. 433), that is to say, from the anterior enterocœl. I have not been able fully to satisfy myself on this point either in Bipinnaria or Brachiolaria, but I would point out that, owing to the fusion of the two cavities (anterior and posterior), it must be almost impossible to determine with certainty from which of them the organ in question is derived.

But whatever may be the case in the larva, its structure and relations in the adult are very striking. In all forms it is a much folded mass of apparently excretory cells, and in Asterids and Echinids, at any rate, if not in Ophiurids (Cuénot and MacBride disagree on this point) it projects into the anterior body-cavity (axial canal) in such a way that its folds may be said to be involutions of this cavity.

It seems to me that we have a good primâ facie case in favour of the homology of the "dorsal sac" and "dorsal

organ" of Echinoderms with the "pericardium" and "proboscis gland" of Enteropneusta respectively.[1] It is true that the observed position of the dorsal sac does not obviously accord with this, but I have endeavoured to show in the foregoing pages what its probable position was in the bilateral ancestor, and that position accords very well with the homology here suggested.

Our present knowledge of the blood-vascular system in Echinoderms is too imperfect to allow of a detailed comparison with that of Enteropneusta, though there is much in the conflicting evidence on the subject which is very suggestive. We may say, however, without much fear of contradiction, that the blood-vessels are simply lacunæ, with no epithelial walls of their own, and that these lacunæ penetrate all through the complex structure of the "dorsal organ"—indeed in Holothurians, where the latter is absent, the lacunæ alone are left in the place which it usually occupies (10).

Again, in Echinid Plutei I have shown that the observed pulsation probably occurs in the raised mass of gelatinous tissue which projects into the floor of the dorsal sac, which gives this mass its only epithelial wall. Now this gelatinous tissue, though not so represented in my drawings (special staining being required to demonstrate it) consists simply of a network of protoplasmic threads, the interstices of which (filled with a watery fluid) may be considered as parts of the original segmentation cavity.

Without further discussion of the very obvious inferences to be drawn from these facts, and the equally obvious gaps in the evidence, I think I may claim to have established a case in favour of the homology of the dorsal sac and dorsal organ of Echinoderms with the pericardium and proboscis gland of Enteropneusta which cannot be lightly set aside; and taking these in connection with the other resemblances between the

[1] The former is apparently suggested by Morgan (22, p. 442), though for "Auricularia" we must read "Bipinnaria," while the latter is suggested by Koehler, on the ground of similarity of structure; but neither offer much evidence.

two groups, we seem to have a chain of evidence of their connection, which though not indeed conclusive—that, embryological evidence alone can never be—is at least as strong as that which binds together any two of the great subdivisions of the Animal Kingdom.

Literature.

1. Agassiz, A.—"Embryology of the Starfish," 'Contrib. Nat. Hist. U.S.,' vol. v, 1864.
2. Barrande, J.—'Système Silurien du centre de la Bohème,' vol. vii, Prague, 1887.
3. Bateson, W.—'Materials for the Study of Variation,' London, 1894.
4. Bury, H.—"The Early Stages in the Development of Antedon rosacea," 'Phil. Trans. Roy. Soc.,' London, 1888, vol. clxxix.
5. Bury, H.—"Studies in the Embryology of Echinoderms," 'Quart. Journ. Micr. Sci.,' April, 1889.
6. Bütschli, O.—"Versuch der Ableitung des Echinoderms aus einer bilateralen Urform," 'Zeitsch. f. w. Zool.,' vol. liii, suppl.
7. Cuénot, L.—"Études Morphologiques sur les Echinodermes," 'Arch. de Biologie,' vol. xi, 1891.
8. Field, G. N.—"The Larva of Asterias vulgaris," 'Quart. Journ. Micr. Sci.,' vol. xxxiv, pt. 2, p. 105, 1894.
9. Götte, A.—"Vergleichende Entwicklungsgeschichte der Comatula mediterranea," 'Arch. f. Mikros. Anat.,' vol. xii, p. 583, 1876.
10. Hérouard, E.—"Recherches sur les Holothuries des Côtes de France," 'Arch. Zool. Exp. et Gén.,' vol. vii, 2 ser., 1889.
11. Lovèn, S.—"Études sur les Echinoidées," 'Köngl. Svenska Vetensk-Akad. Handlungen,' Bd. ii, No. 7, Stockholm, 1875.
12. Lovèn, S.—'Echinologica,' Stockholm, 1892.
13. Ludwig, H.—"Beiträge zur Anatomie der Asteriden," 'Morph. Stud.,' vol. i, p. 150, Leipsic, 1877.
14. Ludwig, H.—"Ueber den primären Steinkanal der Crinoideen," &c. 'Morph. Stud.,' vol. ii, p. 34, Leipsic, 1880–82 (and 'Zeitsch. f. wiss. Zool.,' vol. xxxiv, p. 310, 1880).
15. Ludwig, H.—"Entwicklungsgeschichte der Asterina gibbosa," 'Morph. Stud.,' vol. ii, p. 3 (and 'Zeitsch. f. wiss. Zool.,' vol. xxxvii, 1882).
16. Ludwig, H.—"Zur Entwicklungsgeschichte der Holothurien," 'Sitzungsberichte d. Köngl. Preuss. Akad. d. Wissenschaften,' 3, Berlin, 1891, pt. i, p. 179.
17. Ludwig, H.—"Zur Entwicklungsgeschichte der Holothurien," 'Sitzungsberichte d. Köngl. Preuss. Akad. d. Wissenschaften,' 3, Berlin, 1891, pt. ii, p. 603.

18. MacBride, E. W.—"The Development of the Genital Organs, &c., in Amphiura squamata," 'Quart. Journ. Micr. Sci.,' vol. xxxiv, pt. ii, p. 129.
19. MacBride, E. W.—"The Organogeny of Asterina gibbosa," 'Proc. Roy. Soc.,' vol. liv.
20. MacBride, E. W.—"On Variations in the Larva of Asterina gibbosa," Philos. Soc., Cambridge, May, 1894.
21. Metschnikoff, E.—"Studien über die Entwicklung der Echinodermen und Nemertenen," 'Mém. de l'Acad. Impér. de St. Petersb.,' vol. xiv, ser. 7, No. 8, 1869.
22. Morgan, T. H.—"The Growth and Metamorphosis of Tornaria," 'Journ. Morph.,' vol. v.
23. Morgan, T. H.—"The Development of Balanoglossus," 'Journ. Morph.,' vol. ix.
24. Müller, J.—"Ueber die Larven und die Metamorphose der Echinodermen," 2te Abhandlung, 'Köngl. Akad. d. Wissensch. zu Berlin,' 1848.
25. Neumayr, M.—"Die Stämme des Thierreichs," vol. i, Wien und Prag, 1889.
26. Perrier, E.—'Mémoire sur l'organisation et le developpement de la Comatule de la Mediterranée,' Paris, 1886.
27. Prouho, H.—"Recherches sur le Dorocidaris papillata," 'Arch. d. Zool. Exp. et Gén.,' vol. v, 2nd ser., p. 213, 1888.
28. Sarasin, P. and F.—"Ueber die Anatomie der Echinothuriden und die Phylogenie der Echinodermen," 'Ergebnisse Naturwissensch. Forschungen auf Ceylon,' vol. i, pt. 3, Wiesbaden, 1888.
29. Sars, M.—'Beskrilvelser og Jagtagelser,' &c., Bergen, 1835.
30. Seeliger, O.—"Studien zur Entwicklungsgeschichte der Crinoiden," 'Zool. Jahrb., Abth. f. Morph.,' vol. vi.
31. Selenka, E.—"Keimblätter und Organanlage der Echiniden," 'Zeit. f. wiss Zool.,' vol. xxxiii, p. 39, 1879.
32. Semon, R.—"Die Entwicklung der Synapta digitata, und die Stammesgeschichte der Echinodermen," 'Jen. Zeitsch. f. Naturwiss.,' vol. xxii, new ser., 1884.
33. Semon, R.—"Die Homologien innerhalb des Echinodermstammes," 'Morph. Jahrbuch,' vol. xv, 1889.
34. Spengel, J. W.—"Die Enteropneusten des Golfes von Neapel und der angrenzenden Meeresabschnitte," 'Faun. und Flor. des Golfes von Neapel,' No. 18.
35. Théel, H.—"On the Development of Echinocyamus pusillus," 'Nova Acta Reg. Soc. Sc. Upsala,' Ser. iii, 1892.

EXPLANATION OF PLATES 3—9,

Illustrating Mr. Henry Bury's paper "On the Metamorphosis of Echinoderms."

REFERENCE LETTERS.

Ant. B.C. Anterior body-cavity. *R.B.C.* Right body-cavity. *L.B.C.* Left body-cavity. *Dors.* Dorsal. *Vent.* Ventral. *R.* Right side. *L.* Left side. *W.V.R.* Water-vascular ring. The numbers I—V mark the radii; the letters A—E mark the interradii. [In Figs. 3—9 the numbers I—V refer to the ciliated rings.]

FIG. 1.—Auricularia, seen from left side, showing process of left body-cavity running forwards to the hydrocœl. × 75.

FIG. 2.—The same; older specimen, in which the ciliated band is beginning to break up. × 75. (Hydrocœl not quite correctly drawn).

FIG. 3.—Auricularia, from ventral side. The skeleton and "nerve-bands" are omitted, and the breaking up of the ciliated band is shown diagrammatically. × 75.

FIG. 4.—"Stage A" (transition of Auricularia to "pupa"); from ventral side. × 75.

FIG. 5.—"Stage B"; from ventral side. × 75.

FIG. 6.—Diagram of the anterior pole of Auricularia (compare Fig. 3), showing the breaking up of the ciliated band.

FIG. 7.—Diagram of the anterior pole in stage A.

FIG. 8.—Diagram of the anterior pole in stage B.

FIG. 9.—Diagram of the anterior pole in young "pupa." The gradual evolution of the ciliated rings can be followed with the help of the numbers and letters (I, II *a*, *b*, *c*, &c.), the former referring to the ciliated rings of the "pupa," and the latter to the parts of the ciliated band which form them. The references are the same in all the figures (3 to 9).

FIG. 10.—Section of late Auricularia (between Figs. 3 and 4), showing position of polian vesicle, and extension of "ventral horn" of left body-cavity over to the right side. × 400.

FIGS. 11, 12, and 13.—Sections (from a series) through a larva towards the close of stage A, showing the extension of the "ventral horn" ("part of *L.B.C.*") of the left body-cavity round the œsophagus to the dorsal surface. Two other sections intervene between Figs. 11 and 12, and two between Figs. 12 and 13. Only parts of the sections are shown in the last two. × 300.

FIG. 14.—Diagram of the water-vascular system of the "pupa" seen from the dorsal (aboral) side.

Fig. 15.—Longitudinal vertical section through "pupa." × 300.

Fig. 16.—Section through the posterior end of the stomach in stage A, showing the extension of the right body-cavity on to the left side above (dorsal to) the stomach. The arrow marks the approximate position of the dorsal mesentery in preceding sections. Ectoderm and gelatinous tissue omitted. × 540.

Fig. 17.—Lateral view (from left side) of a young larva of Asterias glacialis (?), showing constriction of left enterocœl into anterior body-cavity, hydrocœl, and posterior body-cavity. Free-hand drawing. × 200 (approximately).

Fig. 18.—Ventral view of young "Bipinnaria asterigera," showing the terminal plates on the left of the middle line. × 35.

Fig. 19.—Transverse section through Brachiolaria (Asterias rubens), showing dorsal organ underlying dorsal sac. × 180.

Fig. 20.—Transverse section through "Bipinnaria asterigera," showing depression in centre of hydrocœl. The position of one of the terminal plates is indicated ("terminal"), though the calcareous matter has been dissolved away. × 220.

Fig. 21.—The same larva seen from the left side. The outlines of the body-cavities (dotted lines) have been filled in from sections, and are only approximate. From a decalcified specimen, somewhat shrunk. × 40.

Fig. 22.—Dorsal view of a larva at about the same age. The dorsal mesentery (not seen) runs just to the right of, and parallel to, the line of terminal plates (compare Fig. 20). × 70.

Fig. 23.—Transverse section of a very old specimen of "Bipinnaria asterigera." × 55.

Fig. 24.—View from the left side of the same larva (oral side of adult), reconstructed from sections. The interradial septum dividing the "axial sinus" from the left body-cavity is shown; the other four are omitted.

Fig. 25.—Section of the same larva, through two of the radial pouches of the stomach. × 75.

Fig. 26.—Transverse section through an arm of young Lindia, showing the hepatic cæca. × 40.

Fig. 27.—Ventral view of young larva of Asterias glacialis (?), showing the left body-cavity passing on the dorsal side of the intestine on to the right side. × 100.

Fig. 28.—Transverse section through a larva of Asterias rubens at about the same stage as the last. × 300.

Fig. 29.—Transverse section through a young pluteus of Echinus microtuberculatus, showing the "dorsal sac." × 440.

Fig. 30.—Transverse section through a later pluteus of the same, showing the raised floor of the "dorsal sac." × 900.

Fig. 31.—Longitudinal vertical section of the same. × 900.

Figs. 32*a* and *b*.—Two spines with four points from an old pluteus of Echinus microtuberculatus; *a* is the more typical form. × 260.

Fig. 33.—Spine with six points from the same larva. × 260.

Fig. 34.—Very young Echinus microtuberculatus seen from the side. × 180.

Fig. 35.—Diagrammatic view of the same, from a slightly different aspect, to show the distribution of the two kinds of spines.

Fig. 36.—Aboral pole of an older specimen of the same, with a diameter of about ·75 mm. × 75.

Fig. 37.—Aboral pole of the same, with a diameter of 7 mm. × 23.

Fig. 38.—The same, diameter 10 mm. In this stage the madreporic area has spread all over the first basal plate. × 23.

Fig. 39.—Part of a section through a specimen of Echinus microtuberculatus with a diameter of 3·5 mm., showing the relative positions of the water-tube, &c. The ovary marks the centre of interradius A. × 90.

Fig. 40.—Ventral view of an Ophiurid pluteus (compare 5, fig. 2). The water-tube (not seen from this side) enters the hydrocœl between pouches I and V. × 180.

Fig. 41.—Section of young Ophiurid just before its separation from the remnant of the pluteus. × 500.

Fig. 42.—Ventral view of Ophiurid pluteus showing the anus pushed to the right side. × 300.

Fig. 43.—The same, later stage. × 300.

Fig. 44.—Diagram of the bilateral ancestor, from the left side.

Fig. 45.—The same, dorsal view.

Fig. 46.—Transition to radial stage, dorsal view

Fig. 47.—The same, from the original left side. Atrium and tentacles omitted.

Fig. 48.—Radial stage, from the former left (now oral) side. Atrium and tentacles omitted as before. For the explanation of the line X—Y, see text.

Fig. 49.—The same, lateral view. Half the atrium, and two of the tentacles, are supposed to have been removed.

Fig. 50.—Diagram of hypothetical ancestor of Pelmatozoa; the stalk is greatly exaggerated (the original form being probably sessile) to show how a precocious development of this organ would lead to the conditions seen in the larva of Antedon.

A Criticism of the Cell-Theory; being an Answer to Mr. Sedgwick's Article on the Inadequacy of the Cellular Theory of Development.

By

Gilbert C. Bourne, M.A., F.L.S.,
Fellow of New College, Oxford.

"Jedes Lebendige ist kein Einzelnes, sondern ein Mehrheit; selbst insofern es uns als Individuum erscheint, bleibt es doch eine Versammlung von lebendigen, selbständigen Wesen, die der Idee, der Anlage nach gleich sind, in der Erscheinung aber gleich oder ähnlich, ungleich oder unähnlich werden können. Diese Wesen sind theils ursprünglich schon verbunden, theils finden und vereinigen sie sich. Sie entzweien sich und suchen sich wieder, und bewirken so eine unendliche Production auf alle Weise und nach allen Seiten."—Goethe (1807).

Mr. Adam Sedgwick has of late thrown himself with considerable zeal into the part of a zoological iconoclast, and has displayed an evident relish in battering the idols which, he would fain make us believe, are turning away the minds of men from the true faith, of which there are but few orthodox exponents. Nor may we blame him for his fervour, for an old faith always emerges purer, if not firmer, from the ordeal of sharp antagonism. The idols in question are the developmental law of von Baer and the cell-theory.

Seeing how important a thing it is that a science should be guided by principles capable of being expressed in precise language, it has been a matter of surprise to me that some competent person has not taken up the challenges which Mr. Sedgwick has thrown down. For, if his views are to prevail, two of the fundamental principles of zoology, principles which have hitherto directed and steadied the course of zoological speculation, are taken away from us; and unless some

better and more distinct principles are put in their place, the course of speculation may be expected to be very erratic indeed. It is not without serious misgivings as to my own competence that I, in default of a better champion, take up one of these challenges, and I propose to criticise Mr. Sedgwick's recent article on the inadequacy of the cellular theory of development, leaving for a future occasion the consideration of his earlier article on von Baer's law.

It is to be regretted that Mr. Sedgwick should, in putting forward a view affecting one of the fundamental propositions of biology, have chosen to adopt a controversial method, which cannot but have the effect of weakening his case. And it is still more a pity that he should be so unsparing in abuse of his imaginary opponents, whilst he himself commits the very fault for which he so much blames them. For he lays, in the front of his indictment, a charge of vagueness and unsubstantiality against the supporters of the cellular theory. "We are dealing," he says, "with a kind of phantom which takes different forms in different men's eyes. There is a want of precision about the cell-phantom, as there is also about the layer-phantom, which makes it very difficult to lay either of them. Neither of these theories can be stated in a manner satisfactory to every one. The result is that it is not easy to bring either of them to book."

I shall show, later on, that this charge of vagueness is not altogether justified; what I am at present concerned with is to show that Mr. Sedgwick is as much open to the charge of vagueness as the rest of the zoological world which he castigates.

Read his article through as carefully as one may, one cannot find any definite or precise statement of his own standpoint, saving that he quotes passages from one of his earlier works. The critic, therefore, must be content to infer from the tenor of the whole article, and from particular passages in it, as well as from his previous writings, what Mr. Sedgwick does or does not believe with regard to the cell-theory, and if he is misinterpreted, it is his own fault.

It is probably a fair summary of his position to say that, for the present, he limits his objections to the application of the cell-theory to the process of growth during embryonic development; but that he scarcely conceals his preference for the view that there are no such things as discrete cells in the so-called multicellular organism. And as it is necessary, at the outset, to have a perfectly clear idea of his meaning, I will quote passages from the work to which he refers in his opening paragraph, assuming that what he stated then he is prepared to adhere to now, and that his last article is intended to emphasise the views which he formerly propounded, and to bring fresh evidence in support of them.

On p. 204 of the second part of his account of the development of the Cape species of Peripatus, he says:—"It is becoming more and more clear every day that the cells composing animal tissues are not isolated units, but that they are connected with one another. I need only refer to the connection known to exist between connective tissue cells, cartilage cells, epithelial cells, &c. And not only may the cells of one tissue be continuous with one another, but they may also be continuous with the cells of other tissues. . . . It is true that the cells of blood and lymph and the ripe generative cells are completely isolated. But the former, in their first stages of growth, form part of the syncytium, as in all probability do the latter also. This continuity, which for à priori reasons we should expect, has hitherto been regarded as a fact of little morphological importance and relegated to the category of secondary features. The ovum, it is said, segments into completely isolated cells, and the connection between them is a secondary feature acquired late in development. It has always been considered that the first stage in the evolution of the Metazoa was a colonial Protozoon, i. e. a mass of perfectly isolated unicellular organisms, derived by complete division from a single cell. Now while I do not wish to exalt the facts of the cleavage and early development of Peripatus to a position of undue importance, or to maintain that of themselves they are sufficient to destroy this conception of the origin and

structure of a Metazoon, I think I am justified in pointing out that, if they are found to be of general application, our ideas on these subjects will have to undergo considerable modification. The ancestral metazoon will no longer be looked upon as a colonial protozoon, but rather as having the nature of a multinucleated infusorian, with a mouth leading into a central vacuolated tract of protoplasm. The continuity between the various cells of the adult—the connections between the nerves and muscles and sensory epithelium, receive an adequate morphological explanation, being due to a primitive continuity which has never been broken. In short, if these facts are generally applicable, development can no longer be looked upon as being essentially the formation of a number of units from a single primitive unit, and the co-ordination and modification of these units into a harmonious whole. But it must rather be regarded as a multiplication of nuclei and a specialisation of tracts and vacuoles in a continuous mass of vacuolated protoplasm."

This is a temperate and lucid statement of a suggestion which is still worthy of serious consideration, the more so since it had been shown, but a short time previous, that protoplasmic continuity between the tissue-cells of plants is of very general occurrence, if not the rule. And, as a historical fact, the continuity of protoplasm was a phenomenon familiar to animal histologists long before it was proved for vegetable tissues; indeed there were authors who, before Mr. Walter Gardiner's researches were published, were disposed to regard protoplasmic continuity as a characteristic of animal organisation, discontinuity as a characteristic of vegetable organisation.

I have quoted at length because Mr. Sedgwick from being temperate has become intemperate, and from being lucid he has become obscure; so that, were I to deal only with his latest utterances, I should be quite at a loss to know what his maturer views might be.

What follows, then, may be taken to be a not unfair statement of his position. That from the connection known to

exist between some cells composing adult tissues, there is an antecedent probability that similar connections exist between all cells composing all tissues; and this probability is heightened by observations made on the development of Peripatus, by the fact that the so-called mesenchyme cells in Avian and Selachian embryos are continuous, and not isolated, as was once supposed, and by a study of the developing nerves of Elasmobranchs. And that it follows from this that the morphological concept of a cell, so far from being of primary, is altogether of secondary importance, and that progress in the knowledge of structure is impossible so long as men persistently regard cells as the fundamental structural units on which the phenomena manifested by organised beings depend. The true method of enquiry must be a study of the growth, extension, vacuolation and specialisation of the living substance—protoplasm.

It is in this sense that I propose to deal with Mr. Sedgwick's views, and he will pardon me if I have misinterpreted them. At any rate, I have done my best to understand them.

I would wish to show, in the first place, that there is very slender ground for the accusations which Mr. Sedgwick levels, in an unsparing manner, against his zoological contemporaries. He goes so far as to say that their eyes are blinded by theory to the most patent facts, and that "they are constrained by this theory,"—the cell theory,—"with which their minds are saturated, not only to see things which do not exist, but actually to figure them." This is abuse and not argument; if Mr. Sedgwick were to remember the qualifying sentence in his writings of 1886, "if they are of general application," he would recognise that there is little occasion for accusing zoologists of perversely ignoring the views which he then set forth.

For, in fact, the phenomena to which he draws our attention have received their due meed of recognition from the time that the cellular structure of tissues was first studied. More recent researches have enlarged our knowledge of protoplasmic continuity, but it is still a phenomenon far from being

of such universal application as to constrain us to abandon that very useful morphological concept—a cell.

For some years past the study of cells, of their ultimate structure, of their chemical and physical properties, of phenomena which accompany their growth and division, has been carried on with a minuteness which a short time ago was undreamt of. And attention has been directed, not only to the cells composing adult tissues, but in the most marked degree to the successive formation of cells from the primitive unit, the oosperm, and to the fate which each subsequently undergoes in the course of development. In place of the off-hand statements of older embryologists, that the ovum divides into two, four, eight, sixteen segments, and so forth, we have the most accurate and minute accounts of the successive formation of cells, of the place which each occupies in the developing embryo, of its parentage and of its progeny, and of the share taken by the last named in the building up of the adult tissues. In short, we have a number of cell-lineages, which show that in a number of animals, some of which are widely separate from one another, the formation of cells from the ovum follows courses which are either identical or so closely similar that the differences excite our wonder far less than the similarities. So minute are these investigations that every karyokinetic figure has been followed in every cell, up to a stage where their number becomes bewildering.

I refer, of course, to the remarkable series of observations which were begun by Selenka, Arnold Lang, Hallez, Blochmann, and others, and have been carried to the highest perfection by von Wistinghausen, E. B. Wilson, Heymons, and Lillie.

It would be impossible, in such an essay as this, to deal adequately with the results obtained by these authors; and it is unnecessary, since their works are within reach of everyone. It is enough to say here that a perusal of them does not tend to diminish the importance which we have been accustomed to attribute to the cell in developmental processes.

Nothing can be more clear than the fact that, in Nereis or

in Unio, there result from the division of the ovum separate protoplasmic corpuscles, as distinct from one another as one room in a house is distinct from another, each of which is not only separate, but contains within itself definite, and probably limited, qualities (at least at stages beyond eight or sixteen cells). One might almost say that, after the earliest stages, each blastomere has a definite task allotted to it, which it faithfully and punctually performs, according to a prescribed course. To each, it might be said in figurative language, is given material, which it must place, not anywhere, but in one particular part of the edifice.

In considering these very remarkable researches, it is not sufficient, for the present purpose, to say that no connection between the blastomeres was observed. Such connections may have existed and have been overlooked; as the connections, which undoubtedly exist, between plant cells were for a long time overlooked. But, a priori, such connections are improbable. For, as has been said, the qualities of each blastomere are limited. Each is specialised before any form changes become visible; each plays one part, and one part only in tissue formation. If their protoplasm were continuous, being made so by uniting strands, then, as Mr. Sedgwick has expressed it, the molecular constitution of any part would in time spread through the whole mass. But the molecular constitution of the blastomeres must be different, for their manifestations are different, and we may possibly see, in this case, some explanation, obscure though it may be, of the isolation of the form elements from one another.

Further than this, there is objective proof that the cells constituting the early embryos of these forms are separate. They exhibit remarkable shiftings of position, which render the existence of connecting strands of protoplasm highly improbable, and the migrations of some cells—e. g. those in Nereis named $c^{1.5.}$ and $d^{1.5.}$ by Wilson—are of such an extent that, if there were protoplasmic continuity, they would be impossible.

It is no exaggeration to say that this is evidence which

effectually disposes of the idea that a syncytial theory of animal organisation is of general application.

It does more than this, it shows that there are not a few instances in which cells possess a morphological and physiological significance greater than was at one time supposed.

There are numerous other cases in which, at an early stage of development, cells wander far from the position in which they originated, and become placed so far from the parent cells from which they sprung, that any idea of protoplasmic continuity is impossible. As examples I may mention: the outer layer cells of Cornacuspongiæ and Silicispongiæ, which, as Maas has shown, go through remarkable migrations; the mesoblast of Callianira bialata, Beroe and Cydippe, as described by Metschnikoff, whose statements are confirmed by observations made (but unfortunately not published) by Mr. Riches on Hormiphora plumosa; the lower endoderm cells of Discocœlis, Eurylepta, and Leptoplana, as described by Lang, Hallez, and Selenka.

In short, the evidence is overwhelming, and it must be taken to be very clearly established that there are numerous cases in which there is not "a primitive continuity which has never been broken."

It is apparent, then, that morphologists have been amply justified in refusing to recognise Mr. Sedgwick's views as to the syncytial nature of animals, and there is no justification for the strong language which he uses towards them on account of their refusal.

It is, on the other hand, quite possible that the frequency of the occurrence of protoplasmic continuity between developing tissue-cells may have been overlooked or ignored by a few authors, and that those who have done so have been led into the error of attributing too great and too fundamental importance to the cell as an independent vital unit (Lebenseinheit).

But, in point of fact, I am unable to find, in the writings of any reputable biologist, any statement to the effect that an organism is composed of independent and isolated units. One may, it is true, find passages here and there which, when

removed from the context, might be made to bear such an interpretation. I have questioned my pupils with regard to such passages, and I find that they do in fact put such an interpretation upon them. For instance, in Waller's 'Introduction to Human Physiology' the following passage occurs on page 2: "The organism is a community; its individuals are cells; groups of its individuals are organs." Here we have an example of the danger of the too free use of illustrative language. In every illustration there lurks a fallacy. The fallacy may not have been present to the mind of the author; but if the illustration alone is used, without a lucid explanation of its meaning, the fallacy may be the one thing which impresses itself on the minds of his readers. In this case there is a fallacy in the analogy, so often made use of for purposes of popular exposition, between an organism and a community. If the analogy is used without the necessary reservations it leads to confusion, for the reader is only too prone to transfer to the organic unit the idea of the individual isolated man, who is the social unit. The organic unit may in some cases be individual and isolated, but in the great majority of instances it has lost, wholly or partially, its individuality, and is not isolated. It becomes a subordinate part of a higher individality, which in its turn may be subordinate to an individuality of a still higher order. This has been explained in the most lucid and masterly manner by Häckel, in his 'Allgemeine Anatomie der Organismen,' published in 1866; and nobody who has carefully studied that work can fail to have a clear understanding of the subject. Yet it is to Häckel that the doctrine of a cell-republic is often attributed! Clearly by those persons only who have not read his works. For he insists, over and over again, upon a distinction (which since the researches of Mr. Walter Gardiner no longer holds good) between the organisation of plants and that of animals, namely, that the special characteristic of plants lies in the preponderance of the perfected and differentiated individuals of the first order—the cells or plastids. "Der wesentliche tectologische Character der Pflanzen liegt in der vorwiegenden Ausbildung und Differ-

enzirung der Individuen erster Ordnung, der Plastiden" (op. cit., p. 222). Of animals he says, on the contrary, "Der wesentliche tectologische character der Thiere liegt sowohl in der verwickelteren Zusammensetzung der Thierleibes aus weit differenzirten Individuen verschiedener Ordnung, als auch besonders in der verschiedenartigsten Ausbildung der Individuen zweiter Ordnung, der Organe, welche viel mannichfaltiger, als bei den Pflanzen und Protisten, differenzirt und polymorph sind. Die Plastiden, die Individuen erster Ordnung, sind bei Thieren allermeist Zellen, und zwar meistens Nacktzellen (ohne Membran) weniger Hautzellen (mit Membran). Sehr häufig, und allgemein in den entwickelten Personen, vereinigen sich bei den Thieren mehrere Nacktzellen zur Bildung von Zellstöcken (Nervefäsern, Muskelfäsern), was bei den Pflanzen nur bei der Bildung der Milchsaftgefässe und der Spiralgefässe geschieht. Daher verliert bei den Thieren stets wenigstens ein Theil Zellen ihre individuelle Selbständigkeit, während sie dieselbe in den Pflanzen meist behalten."

The last sentence, which I have put in italics, shows most clearly that, as long ago as 1866, Häckel did not regard the animal organism as a community, whose individuals are cells; and it is the fact that he applied the term "cell-republic" to plants, intending thereby to emphasise the difference which he believed to exist between vegetable and animal organisation.

So that, as a matter of history, whilst plants used to be considered to be colonies of independent life units, animals were not. A certain exchange of opinion seems to have taken place more recently. Some few zoologists and animal physiologists, borrowing from Häckel the term cell-republic, have thoughtlessly applied it, with all its implications, to animal organisation, whilst botanists, influenced by Mr. Walter Gardiner's researches, have insisted more and more upon the individuality of the plant as a whole, and the subordination of its component parts, the cells. None the less, the facts of cell fusion and cell communication have never been wholly overlooked by zoologists, and recent years have brought to

light facts, such as the continuity of cartilage cells, which were unsuspected when Häckel wrote.

I am therefore far from being satisfied that the independent-life-unit theory has had such a dominant influence as Mr. Sedgwick would have us believe; and I am quite certain that the picture which he draws of the teaching given to every student of biology is a travesty of the truth.

Biology includes botany as well as zoology, and if we were to allow (which I do not) that zoologists generally have become as narrow in their conceptions of the processes of development as Mr. Sedgwick says, it is quite certain that botanists have not. And as all students of biology are—or if they are not, they ought to be—put through a course of elementary botany as well as of zoology (in many schools the subjects are combined), grave blame must be imputed to those teachers who have, in the later stages of their education, warped the liberal conceptions which they must have formed on the subject of organic growth and development. For I take it that, after a study of Mucor, Vaucheria, and the Myxomycetes, there is no student so dull but he will have imbibed ideas respecting cell growth which impel him to ask the question which as Mr. Sedgwick says it is so difficult to find an answer to—"What, after all, is a cell?" If, when he asks this question, he is told that the cell is an isolated corpuscle of protoplasm, the unit of vitality, and that there is "a most fundamental distinction" between unicellular and multicellular organisms, and so forth, the student may go on his way rejoicing, for that he has at last been given a clear and tangible statement; but none the less he will have been started on a very wrong path. I have not a widespread experience of zoological teaching, but I know, at least, that Professor Lankester's pupils are not started on that path. The truth is, and, if I am not much mistaken, zoologists and botanists alike have long been possessed of it, that there is no fundamental but only a formal distinction between unicellular and multicellular organisms; that the cell is a form concept founded on a very wide basis of experience, whereby we can conveniently

interpret to our minds one of the most universal of organic phenomena, viz. the splitting up of protoplasmic masses during growth into a number of more or less distinct corpuscles.

It will not be out of place if I quote here a passage from von Sach's 'Vorlesungen über Pflanzenphysiologie' (English edition, translated by H. Marshall Ward, 1887, p. 73). "To many the cell is always an independent living being, which sometimes exists for itself alone, and sometimes becomes 'joined with others'—millions of its like, in order to form a cell colony, or as Häckel has named it for the plant particularly, a cell republic. To others again, to whom the author of this book also belongs, cell-formation is a phenomenon very general, it is true, in organic life, but still only of secondary significance; at all events it is merely one of the numerous expressions of the formative forces which reside in all matter, in the highest degree, however, in organic substance."

That this is a great limitation of the cell theory, both as propounded by its authors and as held by many zoologists, is not to be denied; and Mr. Sedgwick might well be content if some such statement were made the established doctrine as regards cells. It appears to me that some such limited statement is necessary if we are to have any proposition universally applicable to organic structure; but with this reservation, that I cannot regard as of secondary significance that which all experience shows to be the expression par excellence of organic growth.

In admitting this much, a large part of Mr. Sedgwick's demand is conceded, for it is not to be denied that the cell theory has been very differently and much more dogmatically stated by quite recent authors.

We have, for instance, Dr. Oscar Hertwig's recent work, 'Die Zelle und die Gewebe.' He begins dogmatically enough by saying, "Thiere und Pflanzen, so verschiedenartig in ihren äusseren Erscheinung, stimmen in den Grundlagen ihres anatomischen Aufbaues überein; denn beide sind aus gleichartigen, meist nur mikroskopisch wahrnehmbaren Elementareinheiten zusammengesetzt. . . . Denn die Zellen, in

welche der Anatom die pflanzlichen und thierischen Organismen zerlegt, sind die Träger der Lebensfunctionen, sie sind, wie Virchow sich ausgedrückt hat die 'Lebenseinheiten.' Von diesem Gesichtspunkt aus betrachtet, erscheint der Gesammtlebensprocess eines zusammengesetzten Organismus nichts Anderes zu sein als das höchst verwickelte Resultat der einzelnen Lebensprocesse seiner zahlreichen, verschieden functionirenden Zellen." The whole book is written "von diesem Gesichtspunkt aus," and, admirable as it is, there is reason to think that its value is somewhat impaired by the excessive value attributed to the cell as an independent vital unit.

In passing, I may remark that this passage of O. Hertwig's gives a very precise and definite statement of the cell theory, as it is held now, by a very great authority; and a reference to older works would have shown Mr. Sedgwick that, so stated, it is practically the same as what its authors stated.[1]

For the original words of Schwann are these: "The elementary parts of all tissues are formed of cells in an analogous though very diversified manner, so that it may be asserted that there is one universal principle of development for the elementary parts of organisms, however different, and that this principle is the formation of cells. . . . . In inferior plants any given cell may be separated from the plant and can grow alone. So that here are whole plants consisting of cells which can be positively proved to have independent vitality. Now, as all cells grow according to the same laws, and consequently the cause of growth cannot in one case lie in the cell and in another in the whole organism, and since it may be further proved that some cells, which do not differ from the rest in their mode of growth, are developed independently, we must ascribe to all cells an independent vitality; that is such combinations of molecules as occur in any single cell are capable of setting free the power by which it is enabled to take up fresh molecules. The cause of nutrition and growth

[1] "I am not concerned with what its authors held."—Mr. Sedgwick, op. cit., p. 88.

resides, not in the organism as a whole, but in the separate elementary parts, the cells."

The definitions of Hertwig are a re-statement in other words of the salient features of the theory of Schwann, and it is an error to speak of an unsubstantial cell phantom. Nor is there any unsubstantiality about the cellular theory of development, which, I may remind my readers, originated with Remak. The cellular theory of development, taking as its starting point the conclusions of Schleiden and Schwann that all organisms are cells or composed of an aggregate of cells, states that every cell is formed by the division of a pre-existing cell, not as Schwann had supposed, by differentiation within a structureless cytoblastema.[1] Hence Virchow's well-known aphorism, "omnis cellula e cellulâ," which, besides denying abiogenesis, expresses the cellular theory of development as succintly as possible.

It would have been a great advantage to his own argument, and also to his critic, if Mr. Sedgwick had given the clear and authoritative expositions of the cellular theory which lay ready to hand, instead of confusing the issue by a whimsical account of his experience of morphological teaching.

Let us now examine the cell-theory, as stated by Hertwig, in the light of our present knowledge of animal and vegetable structure.

It would not be a difficult task to demonstrate the general truth of Virchow's aphorism. Wherever there is a cell, it may be shown to be the product, and generally the immediate product, of a pre-existing cell. But it would seem that some biologists have added an unwarrantable corollary to Virchow's generalisation, and would say, "Nil nisi cellula e cellulâ." Now from a certain aspect this might be considered true; everything depends on the question as to what is a cell?

Hertwig has pointed out, with much truth, that our present conception of a cell is inseparably connected with our conception of protoplasm. We are still very far from under-

[1] Mr. Sedgwick appears to have leanings towards a cytoblastema, as I shall show further on.

standing the structure of protoplasm, and it might be said that, if we know nothing of the component, it is useless to make assertions about the compost; but it will at least be useful to criticise the attempts which have been made.

Hertwig gives this definition, which is the same as that originally given by Max Schulze. A cell is a corpuscle of protoplasm in which is contained a specially organised constituent, the nucleus. (Die Zelle ist ein klümpchen von Protoplasma, das in seinen Innern einen besonders geformten Bestandtheil, den Kern (Nucleus) einschliesst.) This at first sight seems satisfactory enough, but the more one examines it, the less satisfactory does it appear, in view of the different kinds of organisms which are usually described as single cells.

If a corpuscle containing a nucleus is a cell, is a corpuscle containing two or more nuclei also a cell? And still more, is a large mass of protoplasm containing many nuclei to be regarded as a cell? Such a mass, I mean, as Botrydium, Caulerpa, or Codium, or even Pelomyxa. By many authors these organisms are regarded as single multinucleate cells, but I am far from being convinced that this is a right view of the case.[1]

If there is one thing more than another which has come into prominence as the result of recent research, both botanical and zoological, it is the fundamental importance of the nucleus to cell life. So many minute organisms, which at one

[1] With regard to the argument which follows, I would remind my readers that Häckel, thirty years ago, clearly expressed the view which I am now urging (see his "Allgemeine Anatomie den Organismen," forming the first part of the 'Generelle Morphologie,' p. 296). "Es muss hierbei ausdrücklich errinert werden, dass wir unter eine Zelle nur einen Plasma-Klumpen mit einem Kerne verstehen können. Der häufig gebrauchte Ausdruck einer 'mehrkernigen Zelle' ist eine Contradictio in adjecto, da ja eben nur die Einheit des Kerns die individuelle Einheit der Zelle als eines Elementar-Organismus bedingt. Jeder Plasmaklumpen, der mehr als einen Kern umschliesst, möge er nun von einer Membran umhüllt sein oder nicht, ist eine Vielheit von Zellen, und wenn diese Vielheit eine bestimmte einheitliche Form besitzt, so haben wir sie als Zellenstock zu dem Range eines Organes erster Ordnung zu erheben." This view, however, has been controverted by many authorities, as will appear further on.

time were believed to be non-nucleate, have since been shown to contain nuclei, or at any rate nuclear matter, that we are tolerably well justified in saying that the nucleus, or its equivalent, is an essential constituent of the cell. At all events we know that division of the nuclear substance, whether mitotic or amitotic, is all-important as a prelude to and accompaniment of cell division. The experiments of Gruber and Verworn show that if Amœbæ are artificially divided, the parts cut off will regenerate and lead an independent existence if they contain nuclear matter, but if they do not, they soon perish. Fragmentation of the nucleus—by which is produced a so-called multinucleate condition, often of considerable duration—is a prelude to spore formation, i. e. to the division of the cell into many parts. Mitotic division is highly characteristic of division of the cell into two parts. It is very difficult to draw distinctions, but it is worth consideration whether the temporary multinucleate condition ending in multiple fission, which is common in protozoa, has not a different value to the permanently multinucleate condition of some plants and animals, which are generally called unicellular. In the one case (e. g. Podophrya, Thalassicolla, Actinosphærium) division or fragmentation of the nucleus leads, sooner or later, to the separation of cells, each containing a fragment of the original nucleus. In the Cœloblastæ (Siphoneæ, e. g. Caulerpa) the repeated division of the nucleus is not followed by any cell division, but the organism is throughout life a mass of continuous undivided protoplasm. The plant, as von Sachs says, is of considerable size, develops roots, even leaf-forming shoots, and in its protoplasm hundreds and thousands of cell nuclei are contained, which with advancing growth are multiplied by division, and obtain a definite arrangement within the protoplasm. And, as in the case in cellular plants, the nuclei are specially aggregated at the growing points. The whole behaviour is just that of a multicellular plant, but there are no partition walls.

It is stretching the point very far to call this a single cell. And, in fact, it is an inconsistency to do so, for where, by an

essentially similar process, a continuous sheet of protoplasm containing many nuclei is formed as a tissue-constituent of a multicellular animal or plant, we do not call the whole multinuclear tract a single cell—we call it a syncytium, or take some roundabout way of describing it. Such a case is the formation of the endosperm in the embryo-sac of Phanerogams. By repeated mitotic division of the nucleus and growth of the surrounding cytoplasm, a tract of continuous protoplasm is formed, containing many nuclei. At a later stage partitions are formed and the mass is divided up into cells, but for a period the endosperm has a structure which recalls that of the Cœloblastæ. Can we say that the condition in the endosperm is to be regarded as multicellular because it is not permanent, and that the condition in the Cœloblastæ is to be regarded as unicellular because it is permanent? If this is allowed the consequences are far-reaching, for it follows that the multinuclear phase in Actinosphærium and other Protozoa is also multicellular, because not permanent.

Take, again, the case of the Mycetozoa. The plasmodium of Badhamia or Fuligo is not unicellular, for it is formed by the union of many cells: it is not called multicellular, because there are no cell divisions: yet we draw, rightly enough, a distinction between the plasmodium, where cell bodies fuse but the nuclei do not unite, and the single cell resulting from conjugation, where the nuclei do unite.

A survey of the facts must lead to the conclusion that there is an intermediate phase between the unicellular and the multicellular condition, which is the multinucleate but non-cellular condition,[1] and that there is no fundamental distinction

[1] The term non-cellular does not exactly represent the condition which it is intended to describe. Yet, if one adheres to existing nomenclature, it is difficult to find a substitute. The term "cell," though founded on an erroneous conception, is so firmly established in biological language that it would probably be impossible to eject it. Yet if one were to make general use of the Greek equivalent κύτις (literally a little box), which has already come into such favour as to have respectable claims on our attention, one might adopt much more exact expressions. Thus the uninucleate Protozoa might be said to exhibit a monocytial condition, multicellular organisms a

between Protozoa as unicellular, and Metazoa as multicellular organisms. I should hardly have thought it worth while to insist upon this had not Mr. Sedgwick written "that an organism may consist of one cell or of several cells in association with one another. We draw the most fundamental distinction between the two kinds of organism, and we divide the animal kingdom into two great groups to receive them. As a proof of the importance which we attach to this feature of organisation we assert that a man is nearer, morphologically, to a tapeworm than a tapeworm is to a paramœcium."

Botanists, who have the great advantage of studying the physiology concurrently with the morphology of their subject, make no fundamental division into Protophyta and Metaphyta. For them, unicellular plants, hypopolycytial plants, Fungi and Algæ are alike Thallophyta, and a passage from Goebel may serve to illustrate the point of view which leads them to classify together organisms which, from the point of view of "independent life units," would appear widely separate. "From this initial stage"—a single small cell—"the process of development may advance, yet still within the limits of a single cell, and whilst the cell increases in size, often reaching dimensions without parallel in the vegetable kingdom, either the differentiation of the cell-contents or that of the external form, as shown by the branching, may make most rapid progress. In other cases the growth of the cells is accompanied by cell-division, the thallus becoming multicellular, and the single cell producing, according to the nature of the plant, a cell row, or a cellular filament, a cell surface or simple tissue layer, or lastly a cell mass increasing in every direction."

polycytial condition, and the so-called non-cellular condition of Cœloblastæ and Opalina might appropriately be called hypopolycytial, the preposition ὑπο being used in a modifying sense, as expressing the intermediate stage between one and many. The term syncytial, which is now used in a loose sense, is strictly applicable to the early condition of the plasmodia of the Myxomycetes, which are formed by the fusion of many units in a monocytial condition, and are therefore different from organisms which exhibit a hypopolycytial condition. In later stages the nuclei of the plasmodia multiply by division; thus the hypopolycytial is added to the syncytial condition.

Although in this passage, which is descriptive of Thallophytes, Goebel attaches too much importance, as I think, to the continuity of a vesicle as determining the unicellularity of a plant, he shows clearly enough that he regards the growth and mode of extension of the protoplasm, not its division into cells, as the feature of fundamental importance.

There is the further property in plants that continuity between the cells of highly organised multicellular plants has been shown to be of very general, if not universal, occurrence. And if complete separation were to be insisted upon as a characteristic of a cell, any given Angiosperm, or other highly organised plant, could no longer be considered as an aggregate of life units, but rather as a conjunct mass of protoplasm, imperfectly broken up into corpuscles, in each of which there is a nucleus. It is but a step from the much-branched, multinucleate Cœloblastæ, which have no partitions, to the formation of incomplete partitions, breaking up the protoplasm into small masses, which remain, however, linked with one another, and so preserve an original continuity similar to that of the Cœloblastæ, which has only apparently but never actually been broken.

So much has this idea impressed itself on the minds of some observers, that Hofmeister suggested that the creeping motion of the plasmodia of the Myxomycetes and their later transformation into fructification, is representative of the simplest type of growth, even for more highly organised plants. This opinion has been quoted with approval by von Sachs, who, before even the continuity of the protoplasm of plant cells was established, wrote that "fundamentally every plant, however highly organised, is a protoplasmic body, coherent in itself, which, clothed without by a cell-wall and traversed internally by innumerable partitions, grows; and it appears that the more vigorously this formation of chambers and walls proceeds with the nutrition of the protoplasm, the higher also is the development attained by the total organisation."

Expressed in this way, the phenomenon of cell-formation is represented to us as being nothing more than a particular

manifestation of growth, and Mr. Sedgwick may contend that his views are thereby conceded, and that the ancestral metazoon may, on this aspect, be considered as "a multinucleate infusorian with a mouth leading into a central vacuolated mass of protoplasm." There may be truth in the contention, yet none the less we may hold fast to the concept of a cell, as I shall attempt to show further on. And it may be observed in passing that Mr. Walter Gardiner, in describing and emphasising the continuity of protoplasm in plants, expressly stated "that the presence of minute perforations of the cell-wall need not lead to any modification of our general ideas as to the mechanism of the cell," a proposition which most reflective persons will be cordially inclined to agree with. For this much is certain, that the formation of cells is not merely the expression of one out of many formative processes which reside in organic matter, but is the formative process, par excellence, which obtains both in animal and vegetable tissues.

Thus far I have endeavoured to show that the independent-life-unit theory has not held the minds of zoologists in an iron bondage, much less the minds of biologists, for, when reference is made to biologists, botanists must be taken into equal account with zoologists.

It is, however, arguable that, whatever botanists have thought, zoologists have not followed their example, but have publicly maintained a complete adherence to the independent-life-unit theory in its most limited form, whatever reservations they may privately have made in their own minds.

But it may be doubted whether the argument holds good. I have already shown that passages which seem to state most dogmatically that cells are separate individuals, prove on examination to be nothing more than illustrations; and it is to be remembered that ideas founded on botanical evidence must always be reflected on the minds of zoologists, and one may certainly say that conceptions of animal structure have of late years been considerably modified by the light thrown upon organic structure in general by botanical investigation. Some zoologists may possibly have given too little attention to

growth without division into cells, because there are not in the animal kingdom any such striking instances of massive growth without cell division as are exhibited by the Cœloblastæ, especially if we leave out of consideration the Mycetozoa, as belonging to the debateable territory between the two kingdoms. Nevertheless, we have instances of growth and mitotic nuclear division, unaccompanied by cell division, which are not apparently a mere prelude to division. Take the single instance of Opalina ranarum. Because this organism is microscopic, and may be described, without offence to our sense of proportion, as a corpuscle, it is invariably called unicellular. Yet in essential features it resembles one of the Cœloblastæ. It contains numerous nuclei, which divide mitotically, and their division is an accompaniment of the growth of the mature organism. The multinucleate mature condition is of considerable duration. In the reproductive process this multinucleate corpuscle divides repeatedly, until a number of small offspring are formed, each containing several, usually four or five, nuclei. The minute product of fission then encysts, and it is remarkable that either during or immediately after encystment the several nuclei break up, and a single new nucleus is formed,—presumably it is constituted out of the chromatin of the several nuclei. The form which emerges from the cyst grows, and growth is accompanied by repeated mitotic division of the nucleus till the mature condition is reached. The whole history reminds one of that of a Mycetozoon, except that the young do not fuse to form a plasmodium, but simply grow up; in this respect Opalina resembles the Cœloblastæ, differing from them, however, in the fact that the whole organism is concerned in reproduction, not a special part. Although it has, as he remarks, a distinct "development," Zeller, who first followed its life history, has no doubt that Opalina is a single cell.

Now the multinucleate condition is far from uncommon in the Protozoa, and it may almost be said to be the rule in the Ciliata, if we regard macronucleus and micronucleus as two separate nuclei. But putting aside this phenomenon, the significance of which we do not yet clearly understand, there

are several Ciliata which have as many as one hundred nuclei, e. g. Holophrya oblonga, Lagynus elongatus, and Uroleptus roscovianus.[1] I do not include as multinuclear those forms in which, as in Trachelocerca phœnicopterus or Chœnia teres, the chromatin is scattered throughout the protoplasm in the form of minute granules. Those Protozoa only may be considered multinucleate in which there are several well-defined aggregations of chromatin. And even if the Ciliata above mentioned may not be considered truly multinucleate, but to possess only a fragmented nucleus, there can be no doubt about some Amœbæ, e. g. Amœba quarta and others described by Gruber.[2] In the last-named the multinuclear state is constant; as Gruber says, "es sich nicht etwa um vorübergehende Entwicklungszustände handelt." He watched these Amœbæ for a long period, expecting that the large number of nuclei would at last find its explanation in reproduction by multiple fission, but he was unable to observe any such culmination. Dr. Gruber is a great authority, and he, equally with Zeller and others, is quite positive that the multinuclear Protozoa are truly unicellular. His reasons are, that closely allied species are uninuclear, and that the protoplasmic body is continuous—contained in the case of Ciliata by a single cuticular coat. But even he admits that the only reasonable interpretation of the multinuclear condition is that it is a prelude to reproduction, that is, to cell division.[3] It is, therefore, a condition intermediate between the unicellular and the multicellular condition, or, as I should like to call it, a hypopolycytial condition, and nothing more need be affirmed of it.

Zeller is quite precise as to his reasons for regarding Opalina as unicellular. "Die kleinsten Thierchen aller bekannten Opalinen, so wie sie von Neuem sich zu entwickeln beginnen, besitzen nur einen einfachen Kern und entsprechen

[1] Maupas, "Études des Infusoires ciliés," 'Arch. Zool. exper. et gen.' (2), i, 1883.

[2] 'Zeit. für wiss. Zool.,' xli, p. 186.

[3] Aug. Gruber, "Ueber vielkernige Protozoa," 'Biol. Centralblatt,' iv, p. 710.

unzweifelhaft, wie Engelmann schon für die von ihm untersuchte Art nachgeweisen hat, 'morphologisch vollständig einer einzigen Zelle.' Aber auch mit der weiteren Entwicklung ändert sich daran nichts. Mag die Zellhaut zu einer aus vielen einzeln zerlegbaren Bändern bestehenden muskulöses Hülle werden und mag der Kern in zwei Kerne zerfallen, wie in O. similis und O. caudata, oder durch fortgestzte Theilungen eine schliesslich sehr grosse Menge von Kernen aus sich hervorgehen lassen, wie in O. ranarum, O. obtrigona und O. dimidiata, die protoplasmische Körpersubstanz selbst zeigt keine weitere Veränderung als die der Massenzunahme und blebt, wie auch Engelmann hervorhebt, 'Zeitlebens eine einzige zusammenhängender Masse, wie von eine einzigen Zelle.'" I have put the last passage in italics, because it expresses most clearly why Zeller and other authors regard multinucleate forms as unicellular, namely because the protoplasm shows no other change than increase in size, and because it remains, its life long, a single continuous mass. The same argument leads many to regard the Cœloblastæ as unicellular. The continuity of the protoplasm, then, is the test of unicellularity.

If anybody accepts this, he cannot escape from its logical consequences. Not only are multinucleate Protozoa and Cœloblastæ unicellular, but also the whole kingdom of plants, for their protoplasm is continuous: the developing Peripatus is unicellular, for its protoplasm is continuous; the epithelial cells of many animals, as Max Schulze, Pfitzner, Klein, Paulicki, Th. Cohn, and others have shown, are united by fine protoplasmic processes much as are the cells of plants, therefore the epithelia are unicellular, for their protoplasm is continuous. The same may be said for muscle cells (Werner and Klecki), for connective tissue, for bone cells, for the developing mesoblast of Vertebrata (teste Sedgwick, Assheton, and others), for the mesoblast (mesenchyme) of trochospheres and Molluscan larvæ (see particularly von Erlanger), and for many other tissues.

Thus the inevitable result of an argument which is meant

by those who use it to tighten the bonds of the cell-theory is to loosen them altogether, and to hand us over unbound to Mr. Sedgwick, who would fetter us once more with a new doctrine, viz. there is no cell, all organisation is a specialisation of tracts and vacuoles in a continuous mass of vacuolated protoplasm.

We do not want to be bound, at least I do not, and if we are to be free we must take refuge in some such lax but comprehensive statement as that of von Sachs, viz. that cell formation is a phenomenon very general in organic life ; but even if we must regard it as only of secondary significance, it is the characteristic expression of the formative forces which reside in organic substance.

Now this statement affirms the existence of cells, and it is necessary to arrive at some understanding as to what is a cell ; what properties are connoted by this term ?

It has become abundantly evident in the course of this argument, that whatever other attributes may be affirmed of the cell, the possession of a nucleus is one of the most important. It is impossible to disagree with Pfitzner when he writes, "Wenn wir aber den Kern überall und zwar immer und in allen Stadien als durchaus selbständiges Gebilde finden, so ergiebt sich daraus dass er fur das Bestehen der Zelle als solchen ein Organ von wiel fundamentaler Bedeutung ist als wir bisher geneigt werden anzunehmen." This is also the view of O. Hertwig, and it is no new one, for Max Schulze insisted upon it, and Häckel wrote in 1866, "Ein Plasmaklumpen ohne Kern ist keine Zelle mehr."

But can we follow Pfitzner when he goes further and says, "bei einer so ausserordentlich Konstanz in der ganze Reihe der Thierformen, von den Protozoen bis zu dem Menschen, kann ich nicht umhin auzunehmen dass überhaupt die ganze Existenz eine Zelle als biologische Einheit an das Vorhandsein eines centralen Körpers, von komplicirten inneren Bau, gebunden ist, dass also die Chromatinstrukturen nicht etwas sekundären erworbenes, sondern die Grundbedingung vitaler Existenz der Zelle darstellen. Und weiter folge ich hieraus

das der als Karyokinese bezeichnete Vorgang nicht ein specielle Kerntheilungsmodus, sondern der Kerntheilungsmodus κατ' ἐξοχήν ist"?

I think not. Particles of chromatin scattered through the protoplasm do not constitute a nucleus any more than a heap of bricks constitutes a house. Under such a view, Ciliata like Trachelocerca phænicopterus and Chænia teres would not be cells, for they have no central nucleus of complex structure, nor have Oscillaria and Bacterium, in which chromatin granules have been discovered. Though the case of Holosticha scutellum, in which scattered nuclei (chromatin particles) unite and fuse to form a single central body or nucleus previous to division, may help to clear our ideas, it is evident that the demand for a central organised constituent is more than the cell conception can bear, especially if the demand carries with it a further demand for the universality of mitotic division in nuclei.

In short, before we could accept Hertwig's definition of a cell, we should have to ask and answer the question, What is a nucleus?

Here I may stop to ask whether it is worth while to discuss the grounds of a definition which, when made, could not be acceptable to the mind of everyone. An argument about definitions would soon land one in the regions of scholasticism, and I have no desire to enter into subtleties which would tax the powers of a Duns Scotus. To give an answer which shall be beyond all cavil to the question, What is a nucleus? would be about as easy as to answer how many angels can dance on the point of a needle.

The truth is that it is the attempt to frame short concise definitions, applicable without exception to whole classes of phenomena, which leads to trouble. The concepts of biology may and should correspond with the phenomena we observe, but they can very seldom be made into universal propositions. There is no place in the science for definitions as exact and universal as those of geometry. The qualities of a nucleus are not to be defined like those of a point or a line. Such propo-

sitions as we may make are but resting-places for our minds as we ascend the mazy scale of organisation. To attempt to form definitions, to predicate the precise attributes of whole classes of phenomena, is to run counter to the very genius of the subject. For what do we mean by evolution if not that life is labile, never resting, protean in its variety? And how can we express this but in an incomplete way, contenting ourselves with particulars, and trying to show that the stream, though it flows in many tortuous channels, is one stream nevertheless.

Cells and nuclei are protean in their variety, and since we very rightly insist on objective study as a preliminary to the understanding of them, it is not wonderful that they should give rise to this concept in the mind of one man, and to that concept in the mind of another man, and thus it is not surprising that the theory of cells should be incapable of being stated, as Mr. Sedgwick complains, "in so many words in a manner satisfactory to everyone."

It is fairly obvious that Mr. Sedgwick's quarrel with the cell-theory began with the dissatisfaction which he felt when he discovered that doctrines, which he believed to be of universal application, were in fact contradicted by several instances. But he fell out of Scylla into Charybdis when he supposed that he could reply to a universal affirmative by a universal negative.

There is an old and respectable rule of logic that of two contrary propositions both cannot be true and both may be false, whilst of two subcontrary propositions both may be true but both cannot be false. Had Mr. Sedgwick remembered this, he would not have attempted to overthrow the cell-theory by the statement of a contrary proposition of equally universal import.

The cellular theory of development in the popular form in which it is often presented may be briefly summed up somewhat as follows. The multicellular organism is a colony, consisting of an aggregation of separate elementary parts, viz. cells. The cells are independent life units, and the organism

subsists in its parts and in the harmonious interaction of those parts.

The falsity of this summary is evident when we consider the known facts of vegetable organisation; the development of Peripatus; the union, by means of protoplasmic processes, of epithelial, muscular, and connective-tissue cells; the evidence lately adduced as to the continuity of the mesoblast in Elasmobranchs, Aves and Mammalia, and other well-known instances.

The absolute contrary, as expressed by Mr. Sedgwick, is equally false, viz. that the metazoon is a continuous mass of nucleated vascular protoplasm, subsisting in the unity of its mass. For, as I have shown in the earlier part of this essay, there are unequivocal instances of distinct isolated cells occurring in the embryos of many Metazoa (Nereis, Unio, Umbrella, Leptoplana). Moreover I am convinced, by my own studies on the histology of Cœlenterates, that, whilst there is organic connection between many of the tissue-cells composing these organisms, as was demonstrated long ago by the brothers Hertwig, there are many other cells of which such continuity cannot be affirmed.

To deal clearly with the cell-theory, or rather with the independent-life-unit theory which has grown out of it, we must split it up into as many separate propositions as it contains. These are:

The multicellular organism is an aggregate of elementary parts, viz. cells.

The elementary parts are independent life units.

The harmonious interaction of the independent life units constitutes the organism.

Therefore the multicellular organism is a colony (cell-republic according to Häckel).

It is not necessary to follow the theory further into the consequences which are deducible from these propositions, e. g. that development consists in the separation of numerous individual units from a single primary unit, the ovum. It is obvious that the truth of the first proposition in no way depends on the truth of those which follow, and that, in fact,

the second proposition is an assumption which is made to explain the first. We may make Mr. Sedgwick a present of the last three, whilst we retain and value the first.

The essence of the whole question is this: are we justified in considering the elementary parts of an organism to be independent life units? Before we can answer this, we must inquire why we do consider them to be independent life units?

The answer to this is probably to be found in the aphorism, which commends itself to everybody, that reproduction is discontinuous growth. From the observation that, in unicellular organisms, division of the unit—the cell-corpuscle—leads to the liberation of a new and independent unit, and that in multicellular organisms it is the liberation of an independent unit—the ovum—which constitutes reproduction, it has become a settled conviction in men's minds, that division of a cell-corpuscle means the liberation of a new unit, that is, the setting free of a new independent being. It is this conviction which has led to the belief that the units composing a multicellular organism are in posse independent beings, though in esse subordinate to the whole of which they form a part. This was the argument of Schwann when he wrote the passage which I have quoted on p. 149, and the argument has been taken as conclusive.

But we know now that the power which Schwann and his followers limited to cells is inherent in protoplasmic masses not divided into cells. For instance, if the cell-membrane of a Cœloblastic alga is ruptured, portions of the exuded protoplasm, provided they contain one or more nuclei, may become, after a time, surrounded by a new cell-membrane, grow, and form a new plant.

The experiments of Gruber show also, that portions of Amœbæ artifically separated may, provided that they contain nuclear substance, recover from the operation, and lead an independent existence.

May I ask, in parenthesis, whether there can be a better illustration of the truth of the contention which I have endeavoured to establish above, that whilst a uninucleate cor-

puscle of protoplasm is in esse as also in posse a unit of independent vitality, a multinucleate corpuscle or mass of protoplasm is in posse composed of separate cells (units of independent vitality if one chooses to call them so) whilst still in esse a single unit of independent vitality?

To continue the subject. We now know also that division into cells is not necessarily, though it sometimes may be, division into units of independent vitality, but is often (may we not say generally?) incomplete separation into form elements which may indeed, under certain conditions, be completely separated, and exhibit an independent vitality (Begonia), but under normal conditions participate in the vitality of the whole plant or animal by means of their connections with their fellows. Hence we must conclude, as it seems to me, that the elementary parts of organisms are not independent life units in esse. They may be so in posse in many cases, but as differentiation and specialization progress they lose this power also, and cannot, when separated from the whole of which they form a part, exhibit independent activities.

This consideration leads to the apparent paradox, that the higher the organisation the less conjunct and, at the same time, the less independent are its parts; the lower the organisation the more conjunct, but also the more independent are its parts.

This is a puzzle which has, for years past, exercised the minds of biologists. There is, I believe, but one solution of the difficulty, and it is to be found in the physiological import of cells.

But before we can enter into this question we must finally satisfy ourselves, as far as circumstances allow, about the morphological concept of a cell.

That the cell is a thing cognisable, and that it is not an unreal figment, due to imperfect observation or to hopelessly prejudiced interpretation of our observations, as Mr. Sedgwick would make us believe, I will try to show.

A cell is a "body," and therefore an external cause to which we attribute our sensations. I would submit that, without

prejudice to the metaphysical standpoint, we must conceive that what is capable of giving rise in us to such very distinct sensations, must have a real existence. I am referring now to the component parts of the tissues of higher animals and plants, and not to unicellular organisms.

If, then, the thing has existence, it must have attributes; we must be able to affirm something of it. What we have to affirm is not the attributes of this cell or of that cell, but of cells in general. We have to give expression to a morphological idea, in the sense in which Goethe used the word morphological. Our concept of a cell must be an "Allgemeines bild," the generalised idea of a cell, derived from our experience of many kinds of cells. I have already shown, at sufficient length, that we must now regard something of the nature of a nucleus as an essential component of all cells, but as the concept of a nucleus as a central organised body is not applicable to all cells, I would widen Max Schulze's definition by saying that "a cell is a corpuscle of protoplasm, which contains a specialised element, nuclein." This is a sufficiently comprehensive statement of our "Allgemeines bild," though I cannot pretend that it is not open to objection.

Cells, as thus defined, are not only of various kinds, but they are variously compounded together. We may, by the process of dichotomous division, classify them, according to their relations to other cells, as discrete and concrescent.

By discrete cells, I mean those whose protoplasm is not in union with that of any other corpuscle.

By concrescent cells, I mean corpuscles whose protoplasm is in union with that of other corpuscles.

Discrete cells may further be divided into:

Independent cells, living wholly apart from one another, or separated by an appreciable interval of space, e. g. uninucleate Protozoa, the mature ovum, leucocytes.

Coherent cells, which are in close apposition to others, but not organically in union with them, e. g. the blastomeres of many developing embryos.

Concrescent cells may also be further divided into:

Continuous cells, whose protoplasm is fused but whose nuclei are separate, e.g. Myxomycetes, Cœloblastæ, Opalina.

Conjunct cells, those which having a protoplasmic body of definite outline are united inter se by fine bonds of protoplasm, e.g. vegetable tissue cells, epithelial cells of many animals; mesenchyme cells, &c.

Experience shows us that independent cells may, in process of growth, give rise to coherent cells, continuous cells, conjunct cells, or to all three together, and that coherent, continuous, or conjunct cells may, and in fact do, give rise to independent cells. As thus stated, can there be a better illustration of von Sachs's principle that cell-formation is an accompaniment of growth?

It will be observed that, in adhering to the present terminology, I am obliged to classify organisms usually (though not always) called unicellular as multicellular. I have tried to escape from this necessity, but the limitations of language compel me to it. I should be grateful for a better and more logical definition.

The view of Mr. Sedgwick—if I do not misrepresent him—is this, that there are no coherent cells; that all which I have classified as continuous and conjunct cells are not cells, but tracts of protoplasm; that the only cell, sensu stricto, is the independent cell, and that morphologically and physiologically it is of no consequence.

I have already shown that there are cells which we must regard as coherent. I cannot, for reasons which I will explain directly, consider the independent cell of no consequence, and the difference between us as to conjunct cells is simply this: Are they to be regarded as one or many? I can, perhaps, best express this difference by an illustration.

Is a house to be regarded as one room or composed of separate rooms? A room is a certain portion of space enclosed by walls, ceiling, and floor; but it is also in connection, by means of the door, with other similar rooms. Is it, then, not a separate room, but part of a larger room? Or if I shut the

door is it a room, and if I open the door is it no longer a room? The subject might be argued with much ingenuity, but the final answer is this—that "room" and "cell" are terms which give expressions to certain states of our consciousness, and for practical purposes they are very useful terms indeed. Where distinct states of consciousness are called up, of such a nature as to give rise to ideas of particularity, it is a mere quibble to argue that the apparent parts are actually merged in a whole. A cell is none the less a cell, in the sense of a thing distinct in itself, because it is conjunct with its fellow cell, than my room is the less a room because it has one door opening into an adjoining room and another opening into the passage.

Yet there is something more than a verbal quibble in Mr. Sedgwick's contention. He would have it that in the case of mesenchyme it is incorrect to say that it is a number of stellate cells joined to one another by their processes. For him the correct description is, "a protoplasmic reticulum with nuclei at the nodes." Does he accept the logical consequences of this, and say of the epithelial cells of the Salamander or of unstriped muscle fibres that they are protoplasmic reticula with nuclei at their nodes? And if so, how does he explain the fact that, in the one case and in the other, the elements when absolutely isolated by appropriate methods show a remarkably constant and characteristic form? Were they what he describes, rupture of the internodes of the reticulum would result in amorphous lumps of protoplasm, not in units of characteristic form. It is the constancy of the various forms of cells which convinces morphologists of their individuality as form elements, and all the arguments which Mr. Sedgwick or anybody else may choose to bring forward will not convince the man who goes into a laboratory, makes a few maceration preparations, and studies the results for himself.

Thus a tissue formed of conjunct cells is made up of many and not of one, and as a form concept the cell holds its ground and, pace Mr. Sedgwick, it will continue to hold its ground against all comers.

As a physiological concept it is hardly less useful, though reflection may induce us to abandon the "cell-republic" theory, as, indeed, it has been tacitly abandoned by many. I take it that the scheme of von Sachs very nearly expresses, in general terms, the physiological importance of the cell. An organism is a protoplasmic body, coherent in itself, which grows, and as it grows it is divided by cleavage into innumerable corpuscles, and it appears that the more vigorously this formation of corpuscles proceeds with the nutrition of the organism, the higher also is the development attained by the total organisation. Nor does this statement stand in any contradiction to the original theory of Schwann, from whom I may quote two more passages: "The elementary parts of all tissues are formed of cells, in an analogous though very diversified manner, so that it may be asserted that there is one universal principle of development for the elementary parts of organisms, however different, and that this principle is the formation of cells." And again, he says of the relations of cells to one another, "Each cell is within certain limits an individual, an independent whole. The vital phenomena of one are repeated, entirely or in part, in all the rest. These individuals, however, are not ranged side by side as a mere aggregate, but so operate together in a manner unknown to us, as to produce a harmonious whole." It should be remembered that Schwann regarded cells as so many separate vesicles, and when allowance is made for this error, the second part of the last passage must be allowed to have great significance. The subordination of the parts to the harmonious whole, leading to the loss of individuality of the parts, in animal tissues, was insisted on by Häckel in his 'Generelle Morphologie.' The first of the two sentences which I have quoted from Schwann is even more true to-day than when it was written, for we have got rid of the cell-forming matrix, the cytoblastema; and I would wish to insist on this passage as expressing in the clearest possible language the cell-theory as we understand it to-day.

From this standpoint we can see, obscurely it may be, why

cell-formation accompanies differentiation with growth of the mass, and why specialisation is not possible in continuous tracts of protoplasm. For, as Mr. Sedgwick himself admits, in a continuous mass of protoplasm, changes of molecular constitution in any one part would in time spread through the whole, so that a differentiation of one part would in time be impressed on all the other parts, and physiological division of labour would be out of the question. The fact that in the Protozoa there is differentiation within the limits of a single corpuscle presents no greater difficulty than the fact that in the epithelio-muscular cells of Cœlenterates, or the similar cells in Nematodes, there is differentiation within the limits of the cell.

Again, metabolism in a large mass is greatly facilitated by its being broken up. As von Sachs says, "It is very intelligible that not only the solidity but also the shutting off of various products of metabolism, the conduction of the sap from place to place, and so forth, must attain greater perfection if the whole substance of a plant is divided up by numerous transverse and longitudinal partitions into cell chambers." The same thing applies, mutatis mutandis, to animals, and it is not difficult to see that the difference between holozoic and holophytic nutrition makes it impossible for the animal to grow to a large mass without division into cells, whilst such growth is possible in the case of plants which, like Codium and Caulerpa, live in water, or like Botrydium in damp earth.

It is known that the spaces between epithelial cells which are traversed by the connecting strands of protoplasm, and were formerly supposed to be occupied by a cement substance, are in reality lymph spaces, and this gives us some insight into the importance of the cell structure in animal organisation. The formation of cells with spaces between admits of nutrient fluid being brought to the very threshold of each constituent corpuscle of the organism. (See on this subject Th. Cohn, R. Heidenhain, Paulicki, Nicolas, Werner, and others.)

Whilst the necessities of cohesion, solidity, and transmission of stimuli may explain the conjunct nature of so many tissue

cells, recent researches on cell lineages may perhaps give us a clue to the interpretation of the fact that blastomeres are in so many cases, no more than coherent. For it is noticeable that wherever cell lineages, with marked isolation of the blastomeres, have been described, there is a decided tendency to the precocious development of organs, or, at any rate, to the precocious isolation of the primordia (Anlage) of organs.

It seems probable that the discrete condition of the blastomeres is connected with the fact, to which I alluded in the earlier part of this essay, that they are, from the very outset, specialised. They have each a definite molecular constitution different from the others, and, in figurative language, a limited part to perform, which they could not perform to advantage if they were conjunct with the other blastomeres and shared in their different molecular constitution. But this is a subject which I must leave for a future occasion when I discuss the validity of von Baer's law of development.

I have travelled in this essay over a great deal of ground, and I have necessarily had to touch more lightly on many topics than I should have wished. I hope that I may at least have succeeded in presenting my arguments in a manner which will make them clear to my readers, and that I have not been too discursive. Starting from Mr. Sedgwick's propositions and accusations, I have tried to show what is or was the exact extent and meaning of the cell-theory; I have tried to examine it and show how much was good and how much bad, and I have finally been led to the conclusion—which is not quite what I proposed to myself at the outset—that the cell concept is a valuable expression of our experience of organic life, both morphologically and physiologically, but that in higher organisms cells are much what von Sachs declares them to be, not independent life units (Lebenseinzelheiten), but a phenomenon so general as to be of the highest significance; they are the constant and definite expression of the formative forces which reside in so high a degree in organic matter.

Lest I should appear to have minimised the importance of the cell too much, let me conclude by saying, that nothing

which has appeared above calls into question that great feature of animal and plant development which most impresses the biological student, viz. that organic growth is a cycle, beginning in the single cell, and returning to the single cell again. And therefore, in a limited sense, the cell is par excellence the unit of life. Its growth takes various forms and shows many complexities, but whatever the form, however great the complexity, it is a progress from the state of an independent corpuscle, through a state of many coherent, or continuous, or conjunct, interdependent corpuscles, back again to the state of a single independent corpuscle.

This was the great advance made by Remak on the theory of Schwann, and summed up in Virchow's aphorism, which I believe to be universally true. For Schwann did not hold that cells are the ultimate basis of life: he held that they are formed, as a crystal is formed out of its mother liquor, from a structureless matrix, the cytoblastema. To some such theory Mr. Sedgwick wishes to take us back again, for his "pale and at first sparse reticulum" bears a most suspicious resemblance to the exploded cytoblastema. "The development of nerves," he says, "is not an outgrowth from certain central cells, but is a differentiation of a substance which was already in position." And earlier in his article, referring to the growth and extension of the mesoblast between epiblast and hypoblast, he says: "What are the facts? The space between the layers is never empty. It is always traversed by strands of a pale tissue connecting the various layers, and the growth which does take place between the layers is not a formation of cells but of nuclei, which move away from their place of origin and take up their position in this pale and at first sparse reticulum."

But surely nobody ever affirmed that the space between the layers was empty except in the sense that it is devoid of cellular structures. It is well known that it is filled with a coagulable fluid, and it is worthy of remark that coagulable fluids, treated with the reagents now most in use, frequently form a reticulum of pale non-staining substance. I can speak

from experience, for not long since I was much puzzled by such a reticulum, and had I been less cautious I should have published, as a great morphological discovery, statements which rested on a wholly insufficient basis of experience. The subject requires further investigation, and the most that one can say now is, that it is possible that Mr. Sedgwick, good observer as he is, may have been mistaken. And he will pardon my observing that the things which he states are not "facts." They are his own inferences from his own individual observations, and will require very abundant confirmation before they can take rank as what we agree to regard as "facts." All the "facts" we have at present, i. e. the accumulated observations of hundreds of highly-trained and able observers, are fundamentally opposed to any such account of protoplasmic growth apart from nuclear formation as Mr. Sedgwick gives us. But there is another way of looking at it, namely, that he has only overstated his case, and that the growth of the tissues in question resembles the apparent creeping motion of the plasmodia of the Myxomycetes. That this may be the case is supported by a study of Mr. Assheton's recent account of the growth of the mesoblast and of the inner layer of the epiblast in the embryo of the rabbit. It presents no theoretical difficulties, but it should be remarked that Mr. Assheton figures numerous nuclei at the very edge of the growing part of his reticula, which is consonant with what we know of protoplasmic growth in other cases, but not with Mr. Sedgwick's account.

But if Mr. Sedgwick can prove that the reticulum is there and that it grows and spreads far from the nuclei which subsequently migrate into it, he must not suppose, as he is apparently so ready to assume, that the inveterate prejudice of morphologists will prevent their accepting his conclusions because of their theoretical difficulties. If his case is proved, it will be accepted, but he must prove it up to the hilt.

And if he does prove it, what then? It will be an isolated case, of secondary significance: merely another addition to our experience of the very various phenomena displayed in

organic growth. For thousands of instances point to the fact that normal growth is effected in a very different way, by mitotic division of the nucleus preceding and directing the formation of a discrete or concrescent cell-corpuscle. The recent researches of cytologists are too many, too good of their kind, and too consistent to admit of any other conclusion.

DEC 1895

On the Distribution of Assimilated Iron Compounds, other than Hæmoglobin and Hæmatins, in Animal and Vegetable Cells.

By

A. B. Macallum, M.B., Ph.D.,
Associate-Professor of Physiology, University of Toronto.

With Plates 10—12.

	Page
I. Preliminary Remarks and References to Special Literature of the Subject	175
II. Methods of Study	179
III. General Observations on the Distribution of Assimilated Iron in Highly Specialised Animal and Vegetable Cells	205
In the Nucleus of the Animal Cell	205
In the Nucleus of the Vegetable Cell	211
In the Cytoplasm of the Animal Cell	214
In the Cytoplasm of the Vegetable Cell	227
IV. On the Occurrence of Assimilated Iron in Special Forms of Life	228
In Ascaris mystax	229
In the Larvæ of Chironomus	231
In Protozoa	236
In Fungi	242
In Bacteria	254
In the Cyanophyceæ	261
V. General Remarks	268
VI. Explanation of Figures	271

I. Preliminary Remarks.

In 1891, in a communication to the Royal Society,[1] I described a method by which the presence of iron in the chromatin of

[1] "On the Demonstration of the Presence of Iron in Chromatin by Microchemical Methods," 'Proceedings Roy. Soc.,' vol. l, p. 277.

animal and vegetable cells may be demonstrated micro-chemically, and I referred to the results then obtained with it as indicating, apparently, that iron is always a constituent element of this substance. The interest which the subject had for me led me to continue the investigation with improved methods of research, and I am now consequently in a position to describe a much more extensive series of observations in support of the generalisation, then somewhat tentatively advanced, that iron is a constant constituent of the nuclein substance proper.

From the commencement of the investigation I have been fully aware of its difficulties, and I can, therefore, readily understand that view of the subject which led Gilson to remark that the solution of the question concerned is one "that seems to require more than a single man's activity."[1] The difficulties encountered in the application of the micro-chemical method are, however, very much less formidable than those met with in the employment of the older methods. I have pointed out, in my first paper on this subject, how impossible it is to be certain that the iron revealed by macro-chemical methods in isolated quantities of nuclein is not present through absorption from some other source, but due to a combination obtaining in unisolated living chromatin, and I have indicated that the only way in which the question could be settled definitely is by the employment of micro-chemical methods. I have shown in the succeeding pages of this paper that the acid alcohol upon which Bunge relied to extract the iron of inorganic and albuminate compounds from egg-yolk and other nuclein-holding substances, and leave intact the organic (nucleinic) iron, does not perform this function at all when the substance treated with it is in mass, while it removes the iron of all three classes of compounds from thin sections of tissues, if the time allowed for its action be prolonged. We have, consequently, in a macro-chemical investigation, no means whatever of distinguishing between organic iron on the one hand and the iron of inorganic and albuminate combinations on the other, and we are therefore

[1] "On the Affinity of Nuclein for Iron and other Substances," 'Report British Association for the Advancement of Science,' 1892, p. 778.

forced, more than ever, to depend on micro-chemical methods to determine the relations of assimilated iron to the cell. Objections may be urged against these methods also, based chiefly on the facts that iron, free or combined, contaminates everything, so to speak, and that what is shown to occur in dead chromatin may not be present in the living compound; but these objections at once lose their force when the methods are applied with all due care accompanied by such control experiments as the conditions may suggest.

I have in my former communication made reference to the investigations of Bunge and Zaleski upon iron-holding nucleins. Since 1891 four other investigators have published observations on the occurrence of iron in organic compounds.

Molisch[1] endeavoured to determine the relations of iron in the vegetable cell by means of concentrated aqueous solutions of potash. He found that when vegetable tissues were immersed in this reagent for a day or longer, they gave a reaction for iron not at all obtainable in the fresh tissues, and he explained the result as due to the removal of the iron from a firmly combined ("maskirt") condition to that in which it is readily detectable by ordinary reagents. The firmly combined iron, as shown by this method, was sometimes in the cell wall, sometimes in the cell contents, and sometimes again in both. His results do not call for a fuller description than this, since in a later publication[2] he has stated that his solutions of potash were not free from iron, and he has consequently withdrawn all the conclusions which he previously based on the results obtained with this reagent.

Petit,[3] in investigating the occurrence of iron in barley, employed Bunge's method to separate the inorganic and albuminate from the organic iron, using for that purpose a 1 per cent. solution of hydrochloric acid in absolute alcohol.

[1] 'Die Pflanze in ihren Beziehungen zum Eisen,' Jena, 1892.

[2] "Bemerkung über den Nachweis von maskirterm Eisen," 'Berichte der deutschen bot. Gesell.,' vol. xi, 1893, p. 73.

[3] "Distribution et l'état du fer dans l'orge," 'Comptes Rendus,' vol. cxv, p. 246, 1892.

The dried and finely pulverised barley was put, with the acid alcohol, in a Soxhlet extraction apparatus and heat was applied for six hours, during which time the reagent was renewed once, but the second liquid extracted no iron. The result was the same when the strength of the acid in the solution was 2·5 per cent. From his experiments he concludes that nearly all the iron is combined with nuclein (à l'état de nucléine) and exclusively contained in the tegmen and embryo of the barley grain. In a second publication[1] he describes the separation of an iron-holding nuclein from the malt-combs (touraillons) of barley, free from sulphur and in which the iron amounted to 0·195 per cent. The separation was made by extracting the pulverised matter with a 1 per cent. solution of potash at 60° C. for some minutes, and filtering off under pressure the brown liquid, which was then neutralised with dilute hydrochloric acid. The precipitate formed was washed by decantation with water, then with alcohol and ether, and finally dried over sulphuric acid.

Gilson[2] found iron in the nucleinic elements, not only when ammonium sulphide, according to my method of using it, was employed, but also after treatment with other reagents and in nuclei which, without such treatment, gave no reaction for iron with the ordinary methods of demonstration. He specially mentions sulphuric acid and sulphurous anhydride as giving the best results, although others, among which he includes saline solutions, produce the same effects. He is, however, inclined to regard the iron demonstrated in the nuclein as due to a combination which is formed only after death, and similar to that which dead nuclein effects with many other substances, especially colouring matters. He showed that dead nuclein has a very strong affinity for iron compounds, the nuclei of freshly extracted cells absorbing from a 0·05 per cent. solution of ferrous sulphate more iron than could be demonstrated in them when simply treated with sulphuric acid; and he maintains it is extremely difficult to ascertain whether nuclein in a living

[1] "Sur une nucléine végétale," 'Comptes Rendus,' vol. cxvi, p. 995, 1893.

[2] Loc. cit.

condition contains iron, or contains it only after death, deriving it by absorption from the blood or other surrounding fluids, or even out of the reagents themselves, if these are not absolutely free from iron. In his remarks upon my methods he states that Bunge's fluid, upon which I relied to extract the iron of inorganic and albuminate combinations from sections of tissues, does not take away the iron artificially combined with dead nuclein even after six days.

Hammarsten[1] has isolated from the pancreas of the ox an iron-holding nucleo-proteid containing 4·48 per cent. of phosphorus.

II. Methods of Study.

In my first communication on the method of demonstrating micro-chemically the occurence of "masked" iron, the reagent whose use I described was called, in a general way, ammonium sulphide. This is a term that is properly applicable only to the diammonium compound represented by the formula $(NH_4)_2S$, but it is sometimes given to solutions which contain either ammonium hydrogen sulphide (NH_4HS), or polysulphides of ammonium, or to mixtures of diammonium sulphide and ammonium hydrogen sulphide. At the time I was unable to determine which of the two latter is the most effective as a reagent in liberating the iron from the chromatin, since either, when recently prepared, gave, with cellular elements from the same piece of tissue, reactions in which differences in intensity were not noticeable, and, while uncertain upon this point, I felt justified in adopting the generic term "ammonium sulphide" to designate a reagent which might be held to indicate either of the two compounds.

About two years ago I gave further attention to the question whether one form of the reagent is more efficient than the other in this respect, and the results of a series of experiments made since then have led me to the conclusion that ammonium

[1] "Zur Kenntniss der Nucleo-proteïde," 'Zeit. für Physiol. Chemie,' vol. xix, 1894, p. 19.

hydrogen sulphide is more active than the diammonium salt, and that none of the polysulphides of ammonium have any action whatever on iron in its "masked" form. These experiments have been controlled by others made with these reagents upon solutions of potassium ferrocyanide.[1] Ammonium sulphide, when mixed with a solution of the latter salt and the mixture kept at a temperature of 30—50° C. for one or more days, will liberate the iron from its combination and precipitate it as sulphide, the amount so liberated depending on the strengths of the solutions forming the mixture, on the temperature and on the time during which the reaction is allowed to go on. A lower temperature will suffice when the time is prolonged. By paying due attention to all the conditions, it is possible to liberate, as sulphide, all the iron of such solutions. In this ammonium hydrogen sulphide is more active than diammonium sulphide, the amount of the sulphide formed being a measure of the activity of either reagent.[2] These experiments have, in all cases, given results which correspond with those obtained with the two sulphides upon the chromatin of isolated cells, but it was not possible in the latter case to estimate the effects as definitely. I found that of two slide preparations of isolated cells, one made with ammonium hydrogen sulphide, the other with diammonium sulphide, the former as a rule gave the

[1] I have not found any reference to the action of ammonium sulphide on solutions of ferrocyanides in the literature of chemistry, although, on the presumption that some such reference exists, I made diligent search for it.

[2] The results of one experiment upon this point may be mentioned. The glass-stoppered cylinder *a* contained 10 c.c. of a 10 per cent. solution of potassic ferrocyanide and 10 c.c. of ammonium hydrogen sulphide made from an ammonia solution of 0·96 sp. gr., while to a similar cylinder *b*, with like quantities of the same solutions, 10 c.c. of dilute ammonia were added. At the end of twenty-four hours' stay in a warm oven with a temperature of 40° C., the precipitates were filtered off with iron-free filters, washed with water containing hydrogen sulphide in solution, dissolved in dilute sulphuric acid solutions, and, after care had been taken to reduce all the iron to the ferrous condition, the amount of the metal in each case was estimated by titration with a standardised permanganate solution. Results: the precipitate in *a* contained 0·0113 grm. iron, while the iron of the precipitate in *b* amounted to 0·0025 grm.

maximum reaction in about ten days, while the latter manifested a reaction of moderate intensity at the end of that time, which, with a longer stay in the warm oven, did not become more marked. In the case of vegetable cells the reactions were more quickly obtained and the differences in intensity greater. This is illustrated in figs. 14, 15, and 16, representing preparations of cells of the ovary of Erythronium americanum, in which the reagents used had been made from dilute solutions of ammonia (of 0·96 sp. gr.). Fig. 15 indicates the depth of the reaction with ammonium hydrogen sulphide at the end of twenty-four hours, the intensity attaining in another cell in ninety hours the degree represented in fig. 16, while in fig. 14 is shown how far the reaction had progressed with diammonium sulphide in forty-eight hours. In the latter case the reaction did not become more marked even on the eighth day. Similar results were obtained in all the experiments of this character, demonstrating that ammonium hydrogen sulphide is more effective in liberating iron from organic combinations than is the diammonium compound.

In the earlier stages of the investigation the reagent was made from strong solutions of ammonia of sp. gr. 0·88; but when thus prepared it deteriorates rapidly and becomes yellow from the formation of polysulphides. Spoiled or unsuccessful preparations were consequently frequently obtained. Sometimes, also, difficulties were experienced in determining whether, in the preparation of the reagent, the saturation of the strong ammonia with sulphuretted hydrogen was complete. For this reason, and also because dilute solutions of ammonium hydrogen sulphide are less unpleasant in every way, I began to use the latter, and found that it gives results not less decided than those obtained with the stronger solutions. The dilute solutions offer other advantages, for when made from pure ammonia of 0·96 sp. gr., they retain their potency for three weeks or longer, especially if kept in a bottle with a well-fitted glass stopper, and in a cool place. The smaller the amount of air in the bottle and the less frequently the stopper is removed, the longer does the reagent retain its strength.

During the last two years the dilute reagent has, in consequence of these facts, been exclusively employed.

The glycerine used was chemically pure.[1] It gave the best results when diluted with an equal volume of distilled water. In making the preparations, the cellular elements were teased out on the slide in a drop of the dilute glycerine, and over this, after thorough admixture with two drops of the dilute solution of ammonium hydrogen sulphide, a cover-glass of 16—22 mm. square was placed. The teasing-out process was done in each case with a clean pair of goose-quill points. Every care was taken to prevent the occurrence of impurities in the preparations. The excess of the glycerine and sulphide mixture is at first uncovered, but if the slide be put in a warm oven with a temperature of 60° C., the mixture rapidly concentrates and in a few minutes is wholly under the cover-glass. When the solution of ammonium hydrogen sulphide is deteriorated, a deposit of sulphur forms at the edges of the cover-glass and the mixture under the latter becomes yellow through the production of polysulphides of ammonium. Such preparations never yield anything of value. On the other hand, when the fluid under the cover-glass remains colourless and free sulphur does not form at the margins, the preparation, if kept at a temperature of 55—60° C. for a period of from two to fifteen days, is almost always successful. Sometimes at the end of one, two, or three days the mixture is further concentrated and has receded from one edge of the cover-glass. This is remedied

[1] Molisch ('Die Pflanze in ihren Beziehungen zum Eisen,' p. 107) states that the glycerine of commerce—even the purest—contains traces of iron. I have not found this to be the case with Price's glycerine, quantities of which, when mixed with ammonium hydrogen sulphide or diammonium sulphide, gave not the slightest reaction or precipitate, even after two weeks, and whenever portions of the stock supply used were evaporated at a low heat in a platinum dish no appreciable residue was left, and not a trace of iron or lead was detected. I found that in some samples of glycerine of other manufacture the sulphide gave no immediate reaction, but at the end of a week, or later, a small precipitate, composed partly of sulphide of iron, was at the bottom of the test-tube. A similar precipitate was obtained in portions of the stock supply of Price's glycerine only when traces of an iron salt were added.

by placing at the dry side of the cover-glass a drop of a mixture of one part of dilute glycerine and two of ammonium hydrogen sulphide, the drop so placed running under the cover, after which the preparation is replaced in the warm oven and in the end usually proves successful. I have found that when the isolated cellular elements are not very numerous and uniformly distributed under the cover-glass, evaporation rarely goes so far as to render a resort to this remedy necessary; but when the tissues are only partially teased, and fragments tilt or elevate the cover-glass, the mixture concentrates, the preparation dries at one side, and the sulphide is largely converted into polysulphide.

The solutions of ammonia used in the preparation of the reagents were chemically pure, and in this respect, as well as in the cleanliness of the slides and covers, I paid due regard to the suspicion that there possibly exists a ferrous sulpho-hydrate (FeS_2H_2), soluble to a certain extent in solutions of ammonium hydrogen sulphide, the presence of which in the glycerine and sulphide mixture of my preparations might, through its diffusion into the nuclei and precipitation therein as ferrous sulphide (FeS), give confusing results. That no such compound existed in my reagents was shown repeatedly by allowing mixtures of the sulphide and glycerine to stand for weeks, when all the ammonium hydrogen sulphide was converted into the diammonium salt, or into polysulphides of ammonium, in the presence of which it would appear that the supposed existence of ferrous sulpho-hydrate is impossible. In these experiments no iron was found, nor did the mixtures in the end lose any of their transparency,—a result which tells against the possibility of any such iron compound existing in the mixtures employed upon teased-out cells. The cover-glasses and slides were cleaned in solutions of hydrochloric acid to remove any adherent compounds of iron, and afterwards passed through distilled water and alcohol. The bottles in which the solutions of ammonium hydrogen sulphide were kept were also, first of all, cleansed in the same way.

Nothing was gained by making "stock" mixtures, in the

proper proportions, of glycerine and ammonium hydrogen sulphide, for in such the reagent is more rapidly converted into the non-active form than when it is kept separate. Apparently also in "stock" mixtures the polysulphides are very rapidly formed, the fluids becoming deep yellow in twenty-four hours or less, although the sulphide used may be nearly colourless. In summer the change of colour is rapid. That it is due in part at least to the formation of polysulphides, appears to follow from the fact that drops of the mixture, when allowed to remain uncovered on the slide for a few minutes, quickly become milky in appearance from the precipitation of free sulphur. The mixtures retain a part of their strength during the first two or three days, after which they become useless.

The tissues which were teased out for treatment were always hardened in alcohol wholly free from iron in solution. Latterly I have employed for this purpose redistilled methylated spirit. I have not used in this connection material fixed with any of the mineral hardening reagents, since the latter frequently contain iron, the presence of which in dying cells and tissues might be held to contribute, under the influence of the hardening reagent, to the formation of firm organic compounds of iron. Some mineral reagents, moreover—as, for example, corrosive sublimate and osmic acid—are difficult to remove from the tissues upon which they have been allowed to act, and their presence in preparations treated with ammonium sulphide, which forms sulphides with these metals, gives appearances obscuring, in a greater or less degree, the occurrence of iron compounds.

To facilitate the teasing-out I frequently used sections made with a clean steel knife[1] covered with absolute alcohol, the cells of such sections readily separating, and yielding sometimes a number of free nuclei. In order to determine whether iron in an inorganic or albuminate form is present, and to what

[1] In my earlier paper (loc. cit.) I pointed out that the knife so used gives no iron to the preparation. All my observations for the last two and a half years have in no way called in question the correctness of this contention.

extent, it was my practice to allow the section to lie in the glycerine and sulphide mixture for a few minutes before teasing it out, the iron of these forms of combination giving an immediate reaction on the penetration of the reagent. The removal of all iron of this description is necessary, since its presence may give confusing results in teased-out cells. For this purpose I have used Bunge's fluid, in which the sections were kept for about an hour with the reagent at a temperature of 55° C., the subsequent treatment with alcohol and ammonium hydrogen sulphide in all cases showing that the inorganic and albuminate iron had been thereby removed.[1] Sections so treated were teased out and mounted in the glycerine and sulphide mixture in the usual way.

The disadvantages connected with the use of ammonium hydrogen sulphide to demonstrate the presence of "masked" iron are that it effects, in the animal cell at least, structural changes, that it is not successful on large nuclei or on nuclei of large cells, and that it requires a great expenditure of time. In regard to the structural changes it is obvious that, however well hardened or well fixed cellular elements may be through the action of alcohol, ammonium hydrogen sulphide or diammonium sulphide must, when heat is applied, sometimes alter, to a greater or less degree, the structure of the cell, and especially of its nucleus. This is quite evident when we compare such preparations with others in which the "masked" iron has been liberated by the use of sulphuric acid alcohol, and subsequently treated with the sulphide. Figs. 23 and 24 illustrate the differences obtained with the two methods, the former representing liver-cells of Necturus lateralis treated for ten days at 55° C. with the glycerine and sulphide mixture, while the latter was drawn from a section of the same material after it had been acted on by sulphuric acid alcohol for seven hours at 35° C., and then with the glycerine and sulphide mixture. The first difference to be noted between the

[1] In regard to the capacity of Bunge's fluid for extracting iron of all forms of combination, see the description of the properties of hydrochloric acid alcohol as given below.

preparations represented is that of the iron reaction illustrated. This is partly due to the fact that in one preparation the ammonium hydrogen sulphide has not liberated all the iron of the chromatin, but partly also to the fact that the reagent has caused the delicate chromatin elements to become swollen, thereby rendering the iron reaction more diffuse and less marked. The effect on the cytoplasm is not less striking. It is, however, chiefly with concentrated solutions of ammonium hydrogen sulphide that preparations of animal nuclei exhibit this phenomenon. Solutions of the reagent made from ammonia of 0·96 specific gravity do not as readily produce this change, and in many cases none at all may be shown. When the reagent is fresh the reaction is quickly obtained, sometimes in two or three days, and then no swelling of the nuclear network occurs; but when it is not fresh, or when it gives an odour of ammonia, the reaction is slowly obtained, and the prolonged application necessary in order to bring out this result, aided perhaps by the ammonia, causes a swelling of the chromatic elements.

The slowness with which the reaction comes out is not wholly a disadvantage, for by this means one may determine whether the iron demonstrated is derived from other than inorganic or albuminate compounds. With the exception of hæmoglobin, hæmatin, and the compound found in yolk-spherules, the organic combinations in which the iron is "masked" are affected very slowly by ammonium hydrogen sulphide, and only when heat is applied; whereas the reaction comes out at once, or after a few minutes at the longest, and without heat, in the case of inorganic and albuminate compounds. The distinction between these and the "masked" compounds is, therefore, very marked. In one of the exceptions mentioned the distinction is not so clear, for when ammonium hydrogen sulphide is added to the fresh yolk of hen's egg it gives a greenish reaction at once, but when the yolk is hardened with alcohol or with heat the reagent gives this result only after several days' application at 50—60° C. On the other hand, the yolk-spherules in Amphibia (Necturus

and Amblystoma), whether hardened or fresh, yield the reaction in a few minutes. Such compounds are of too limited a range of distribution to affect the value of the reagent in making a distinction between the iron compounds. On hæmoglobin and myo-hæmatin (myo-hæmoglobin) the reagent has not the slightest action. I have kept mixtures of the reagent with solutions of hæmoglobin and myo-hæmoglobin for more than a year at a temperature of 55° C., and in no case have I found that iron was liberated from these compounds as sulphide. I have, moreover, mounted in the glycerine and sulphide mixture on the slide finely powdered hæmoglobin which had been coagulated in alcohol, and applied heat to the preparation for weeks without once obtaining the iron in an inorganic form. When, therefore, in preparations of animal tissues which have been hardened in alcohol one obtains with the glycerine and sulphide method after a time an iron reaction, it may reasonably be concluded that the iron so demonstrated is not derived from hæmoglobin in the tissues. One may not, however, exclude hæmatin as a possible source of iron, for although hæmoglobin in all forms will not yield its iron to ammonium hydrogen sulphide, the latter readily liberates the iron of hæmatin, and from a solution of hæmatin in ammoniated alcohol or in dilute ammonia, into which hydrogen sulphide has been passed, part of the iron at ordinary temperatures, but the whole at 50° C., is precipitated as ferrous sulphide, in a few days.[1] Even in a solution of hæmatin in ammoniated alcohol, if kept for several days at the temperature of the room,

[1] The compound formed from the hæmatin in this process of liberating the iron is neither hæmatoporphyrin nor bilirubin. With yellow nitric acid it gives a play of colours in which violet, faint red, and yellow successively appear, the mixture finally becoming colourless, and it yields an absorption spectrum like that of bilirubin. It is insoluble in ether, and soluble in chloroform and hot alcohol. The other properties of this compound are now under investigation. It has one special claim to interest in that it is formed from hæmatin by a method very much less drastic in its effects than those in which strong sulphuric acid or bromine in glacial acetic acid is used to form hæmatoporphyrin or bilirubin (Nencki and Sieber, 'Monatsh. für Chemie,' vol. ix, p. 115, 1888).

a part of the iron of the hæmatin is precipitated as a greyish-white hydroxide, which, if filtered off, gives at once with ammonium sulphide the greenish-black sulphide reaction. Very weak solutions of hydrochloric and other acids effect the removal of the iron, and if solutions of hæmatin in alcohol are kept for a week or more in contact with solutions of various salts (potassium chlorate and sulphate and sodium chloride and phosphate), decomposition of the hæmatin results, and iron is liberated as an inorganic compound. In all these respects hæmatin behaves like the ferrocyanides, while it differs markedly from hæmoglobin in the same points.

Experiments show, however, how little, if any, of the iron demonstrated in animal cells is derived from hæmatin. Sections of the liver and other organs of Vertebrates, as well as of vegetable tissues, were placed in alcoholic solutions of hæmatin for twenty-four hours, then washed in alcohol for a few minutes, and kept in a quantity of the glycerine and sulphide mixture at a temperature of 35° C. for twenty-four hours. At the end of the latter interval all the sections were blackened, and under the microscope the nuclei were dark green from the ferrous sulphide liberated from the hæmatin absorbed by the chromatin. In order to get this result the sections do not require to be teased out at all. The rapidity with which such a strong reaction is obtained indicates that in ordinary teased-out cells mounted on the slide in the glycerine and sulphide mixture, the deep reactions obtained after several days or after a week are due to a decomposition, not of hæmatin, but of some other compound or compounds.

Ammonium hydrogen sulphide, then, may be regarded as a reagent of very great value in the investigation of "masked" compounds of iron, and it must constitute a final test for this purpose, whenever the accuracy of the other reagents, used also for determining the distribution of assimilated iron compounds in cells, is called in question.

In June, 1891, Mr. R. R. Bensley, while carrying on under my direction a research on the distribution of iron in the ovary of Erythronium americanum, as demonstrated by

the employment of ammonium sulphide, succeeded in obtaining some interesting results which necessitated control experiments based on the removal of all traces of inorganic compounds of iron from the tissues under investigation. For this purpose Bunge's fluid was used, and it was thought that hardened specimens of the ovary, when subjected to its action for a time, would not give, on the addition of ammonium sulphide, any immediate reaction for iron, and that further treatment with the latter reagent in a warm oven for several days would show the presence of iron in the nuclei of their cells, and possibly also in their cytoplasm. Much to our surprise, however, the treatment of the ovary of Erythronium with a quantity of Bunge's fluid for two weeks at 20° C., and the subsequent application of ammonium sulphide, resulted in the production of a marked reaction for iron, which under the microscope was found confined to the nuclei. I was at first inclined to believe that the iron so shown was due to diffusion into the nuclei of that present in an inorganic form in the tissues, and this would appear to be Gilson's view; but repeated experiments have demonstrated the incorrectness of this explanation, and that Bunge's fluid liberates the iron of organic compounds.[1] Experiments were also made on animal tissues and similar results were obtained. The liberation of the iron is to be attributed to the hydrochloric acid, the only active part of the reagent. This conclusion suggested a number of experiments, all based on the principle that whatever proper-

[1] Gilson's statement is difficult to interpret. He does not say whether he applied the reagent to sections of tissues or to the latter in mass, and at what temperature it was allowed to act. He appears to regard the iron absorbed by dead nuclein as combined with the latter, and he remarks, in reference to my statement that Bunge's fluid removes all inorganic and albuminate iron from sections after treatment with it for ten hours: "but I have observed that Bunge's liquid does not take away the iron artificially combined with dead nuclein after six days." I can explain his statement only on the supposition that he used the reagent on the tissues in mass, and that he thereby obtained the same results that I did under similar circumstances; in other words, the iron "artificially combined with dead nuclein" was in reality iron liberated by Bunge's fluid from its masked condition in the chromatin and retained by the latter.

ties hydrochloric acid may have in this respect are possessed, in a greater or less degree, by other mineral acids, whether in dilute aqueous solutions or in alcohol, and the results were of such a character as to induce me to employ these reagents on all species of cells in which the distribution of iron had been determined with ammonium hydrogen sulphide.

The more serviceable of these were found to be sulphuric acid and nitric acid dissolved in alcohol of 95 per cent. strength. The former was prepared by adding four volumes of the strong acid to one hundred of alcohol, while the latter contained three volumes of the acid (of 1·4 sp. gr.) in one hundred of alcohol.

The chemicals used in the preparation of these reagents were free from traces of iron, and care was taken to have all bottles and vessels used to hold them also free from adherent iron compounds. It was, of course, impossible to provide against the iron in the glass, but I am not certain that the reagents derived any from this source, even in infinitesimal quantities. The alcohol used contained not a trace of iron. During the last eighteen months re-distilled methylated spirit was found to be in every way as serviceable as the pure ethyl alcohol used earlier in the investigation.

The alcohol of these reagents largely prevents the occurrence of digestive changes which the acids effect when, in aqueous solutions, they are allowed to act on tissues for several days, and especially at a slightly elevated temperature. Another important function of the alcohol is to prevent a too rapid extraction of the liberated iron, and thereby also its diffusion from one part of the tissue into another, from nucleus to cell, or from cell to nucleus. Acid alcohols dissolve iron salts more readily than does alcohol alone, but less so than aqueous solutions of the acids. For example, ferrous sulphate is insoluble in absolute alcohol and in strong methylated spirit, but it is soluble in these when they contain a small quantity of sulphuric acid,—not, however, in any way as much so as in distilled water, or in dilute aqueous solutions of sulphuric acid. The smaller the proportion of the acid in the alcohol

the less readily does it dissolve the iron salts, and when used upon tissues acid alcohols have a smaller capacity for extracting the iron salts the longer the reagents are allowed to act, for the liberation of the iron from its " masked " condition entails the neutralisation of the acid, a very gradual process. As a result of this neutralisation the iron salts become less soluble and would pass back into the tissues, but the danger of this happening is minimised or altogether prevented by the property which the chromatin has of retaining the iron that is set free in itself by the acid alcohol. This is shown specially in the case of nitric acid alcohol, for when sections of vegetable tissues are allowed to lie for two weeks in a large quantity of the reagent, the nuclei at the end of that time give as intense a reaction for iron as they do at the end of two days. The result is due to the fact that the tenacity with which the chromatin holds the iron liberated in it counteracts the extractive capacity of the reagent.

The results of the action of nitric and sulphuric acid alcohols differ from those obtained with Bunge's fluid[1] in one important respect. The two former, whether they are used upon the tissues in mass or on sections of the same, leave the iron, on the whole, in the parts in which it is liberated; but when sections of tissues are treated with hydrochloric acid alcohol, the iron is extracted as quickly as it is liberated, and consequently such preparations on treatment with ammonium sulphide give a feeble reaction for iron or none at all. This is most distinctly seen when the temperature is raised, and if the reagent is allowed to act for two or three days under these conditions, no iron, organic or inorganic, is left in the preparations. When the tissues are in mass, on the other hand, the quantity of acid that penetrates the preparations is largely neutralised and extraction takes place very slowly, with the result that teased-out portions of such tissues give a marked reaction for iron, limited, as in preparations obtained with

[1] Bunge's fluid, or hydrochloric acid alcohol, consists of ninety volumes of alcohol of 95 per cent. strength, and ten volumes of a 25 per cent. solution of hydrochloric acid.

the other acid alcohols, to the parts in which treatment with the glycerine and sulphide mixture demonstrates its occurrence.

In describing the properties of hydrochloric acid alcohol, Bunge expressly states that while it extracts inorganic iron it does not remove the iron from the nuclein (hæmatogen) of egg-yolk.[1] This is not quite correct, for when hard-boiled yolk is treated with ammonium sulphide it gives only a feeble reaction for iron, even when kept for twenty-four hours at an elevated temperature; but when it has been acted on by a quantity of Bunge's fluid for a day at 30—35° C., the application of ammonium sulphide, after all traces of the acid have been removed with alcohol, gives an immediate and marked reaction for iron. The iron under such conditions must be in the form of chloride, and as an inorganic compound it should be extracted by the reagent, if Bunge's views concerning the properties of the latter be correct, but this happens only when the quantity of the yolk so treated is very small, and then the whole of the iron is removed in a few days, this fact demonstrating clearly that the reagent in its action makes no distinction between inorganic and organic iron. The latter is in its liberation from the "masked" condition converted into the inorganic form, and it depends on the quantity of yolk used whether or not the extraction may keep pace with this conversion. If the quantity is large, the liberation of the iron from its organic combination entails a diminution of the acidity of the reagent, and at length the extraction of the liberated iron ceases. It commences again only when a fresh quantity of the reagent is substituted for the exhausted fluid.

The results of its action upon the iron-containing nucleo-albumin of yolk are therefore practically similar to those which it gives when applied to animal and vegetable tissues.

The fact that a considerable diminution of the acidity of hydrochloric acid alcohol allows the liberated iron to be retained

[1] "Ueber die Assimilation des Eisens," 'Zeit für Physiol. Chemie,' vol. ix, 1885, p. 49.

in its original position in the cell has led me to try the effects of solutions in which the strength of the acid was less than 1 per cent.,[1] and they have been found, when used upon thin sections of tissues, to give very successful preparations, permitting the iron liberated to be demonstrated as fully as after the employment of either sulphuric or nitric acid alcohol.

The time during which these reagents must be allowed to act on a piece of tissue varies. I prefer to give general statements on this point, because specific directions are impossible in a case where the size of the object, the quantity of the reagent, and the temperature constitute the conditions. Bunge's fluid extracts as readily as it liberates the iron in thin sections of tissue, but when the latter is in mass the reagent requires a length of time which may vary from a week to two months, all depending on the size of the object and on the temperature, which in summer may be that of the room (20°—29° C.), but in the colder seasons that of the warm oven (35° C.). Sulphuric acid alcohol acts more slowly, and consequently requires a longer time for liberating the iron in unsectioned objects, while in sections its action is complete in from one to four days, this depending also on the temperature, the most favourable being 35° C. A longer stay than is just sufficient to liberate all the organic iron results in removing from the sections some of the iron set free, the more being extracted the longer the sections lie in the reagent. When examples of the Protozoa and Protophyta were subjected to

[1] These differences in extractive capacity exhibited by weak and strong alcoholic solutions of hydrochloric acid have apparently not been noted by Petit (loc. cit.), who used the diluted reagent in a Soxhlet apparatus to remove the inorganic iron compounds from barley. As the boiling-point of hydrochloric acid is higher than that of alcohol, it is obvious that little of the former must pass from the 1 per cent. solution at the bottom of the flask as vapour to condense above and act on the substance whose iron is to be extracted, while the alcohol is readily converted into vapour; in other words, the reagent in the upper part of the apparatus must be much more dilute than that in the flask below, and consequently its extractive power must be very feeble. This method is, therefore, open to the objection that it does not ensure the removal of inorganic iron compounds.

the action of the acid alcohol the full effect was obtained at the end of twenty-four hours at the latest, when the temperature was 35° C. With nitric acid alcohol the liberation of the organic iron was rapid, sections of vegetable tissue (Erythronium and Iris) giving, after a stay of ten hours in the reagent at 35° C., an intense reaction with the acid ferrocyanide mixture. At a lower temperature the result was less marked, but the reaction was deeper than that obtained with sections treated with sulphuric acid alcohol for the same length of time and at the same temperature. The process of liberation was usually completed in about thirty-six hours. So little does nitric acid alcohol extract the iron it liberates that in sections of the ovary of Erythronium americanum kept for six weeks in it I found little diminution in that intensity of the iron reaction which sections, placed in the same fluid at the commencement with the others, gave at the end of two days. With sections of animal tissue the intensity of the reaction was less marked with the prolonged stay in the reagent, which, after four or five days' action, slightly alters the cellular structures. When nitric acid alcohol is allowed to act on a section for a longer time than is necessary to set free all its organic iron, diffusion of the iron salts thus formed is apt to occur, especially in vegetable preparations, the cytoplasm giving in such cases a reaction for iron.

That the iron demonstrated after the use of acid alcohols is derived from organic compounds I have shown by numerous experiments. I have found that when thin sections of animal or vegetable tissue are covered with a large quantity of Bunge's fluid and kept for three days at 35° C. or higher, the teased-out cells give no iron reaction when mounted with glycerine and ammonium hydrogen sulphide on the slide, even after two weeks and at 60° C. Furthermore, sections so treated with Bunge's fluid, when subsequently subjected to the action of sulphuric acid alcohol or of nitric acid alcohol, yield no iron reaction whatever. Bunge's fluid, therefore, extracts the iron which the prolonged application of ammonium hydrogen sulphide and glycerine at an elevated temperature liberates and demon-

strates, and with this removal disappears the iron demonstrable after treatment with either of the other acid alcohols. This shows that the iron in such cases cannot be derived from the reagent nor from the glass of the vessel used, and this is emphasised by the results of other experiments. I extracted with Bunge's fluid all the iron from a series of sections of an ovary of Erythronium, and then subjected these to the action of a large quantity of sulphuric acid alcohol for twenty-four hours at 35° C. These gave no iron reaction, while others did so which had not been treated with Bunge's fluid, and which were put in the acid alcohol at the same time. That the absence of an iron reaction was not due to a lack of absorptive capacity on the part of the section, brought about by Bunge's fluid, was proved when such sections were allowed to stay in sulphuric acid alcohol containing a little ferric salt in solution[1] for half an hour. The reaction obtained was marked, and almost wholly confined to the nuclei. These experiments were repeated again and again with sections of animal and vegetable tissues, and the results were always the same, proving that the iron demonstrable after acid alcohol has been used on tissues is derived from the latter, and not from the reagent or the vessel used. These experiments indicate, however, how necessary it is, in investigating the distribution of iron in tissues, that the reagents should be absolutely free from iron, and that, in sections of tissues containing iron in an inorganic or albuminate form, there is danger, when either sulphuric acid alcohol or nitric acid alcohol is used upon them, of its redistribution, and especially of its deposition in those parts of the cell which absorb various compounds readily. In order to guard against this, I found it advisable to steep the sections in a quantity of Bunge's fluid for a time which varied with the temperature at which the reagent was applied, as, for example, for one to two hours at 50°—60° C., but for eight to ten hours at 35° C.

[1] This solution was made in the following way. A quantity of sulphuric acid alcohol was allowed to act on ferric oxide in powder for about a week, when a portion passed into solution as a ferric salt. Of this solution 1 c.c. was taken and added to 10 c.c. of pure sulphuric acid alcohol.

Bunge's fluid extracts very little or no iron from sections when the temperature is below 20° C., but at the higher temperatures stated the extraction is complete at the end of the intervals mentioned, and with a longer action more or less of the "masked" iron is liberated and removed. When a tissue—as, for example, that of the spleen in some animals—contains an excess of iron in an inorganic form, the time of extraction must be prolonged, and the extracting fluid large in quantity. After the inorganic and albuminate iron has been thus removed from a section—a result which may be demonstrated by treatment of the preparation with ammonium sulphide,—it may be subjected to the action of either of the two other acid alcohols to liberate that portion of the "masked" iron as yet unaffected.

The acid alcohols do not readily attack and liberate the iron of hæmoglobin and hæmatin except at a high temperature. Of this fact I have convinced myself by numerous experiments on hæmoglobin, whether prepared from alcohol material or from that coagulated by heat. A quantity of it, in a powdered form, put into a flask and covered with a quantity of Bunge's fluid, was heated for twenty minutes, and the fluid then, after filtration through a filter free from iron, was neutralised and treated with ammonium sulphide. The mixture gave no immediate evidence of the presence of iron, but when the test-tube containing it was put aside for twenty-four hours, a dark-green sediment made its appearance, and this was shown to be sulphide of iron when it was separated on an iron-free filter and treated with a quantity of an acid ferrocyanide mixture. This iron was, in great part, derived from the hæmoglobin and hæmatin, as well as from organic combinations present in the leucocytes and plasma, and but little had its source in the inorganic and albuminate compounds of the same, a fact shown by further experiments on the powder which had once been acted upon by boiling Bunge's fluid. The extract made with a fresh quantity of the reagent gave, on neutralisation and on the addition of ammonium sulphide, the same evidence of the presence of iron that was obtained in the first experiment. A

third, fourth, and fifth extraction resulted in the same way. When, on the other hand, a quantity of crystallised hæmoglobin was acted upon by the reagent for forty-eight hours at 35° C., the filtered fluid, tested for iron in the manner described, gave a scarcely appreciable evidence of the presence of the metal. The iron, therefore, which is found in animal tissues after the use of Bunge's fluid at either 35° or 50° C. for short intervals cannot very well be supposed to be derived, in any appreciable quantity, from the hæmoglobin in them, and as ammonium hydrogen sulphide does not affect the iron of the pigment, yet reveals the iron of "masked" combinations of an apparently less firm character, it follows that weak solutions of hydrochloric acid at slightly raised temperatures must attack such combinations more readily than it affects hæmoglobin. This was most clearly shown by results of experiments on hæmoglobin and chromatin with a quantity of Bunge's fluid for twenty-four hours at 35° C. When hæmoglobin alone is thus treated, neither the powder nor the extract gives any appreciable indication of free iron, but the latter is readily demonstrable in chromatin, or in mixtures of chromatin and hæmoglobin, after similar treatment. Since the iron in hæmoglobin is not affected to any perceptible degree by treatment with the reagent for twenty-four hours at 35° C., one may postulate that it is as little affected by treatment with either of the other two acid alcohols at the same temperature, and experiments with these have given results which bear out this conclusion.

The substance chlorophyll, the relations of which to iron, though generally recognised, have not been definitely determined, is, as is well known, an abundant constituent of the cells in many vegetable forms, and, therefore, a brief discussion of the possibility that this substance is the source of the iron demonstrated in vegetable cells, is necessary.

Some of the more recent investigators of this substance have made conflicting statements on the question of the presence of iron in the molecule. Adolph Hansen[1] found it to contain

[1] 'Die Farbstoffe des Chlorophylls,' Darmstadt, 1889, p. 58.

iron, while Emich, at the request of Molisch,[1] examined a quantity of pure chlorophyll and found it free from iron. Molisch also made observations on the subject, and determined that, after every care was taken to prevent contamination with iron salts through impure extracting fluids, the ash of chlorophyll gave not the slightest reaction for the metal. Gautier[2] also claims that it does not contain iron. Schunk,[3] on the other hand, found ferric oxide in the ash of phylloxanthin, one of the decomposition products of chlorophyll, even after that compound had been treated with acids and after repeated solution of it in ether.

The material from chlorophyll-holding organisms was, in all cases, thoroughly freed from that substance before the disposition of the iron in it was examined. Chorophyll, however, has not in any of my preparations yielded any evidence that it contains iron, nor does its presence or absence at all affect the question of the occurrence of iron in other compounds in the cell. This is very distinctly shown when one compares the results, obtained from experiments on vegetable cells holding chlorophyll, with those determining the distribution of iron in Fungi and in Monotropa uniflora and Corallorhiza multiflora, which are destitute of chlorophyll. In the two latter the disposition of the assimilated iron is as it is in the chlorophyll-holding Phanerogamous plants, and consequently one may dismiss the objection that the pigment constitutes the source of the iron demonstrated by my methods in the nuclei of vegetable cells. It may be proved also from Monotropa[4]

[1] Op. cit., p. 87.

[2] 'Chemie Biologique,' Paris, 1892, p. 20.

[3] "Contributions to the Chemistry of Chlorophyll," No. 4, 'Proceedings Roy. Soc.,' vol. l, 1891, p. 302.

[4] The importance of Monotropa material for control purposes renders a short description of the methods of preparation employed upon it necessary. This plant, when hardened in alcohol, blackens more or less through the production on the part of the dying cells of a dark greenish-blue pigment, but it remains colourless when fixed in solutions of corrosive sublimate, a reagent whose use is, for reasons already mentioned, objectionable when ammonium sulphide is to be employed. To obtain material on which this reagent may be

that none of the iron found in the nuclei is derived from the cytoplasm, for there is very little and often no cytoplasm in the cells of the coats of the ovules in this plant, and yet the nuclei of these give as intense a reaction as those of the ovary of Erythronium, Iris, Hyacinthus, or any form in which the cytoplasm is abundant.

In order to get the best results with the use of the acid alcohols, I have found that the tissues must be well hardened. If the tissues are fresh or imperfectly hardened, the application of acid alcohols for a time sets free the organic iron, but the structure of the cellular elements is more or less changed in such cases by the acids—a change not at all found to occur when the tissues have been carefully hardened. Strong alcohol (90—95 per cent.) was used for this purpose, and it was found to present, over the other hardening reagents, a number of advantages. It can by redistillation be made free from iron, and when it is of absolute strength it neither extracts any of the iron compounds (hæmatins excepted) from tissues, nor allows these to diffuse. There is the important point also, that tissues fixed with it can be subjected to all the reactions for iron, without incurring the risk of complications due to the deposition of iron or other metallic salts, which occur when other hardening reagents are used. In this way one may treat pieces of a tissue with ammonium hydrogen sulphide and with the acid alcohols, and thus allow the methods to control each other. When, on the other hand, it was not necessary to use ammonium hydrogen sulphide on the tissues, other hardening reagents were employed, but only such as did not, by their presence in the tissues, interfere with or obscure the demonstration of the iron. Saturated solutions of corrosive sublimate and ½ per

used advantageously, parts of the fresh plant are thrown into boiling distilled water, and those which remain uncoloured at the end of ten minutes are further hardened for several days in absolute alcohol. I have often treated material so prepared with the warm glycerine and sulphide mixture for from four to ten days, and then with an acid ferrocyanide solution converted the ferrous sulphide demonstrated into Prussian blue. Such preparations are probably the most instructive obtainable in regard to the question of the relation of iron to the vegetable cell.

cent. solutions of osmic acid were found serviceable, the latter reagent also having been used in the combination known as Flemming's fluid.

The corrosive sublimate solution was allowed to act on the preparations of tissue for about ten minutes, after which they were washed for a few minutes in distilled water, and then in 50 per cent. alcohol. The hardening was completed with alcohol of 70 and 90 per cent. strengths in the usual way. When Flemming's fluid was used the tissue was not allowed to lie in it for more than half an hour, while for the osmic acid solution not more than ten minutes were given, and the fixation was carried on further with alcohol of 50, 70, and 95 per cent. strengths. Preparations, whether made with corrosive sublimate or with osmic acid solutions, retain, even after careful washing, traces of the metallic salt of the reagent used, and the black or dark reaction which they give with ammonium hydrogen sulphide, in consequence of the presence of such metals, interferes with the proper demonstration of the distribution of iron by that reagent. On this material the acid alcohols only were used, and the preparations were subsequently treated with the acid ferrocyanide mixture, the Prussian blue reaction obtained not having been in the least affected by the presence of minute quantities of the metallic salts of the hardening reagents. The latter were free from iron salts, a fact of which I convinced myself by qualitative analyses.

To the use of all hardening reagents other than alcohol there are objections. Those which contain an acid may assist in the diffusion of iron salts in the tissues, and cause the deposition of these in some other parts than those in which they originally were held. Further, the acids of some of the reagents (e. g. acetic acid in Flemming's fluid) may liberate the organic iron, which cannot in such a case be distinguished from the iron of inorganic or albuminate combinations. For these reasons I have used acid hardening reagents but occasionally, and then the time allowed for their action was short, in order to reduce to a minimum the risk of liberating organic iron, and of the diffusion of iron salts through the tissues. Against corrosive

sublimate as a hardening reagent, which I have frequently used, it may be urged that it possibly assists in the diffusion through the tissues of the inorganic compounds of iron, and that consequently the distribution of the latter, in preparations thus hardened, may not correspond with that obtaining in the fresh tissue. Where this is not under investigation it is a matter of no importance, for treatment of sections of the tissue with warm Bunge's fluid for a few hours removes such compounds, and the sections so treated may be subjected to the action of the various reagents described to demonstrate the organic iron; but when it is desired to study the distribution of both classes of iron compounds in a tissue, the objection urged would, if well founded, exclude corrosive sublimate as a hardening reagent for this purpose. My experiments in relation to this were made on pieces of the same organ (liver and kidney of guinea-pig and of Amblystoma) hardened with alcohol alone, and with corrosive sublimate and alcohol, and I have found, on comparing the distribution of iron in both series of preparations, that though the possibility of the diffusion of iron salts is not excluded when corrosive sublimate is used, yet no appreciable evidence of it was manifested in the preparations. I have not, however, based my observations in any one case alone upon material hardened in corrosive sublimate, but have used material hardened in alcohol in all cases to control the results obtained when that reagent was used.

When the iron was liberated by acid alcohol the whole of it appeared as a ferric salt in some tissues, while in others a very small portion of it also was set free as a ferrous compound.[1] The latter condition was illustrated in some of the Protozoa. In such preparations all the iron set free is demonstrated, after treatment with ammonium sulphide, as a ferrous salt, and the preparations may then, on being acted upon with a mixture of equal volumes of dilute solutions of hydrochloric acid and potassic ferrocyanide, reveal all their liberated iron as Prussian blue. The iron in the ferrous form is usually so very minute in quantity, if present at all, that it may be

[1] For an explanation of the preponderance of the ferric compound see p. 268.

neglected in the making of permanent preparations. In order to prevent contamination of the sections with iron compounds in the demonstration, the solutions of potassic ferrocyanide were, on all the occasions used, not more than a week old, although I found that those of longer standing, up to the end of two months or so, when filtered carefully, gave preparations which were free from any objectionable characters. The strength employed was 1·5 per cent., and a volume of this was mixed with an equal volume of hydrochloric acid of 0·5 per cent. strength, when the mixture was required.

The sections, after removal from acid alcohol, were first washed in pure alcohol, then in distilled water, after which they were placed in the acid ferrocyanide mixture for not more than five minutes. Again washed carefully in distilled water, they were either dehydrated in alcohol, cleared in oil of cedar, and mounted in benzole balsam, or, before they were put through this course, stained with either safranin or eosin. The staining reagents were of 1 per cent. strength in 30 per cent. alcohol, and the time allowed for the action of the eosin was three minutes, while that for the action of safranin was half an hour. The excess of the stain in either case was removed with alcohol. The advantages given by the use of these stains I have explained in the description of the constituents of the nucleus. Very frequently I have found that a preparation which illustrated, in a remarkable way, some point in the distribution of iron in the cell, became useless through a complete fading out of the blue. The causes of this result are two: exposure of the preparation to the light for a time, and the use of inferior oil of cedar, that is, impure through the presence of minute quantities of water and other matters. I found that when I used old oil of cedar to clear up the sections and to remove all traces of alcohol, the preparations would keep their beauty unimpaired, if placed away from the light in the slide box. In some way the preservation of the blue colour depends on leaving a trace of the oil used in clearing-up upon the section when the balsam is added, but in this quantity allowed to remain there must be no alcohol or

water. When oil of cloves or oil of lavender was used all the preparations faded, for some reason at present unexplainable. The presence of safranin or eosin in the preparation does not influence, in any way, its chances of fading, but if the excess of the stain has not been removed it is apt, while the balsam is hardening, to diffuse, and thereby obscure the finer details of the preparation. That it is not difficult to keep Prussian blue preparations of animal and vegetable tissues, if carefully made, is shown by the fact that I have had now for over two years several hundred of such which retain unimpaired the original intensity of the reaction.

I have always washed the sections with distilled water, before putting them in the acid ferrocyanide mixture, because the presence of acid alcohol, especially that containing nitric acid, causes decomposition of the ferrocyanide and a deposition of Prussian blue in parts of the preparation in which iron did not occur originally. The acid ferrocyanide mixture itself decomposes after twenty minutes with the formation of Prussian blue, but that this is not, even in an infinitesimal part, the source of the blue that obtains in a section during the first five minutes after the mixture is made, was shown by the complete absence of a blue reaction in other sections of the same tissue (*e.g.* cartilage, muscle, ovary of Erythronium) placed in the mixture at the same time without having previously been treated with an acid alcohol. The distribution of the Prussian blue due to such a decomposition is quite different from that which one finds in preparations treated with acid alcohol, but in which this decomposition was avoided, for when one leaves sections of animal or vegetable tissue in the acid ferrocyanide mixture for two hours, the blue colour is uniformly diffused through the section, not localised as it is when the reaction is due to the iron of the tissue.

In the permanent preparations made to illustrate the distribution of iron, and on which no staining reagents were employed, the parts revealed by the transparent blue are not as sharply outlined as they would be if stained with hæmatoxylin for example, owing to the cytoplasmic parts, over or under the

structures coloured blue, obscuring the latter. This may be obviated, especially with high powers, by raising the Abbé condenser to the level of the stage and removing altogether its diaphragm, when the brilliancy of the light in the field of the microscope enhances the blue due to the iron reaction, while it renders more or less obscure the other details of the preparation. It was only in this way that I was able to determine the occurrence of very minute traces of iron in the tissues and, when the sections were stained with safranin, of bodies which gave but a feeble Prussian blue reaction (figs. 45 and 46).

The sections of tissue were made, either by the free hand with a polished steel knife, or by the paraffin or celloidin methods. Care was taken that the knife should not yield a trace of iron to the sections. When the paraffin method was employed the surface of the cutting instrument was dry, but with the other methods it was covered with absolute alcohol. The transference of sections from one fluid to another was done with goose-quill points or with glass needles.

I may not leave this part of the subject without a reference to the potash method for the liberation of "masked" iron, as described by Molisch, but afterwards determined by him to be untrustworthy. I have studied the effect of concentrated solutions of potassium hydrate upon vegetable tissues hardened in alcohol, and have obtained, frequently, evidences of the presence of iron in the cell wall, cytoplasm and nucleus, but the amount thus indicated in the last was always very much less than could be demonstrated by the other methods, while the reagent so altered the nuclei that a determination of the definite relations of the iron observed to the nuclear structures was impossible. My observations have convinced me that a very large part of the iron demonstrable after the use of this reagent is derived from the latter, however pure it may apparently be, and I am, therefore, upon this point in accord with Molisch. One of the readiest ways of proving this is by extracting all the iron from sections of vegetable tissues by keeping them in a quantity of warm Bunge's fluid for several

days and then transferring them to a quantity of a concentrated solution of potassium hydrate for an hour, after which interval the sections give abundant evidence of the presence of iron. That not a trace of iron is left in the sections by Bunge's fluid may be shown by incinerating some of the sections so treated and examining micro-chemically the ash for the presence of iron. Were one able to obtain the reagent absolutely free from iron, its employment for this purpose, limited as it must be through its drastic action on cellular structures, would, however, still be open to objection on the score that it dissolves and redistributes the iron of the tissues.

III. General Observations on the Distribution of Assimilated Iron in highly specialised Animal and Vegetable Cells.

The greater part, and sometimes the whole, of the assimilated iron in the cells of the higher forms of animal life is held in the nucleus, in the chromatin of which it is chiefly found. The chromatin fibrillæ, the chromatin granules, the nodal points of the chromatin network, all exhibit, after the employment of the methods described above, the clearest evidence of the presence of iron. Though no definite comparison is possible, yet, judging by the depth of colour resulting from the Prussian blue reaction in a large number of animal nuclei, one may say that the amount of iron thus demonstrated appears to correspond in all cases with the amount of chromatin present. This is probably best seen after the use of sulphuric acid alcohol, followed by treatment with an acid ferrocyanide solution, the sections thus prepared being compared with others simply stained with a reagent like Ehrlich's hæmatoxylin so employed as to affect the chromatin only. In this case the hæmatoxylin stain in the chromatin is always found to correspond in intensity, in the object stained and in the general distribution of the stain, with the blue reaction obtained in the other sections. If, further, sections

illustrating the Prussian blue reaction, be stained also with safranin, which, when carefully employed, affects only the chromatin, it will be observed that all the elements coloured by the safranin exhibit the blue reaction also, the combination of the red and the blue giving to the chromatin a colour of a violet shade (figs. 46 and 48).

It is not, however, the chromatin alone in the animal nucleus that possesses assimilated iron, for one sees in sections exhibiting the Prussian blue reaction, but more readily in those which have been also stained with safranin, that nucleolar elements possess a light blue colour (figs. 45 and 48 *a*). Some difficulty is experienced in observing this under ordinary conditions, but this is overcome, when homogeneous immersion apochromatic objectives are employed, by withdrawing the diaphragm of the Abbé condenser, the great amount of light thus transmitted causing all the blue parts to appear with remarkable distinctness, and amongst these the nucleolar bodies coloured light blue, while all the other elements are rendered indistinct or invisible. When, however, safranin has also been employed to stain such preparations, the chromatin absorbs it but the nucleolar elements are absolutely unaffected by it, and they thus stand out in marked contrast with the other structures. Such nucleolar bodies take but a faint stain with hæmatoxylin, a fact which, considered in connection with the result of the employment of safranin, would seem to demonstrate that they are not essentially formed of what the cytologist comprehends under the term chromatin. The number of these in a nucleus varies, and the shape and size of each are not constant, while not unfrequently the central portion appears free from iron, the outer or peripheral part, coloured light blue, appearing as an envelope of greater or less thickness for the uncoloured part (fig. 46, *a* and *d*). These bodies are always attached to the chromatin network, and sometimes there appears about them a membrane derived from, and continuous with, the fibrils with which they are connected. This is very distinctly seen in the safranin preparations, the membrane in this case exhibiting a combination

of the blue and red reactions, and thus appearing in sharp contrast with the enclosed nucleolar body coloured light blue.

It is chiefly in the nuclei of the glandular cells that one finds these nucleolar bodies, and they are most distinctly seen in large nuclei, as, for example, those of hepatic and renal cells and of the intestinal epithelium of Necturus lateralis. They are very rarely seen in the nuclei of the muscle fibre and in those of the cutaneous epithelium of the same animal, while they are never present in those of leucocytes or lymph cells, or in those of the red blood-corpuscles. In the search for them in all these elements the greatest assistance is obtained from the employment of eosin, which, in sections exhibiting the Prussian blue reaction, gives these bodies an ochre-red colour, while the parts showing a dark-blue reaction are unstained by it (fig. 47). In the nucleus of the glandular cell which is passing into the mitotic phase, the nucleolar body disappears, apparently by solution into the chromatin threads, for in the nucleus of a renal cell, in which the meridional disposition of the chromatin filaments obtained preparatory to the formation of the loops, I saw, attached to one of the filaments and partly embraced by its substance, what appeared to be the remains of such a body. In later stages of mitosis not the slightest evidence of this body or of its remains can be observed.

Whether the iron which these bodies contain is that of a small quantity of chromatin dissolved in them, I am unable to say. The fact that they take sometimes a very feeble stain with hæmatoxylin, seems to indicate that they may contain a small amount of chromatin. The iron in them is held neither more nor less firmly than in the typical chromatin elements, since in hepatic nuclei containing them, prolonged treatment with ammonium hydrogen sulphide in the warm oven does not result in demonstrating any difference, except in the amount of iron in the one and in the other. The substance which holds the iron does not possess the slightest affinity for safranin, but attracts eosin as no other cellular constituent does, and in these properties, as well as in the

very small amount of iron present, there would appear to be distinctions which separate it from chromatin. My preparations were chiefly obtained from the organs of the fasting animal, and as I did not succeed in my attempts at feeding artificially some examples of Necturus that I had, it is not possible for me to say whether the constitution of the nucleolar bodies is always similar to, or ever different from that described; but in preparations of the liver and other organs of specimens of Amblystoma punctatum killed soon after their capture, or after artificial feeding, the nucleolar bodies appeared to present the characters noted in the cells of the fasting animal, the smaller size of the elements in this case, however, not allowing as clear a view of them as was desired.

In the nuclei of the liver-cells of Necturus, as illustrated in preparations made after the manner described, I frequently found a third element, whose significance is unknown to me. It manifested itself by the red stain which eosin gave it, the nucleolar bodies taking, in contrast, an ochre-red stain. It had no constant shape or form, in some cases being of a filamentous character, in others resembling a localised granular deposit (fig. 47); and when the structures were filamentous, several usually appeared in the same nucleus. The substance forming them did not contain the slightest trace of iron, and therefore appeared to have no relation to the nucleolar bodies or to the chromatin. I have not in any other organ observed similar structures.

The disposition of the iron-holding compound in the nuclei of Amphibian ova deserves special mention. In the ovarian ova, whose nuclei contain no peripheral nucleoli, the iron is distributed as represented in fig. 36, the chromatin in this case forming a fine reticulum, in the trabeculæ of which large granules are found with lateral prolongations. The iron demonstrated in this preparation was set free by sulphuric acid alcohol, but a disposition of iron in the main like this may be found in similar nuclei when the latter are, on removal from the ova, broken into small pieces on the slide in the glycerine and sulphide mixture, and, thus prepared and provided

with a cover-glass, kept for days in a warm oven. This method must be resorted to in order to get the iron reaction, since otherwise the large nuclei may be kept for a month in contact with the reagent in the warm oven without resulting in demonstrating, in the slightest, any iron reaction. In the peripheral nucleoli, when they are present, the amount of the iron, as indicated by the depth of the reaction, is great, but in the remaining elements of such nuclei it is small. When such preparations are examined with a strongly magnifying objective, the chromatin network, as revealed by the iron reaction given, is found to be less distinct, and instead of granules of iron-holding substance arranged at definite positions along the course of the fibrillæ, as in ova much less developed, the iron is now seen to be chiefly confined to beadlets, few in number, sometimes regularly, sometimes irregularly, disposed on the fibrillæ, which, in ammonium hydrogen sulphide preparations, manifest but a feeble greenish tint. There is an inverse relation between the size of the nucleoli and the amount of the chromatin in the network, and an examination of some nuclei in which the formation of the peripheral nucleoli has commenced, and of those in which the development of these bodies is much more advanced, irresistibly suggests that the latter are derived from the chromatin of the network. I have elsewhere[1] pointed out that the solution of the substance of which these nucleoli are composed and its diffusion from the nucleus into the cytoplasm of the ovum are connected with the formation of the yolk-sperules in Amphibia. That a solution of the peripheral nucleoli takes place has been noted by O. Schultze[2] and Born.[3] Schultze found that with the solution of the nucleoli (keimkörperchen) the contents of the nucleus and the substance surrounding the latter were affected in the same way by reagents and staining fluids, and he believed that the dissolved sub-

[1] 'Transactions of the Canadian Institute,' Toronto, vol. i, part 2.

[2] "Untersuchungen über die Reifung und Befruchtung des Amphibieneies," 'Zeit. für wiss. Zool.,' vol xlv, p. 177.

[3] "Die Struktur des Keimbläschens im Ovarialei von Triton taeniatus," 'Arch. für Mikr. Anat.,' vol. xliii, p. 1.

stance diffused from the nucleus of the ovum into the cell-body. Born observed that the nucleoli are always placed as closely as possible to the cell protoplasm, while the chromatin in the development of the nucleus and ovum becomes so finely divided in the karyoplasm that it is stainable with great difficulty, and it is as difficult to demonstrate optically, a condition which continues till the formation or deposition of the yolk-grains (Dotterkörner) commences. In later stages the persisting peripheral nucleoli lose their capacity for absorbing colouring matters.

In support of these observations of Schultze and Born, I may but add that the iron in the cytoplasm of the ovum makes its appearance only after the solution of the peripheral nucleoli commences. The substance forming the peripheral nucleoli does not react with staining reagents as does the chromatin of the nuclear network, and especially with the indigo-carmine staining fluid of Shakespeare and Norris the resulting stains of each are different, the chromatin of the network being coloured red while the nucleoli are stained blue or green, the latter colour obtaining also in the yolk-spherules of such preparations. A further difference is noticeable in the effect that ammonium hydrogen sulphide exercises when applied to these structures for some time at an elevated temperature. In this case the iron of the peripheral nucleoli reacts more readily than that of the chromatin of the network, but less readily than that of the yolk-spherules, which in the ova of Necturus and Amblystoma give a green reaction in a few minutes with the reagent. It would appear as if the iron compound undergoes a change in its transference from the nucleus to the cytoplasm.

The peripheral nucleoli appear to be formed at the nodal points of the chromatin network, if one may judge from preparations of which fig. 34 is an illustration; but there is a possibility that these represent a pathological condition, since they are not common in the ovary when, if they were normal, they should be present in larger numbers. I have, moreover, found that they were accompanied by examples of

another condition which I regard as pathological. In the latter the nuclei were indistinct or disintegrated, their chromatin had disappeared, and the surrounding connective tissue, with its blood-vessels and their red corpuscles even, gave in a few minutes, with warm ammonium hydrogen sulphide, an iron reaction, frequently so deep as to obscure largely the details, while the tissues, a little further away from such examples, and other ova under exactly the same conditions of treatment with the reagent, gave no such reaction. It may possibly be that the chromatin of such disintegrated ova furnished the iron observed thus diffused in the connective tissue and blood-vessels.

In the nuclei of all the higher vegetable organisms the assimilated iron compounds are, on the whole, distributed as they are in the nuclei of the more highly developed animal forms, a fact which may be demonstrated in any Phanerogamous plant, especially readily if its nuclei are large, as is the case in Erythronium americanum. In many of the preparations of the latter form the chromatin filaments were, in the process of teasing-out, partially or almost wholly set free from the nuclei containing them, and to the parts thus set free, as well as to the remainder, the glycerine and sulphide mixture always gave a distinct reaction for iron in a few days (fig. 17). Mitotic figures in such preparations appeared very sharply defined through the iron thus revealed in the chromatin elements. In successful preparations made by this method the reaction for iron is very marked, as much so as in those made with sulphuric acid alcohol; and in this respect there is a contrast between animal and vegetable nuclei, for in the former the glycerine and sulphide mixture brings out, after a longer application and less frequently, a reaction as intense as that which may be obtained after treatment of the nuclei with acid alcohol.

Of nucleoli and nucleolar bodies there are at least three kinds. The reaction for iron given in one variety by the glycerine and sulphide mixture was weak, and it was obtained at the same time that it appeared in the chromatin network or filaments. These are smaller, apparently, in the hardened

than they are in the living cell, for, as a rule, they only partially occupy the cavity in which they lie (figs. 17 and 19). I have in some cases isolated them from their nuclei in the glycerine and sulphide mixture, and the greenish reaction which they gave could, therefore, not have been due to the iron of a compound which diffused from the chromatin elements into them. When the sections were treated with sulphuric acid alcohol and subsequently with the acid ferrocyanide mixture and the eosin solution, the result was usually that of which fig. 42 is a representation. These nucleoli stain intensely with eosin, which also colours very slightly the chromatin network, the blue of the latter thereby becoming violet, but after being thoroughly washed in alcohol the bright blue colour returns; while this treatment makes no difference in the intensity of the stain in the nucleolar bodies. These effects are most distinctly observed when the diaphragm of the Abbé condenser is removed from the field, in which case it is possible to see the most minute of the nucleolar elements, a device that is necessary when the nuclei of ordinary parenchyma cells are under examination.

In the second class are those nucleolar elements which may be found in the cells of the nucellus, and which are composed of chromatin, since they give a deep iron reaction after the employment of any of the methods of treatment for liberating the element, and since, also, they stain in every respect like the chromatin threads. They usually occupy cavities in the nuclei like those which contain the eosinophilous nuclei last described. I regard these as reserve masses of chromatin deposited in the nuclei engaged in the formation of chromatin, which eventually is transferred to the cells of the endosperm. To this subject I propose to refer again.

Nucleoli of the third class are to be found in the nuclei of the embryo-sac (fig. 44, *a* and *b*). They are not present in the mitotic nucleus, but in the retrogressive stage they appear on the course of the filaments as spherical elements enclosing one or more refracting corpuscles and containing but a small amount of iron, which, however, in later stages, when the fila-

ments became thinner and less rich in chromatin, is more abundant. These nucleoli are eventually formed chiefly of chromatin, and in stained preparations appear to contain nearly all the chromatin of the nucleus. When mitosis again commences the filament forms at their expense, the increase in size of the filament keeping pace, apparently, with the decrease in the quantity of chromatin which the nucleoli contain. Finally, before their disappearance, when they contain but a minimal quantity of iron, they take the eosin stain deeply.

All these forms of nucleoli take up safranin from solutions as readily as do the chromatin elements in the same nuclei, and they hold the stain as tenaciously when they are washed with alcohol. They are in this respect different from the eosinophilous nucleoli in the animal cell, which appear to be unrepresented in the vegetable cell.

Of an exceptional character are the nucleoli in Corallorhiza multiflora and in Spirogyra. In these the greater portion of the chromatin in each nucleus forms a single large spherical element unconnected with the chromatin network, which after prolonged treatment with the glycerine and sulphide mixture, gives a pronounced reaction for iron.

I have, on a few occasions only, in preparations illustrating the iron reaction, seen the chromatin localised at points along the course of the filament, and concluded that this was not due to faulty methods of manipulation, for hæmatoxylin and other dyes just as infrequently render such a distribution visible. It was also, with the aid of the acid alcohols, found that in the loops of the mitotic nucleus of the embryo-sac the chromatin is disposed under the membrane enclosing the filament, in such a way as to make the latter appear as a tube of chromatin.

In some of the elongated oval nuclei of the nucellus and of the fibro-vascular bundle of the ovule, Mr. Bensley has observed a point of some interest. This consists in the occurrence in the karyoplasm, amongst the trabeculæ of the chromatin network in one end of such a nucleus, of an iron-holding compound with all the characters of chromatin, and,

in some cases, in such abundance as to obscure the outlines of the trabeculæ. He has found that in the fibro-vascular bundle this end of the nucleus is directed toward the base of the ovule, and is of the opinion, as a result of some investigation of this subject, that the phenomenon in question is connected with the processes of the formation of chromatin, which he regards as taking place here.

The presence of assimilated iron, apart from its occurrence in hæmoglobin and hæmatin, is an exceptional feature in the cytoplasm of the cells of the higher forms of animal life, but the exceptional instances are themselves of a constant character, and comprise, in addition to yolk-holding ova, the cells of yolk-holding embryos, the hæmatoblasts of Vertebrates, and the ferment-forming gland-cells of all descriptions.

The iron in the yolk of Amphibian ova is held in the yolk-spherules, which manifest a strong affinity for dyes, and are usually homogeneous in composition. These give with ammonium hydrogen sulphide a dark green reaction, which makes its appearance sometimes in a few minutes, but at the latest in a few hours, when the preparation is kept warm. The reaction is uniform throughout each spherule. The enclosing cytoplasm does not, before development of the ovum begins, contain any assimilated iron; but in the developing embryo, with the multiplication of the cells and the partition of the yolk, the spherules gradually undergo solution, for they become smaller in size, and then one obtains an iron reaction in the cytoplasm of each cell. The solution of the yolk-spherules may be studied also in preparations made with the carmine-indigo-carmine fluid, the reagent giving, in the earliest stages of the embryo, a green colour to the yolk-spherules, and a red stain to the cytoplasm and nuclei; but in later stages the red colour is rarely obtainable, and both cell and nucleus, the latter especially, are coloured blue-green or dark green. This result is brought about by the solution of the yolk-chromatin in each spherule and the diffusion of the dissolved substance through the cytoplasm and nucleus of each spherule-holding cell, for in those examples of larval Amblystomata which yield pre-

parations giving a dark green or blue-green colour in cell and nucleus after treatment with the reagent mentioned, the cells are found, after the prolonged application of the glycerine and sulphide mixture, to exhibit an iron reaction in the cytoplasm apart from the spherules, and a similar reaction diffused in the karyoplasm independent of that manifested by the chromatin network, the intensity of the reaction corresponding in each case to the depth of the green stain given these elements by indigo-carmine reagent. When in the advanced development of the larval Amblystomata the yolk-spherules disappear from the cytoplasm of the cells, the nuclei and all cells, except those undergoing transformation into striated muscle, lose their capacity for absorbing and retaining the indigo of the fluid of Shakespeare and Norris. This indicates that the yolk chromatin is changed into some other compound, and the prolonged application of the glycerine and sulphide mixture confirms this, for the cytoplasm, except in secreting cells, the striated muscle-fibre, and in the hæmatoblasts and red corpuscles, is destitute of iron compounds, while the nuclei give, much more slowly, and apparently with greater difficulty, a reaction for iron which is, in contrast with what is observed in the earlier stages, confined to the nuclear network and nucleoli. The iron-containing substance is transferred to the nuclei, and with this transference the iron becomes more firmly combined—a process the very reverse apparently of that which is illustrated in the formation of the yolk-spherules, for the iron compound of the latter, though derived from the nucleus of the ovum, is less firmly combined than that of nuclear chromatin giving origin to it.

The yolk-spherules of the hen's egg, as is well known, have characters differing from those of Amphibian ova, but the most marked difference consists in the distribution of the iron-containing compound. The yolk-spherule in the ova of Amblystoma and Necturus is homogeneous, and the iron compound is uniformly distributed through it; but in the hen's egg elements of this character are to be found only in the constituents of the "white" yolk and in some of the "yellow" spherules in

the most peripheral layers of the yolk, while in all the other spherules the distinctive feature is the disposition of the iron compound in a finely granular form. This cannot be determined with fresh yolk, for when treated with ammonium hydrogen sulphide the greater part of it dissolves, and the solution becomes dark green owing to the formation of sulphide of iron. Under the microscope no formed elements can be observed in such a preparation, except those derived chiefly from the "white" region, and it is not possible to ascertain, under these conditions, the relations of the iron-holding nuclein in other parts of the yolk. Another difficulty experienced in dealing with fresh uncoagulated yolk is that, when removed from the egg the spherules disintegrate, the granular contents escaping and obscuring more or less the characters of the other elements. To avoid this the substance of the spherules must be coagulated, and to accomplish this satisfactorily I placed the eggs in boiling water for ten minutes. The spherules were thus fixed in polyhedral form, and, after these had lain in strong alcohol for several days, it was an easy matter to determine the distribution of the iron in them.

The results obtained were according to the variety of spherules examined. In those known as "white" the reaction for iron was very distinctly obtained, but it was wholly confined to their homogeneous spherical bodies. The reaction is, immediately after the application of the glycerine and sulphide mixture, light green, but this becomes deeper after a few days, when the preparation is kept at a temperature of 60° C. The homogeneous elements undoubtedly contain a quantity of nuclein, for they resist the action of artificial gastric juice and dissolve in weak alkalies, while they constitute the only part of the "white" spherules that possesses, like chromatin, the property of absorbing and retaining colouring matters. This was found to be the case specially when the spherules, coagulated by heat, were further treated with Flemming's chrom-osmio-acetic mixture for twenty-four hours, then with alcohol, and finally with a solution of safranin. When the excess of the stain was extracted with alcohol and the spherules mounted in

balsam, it was always found that the spherical elements exhibited an intense stain, while the remaining parts of the spherules were absolutely uncoloured. I found it possible to demonstrate this and the reaction for iron in the same preparation. When the "white" spherules, fixed with heat, were kept in slightly warm sulphuric acid alcohol for twenty-four hours, their spherical elements gave, on treatment with an acid ferrocyanide solution, a Prussian blue reaction, and, when subsequently stained with a safranin solution, became violet. These results show how close is the relationship between the substance composing the spherical elements and chromatin.

A few of the spherical elements in the "white" spherules are not of the character described, for in preparations made with Flemming's fluid one finds, now and then, a spherule in which one or more large droplets of fat are demonstrated by the intensely black reaction of the osmic acid. Apart from the occurrence of these, there is comparatively little fat in the "white" spherules, a fact strikingly shown when a thin section of the hard-boiled yolk, embracing portions of the "white" and "yellow" zones, is submitted to the action of the reagent for twenty-four hours, the "white" then exhibiting a greyish appearance, while the "yellow" area is almost black.

The "yellow" spherules are also richly supplied with the iron-containing compound, but this is quite differently distributed from what it is in the "white" zone. The appearances of these are subject to a great deal of variation. Some contain only large round granules, in others the granules have a punctiform character, while in others again both kinds of elements may be mingled with minute fat droplets. Owing to differences in the specific gravity of the constituents apparently, the granules may be found, in some cases, to be gathered in one portion of the spherule, the remainder of the contents being occupied by a clear, non-granular substance of a firm consistence, a character resulting from heat coagulation. It is in such spherules as these that one determines distinctly how the iron compound is disposed, for, in those in which the granules are uniformly distributed, it is sometimes exceedingly difficult

to decide whether the iron is contained in the granules or in the extra-granular substance, so intimately are these usually intermingled. The granules, whether of the large or of the punctiform variety, always contain an iron compound, while the substance in which they are shown is destitute of this element. In demonstrating this fact the acid alcohols are of the greatest service, the glycerine and sulphide mixture, owing to the large size of the vast majority of the spherules, not being as effective in liberating and demonstrating their iron, but in the smallest spherules the complete reaction may be obtained with the mixture in four or five days. In those spherules which contain, as described, granular and non-granular portions, the granules, closely aggregated as they usually are, appear very prominent by reason of the reaction for iron which they give with both methods of demonstration.

In some of the "yellow" spherules also, after treatment with sulphuric acid alcohol, vesicles of different sizes were observed, each of which appeared to be enveloped by an iron-containing membrane-like structure. Their position near the centre of the spherule often rendered the occurrence of iron in the envelope obscure, owing to the light passing through so many iron-holding granules above and below these vesicles. What the latter contained it is not possible to say, for although fat globules of a similar size can be demonstrated in some spherules when these are subjected to the action of the chrom-osmio-acetic mixture for twenty-four hours, it cannot be demonstrated that the two classes of structures are connected in any way.[1] The difficulty lies in the fact that in order to show the occurrence of iron in the envelope, alcohol in some form must be used, and by this the fat is largely, if not wholly, removed; while in those spherules treated only with osmic acid solutions the black reaction of the globules prevents a demonstration by the Prussian blue reaction of any iron present.

[1] In another paper ("On the Absorption of Iron in the Animal Body," 'Journ. of Physiol.,' xvi, 1894, p. 268) I expressed the view that these vesicles contain fat. After a more extended study of these elements than I was able to make before that paper was published, I am doubtful of this interpretation of their structure.

Apart from the question of the occurrence of fat in such elements, there may be no doubt about the intimate association of the iron-containing substance and the fat in the spherules. Owing, however, to the size of the latter, as well as to the density of the coagulated material in them, the osmic acid used to demonstrate the fat penetrates but slowly, and when, as usually happens, fat droplets stud the periphery of the spherule, little or none of the reagent reaches its interior, which then has only a straw-yellow colour. If, however, a few spherules, coagulated with heat, are kept in a quantity of Flemming's fluid for twenty-four hours, the osmic acid penetrates the spherules in some cases and causes their granules to become brownish-black, a fact which can be most distinctly observed when the cover-glass is pressed down sufficiently to disintegrate the spherules and set the granules free. If the granules are large, the occurrence of fat in them is much less readily demonstrated, possibly because the density of such elements prevents penetration on the part of the osmic acid.

These granules are undoubtedly the source of the greater part of the iron-holding nuclein isolated by Bunge from the yolk,[1] since the "white" yolk is comparatively small in amount. Miescher[2] regarded the nuclein, which he separated from the yolk, as only in part localised in the homogeneous spherical elements in the "white" portion, and he believed that the greater part of it was derived from the granules in the "yellow" spherules, and that none of it exists in a dissolved form, a conclusion fully supported by the facts concerning the localisation of the iron.

In describing the transference of the chromatin of the spherules from the cytoplasm to the nucleus of each cell of the larval Amblystoma, reference was made to an exception in the case of developing muscle-fibre. In the cells undergoing transformation into striated fibres, some of the chromatin dissolved in the cytoplasm finds its way into the nuclei as in other

[1] "Ueber die Assimilation des Eisens," 'Zeit. für Physiol. Chemie,' vol. ix, p. 49, 1885.

[2] "Die Kerngebilde im Dotter des Hühnereis," 'Hoppe-Seyler's Med.-Chem. Untersuchungen,' 1871, p. 502.

cells generally, but the greater part appears to remain in the cytoplasm of the developing fibre, and undergoes a transformation which is one of great interest in connection with the origin of hæmoglobin. In the cytoplasm of the muscle-cells there is an abundance of yolk-spherules which, as in other cells, gradually undergo solution, the dissolved substance diffusing through the cytoplasm. When the striation makes its appearance at one side of the now elongated cell, the dissolved substance passes into the striated area, for ammonium hydrogen sulphide brings out an iron reaction in this part as readily as in the undifferentiated cytoplasm and in the spherules, but confined to the dim bands, the light bands giving no evidence of the presence of the compound. In the fibre from which the spherules have all but disappeared, and in which the striated area embraces nearly the whole of its width, the reaction with ammonium hydrogen sulphide is as distinct and as marked as in the earlier stage, and this is true also of the fibre in its final form. In this stage the iron is quickly liberated by acid alcohols, as well as by ammonium hydrogen sulphide, and its presence may be readily demonstrated by means of these reagents up to the period when all traces of yolk disappear from the cells of the larvæ. After this date the iron compound becomes firmer, or, to speak more accurately, is less readily attacked by acid alcohols or the sulphide reagent, and in the muscle-fibre finally its presence may not be shown by these methods. It is not that the iron is removed from the fibre, but that the compound containing it is transformed, in red muscles, into what is called myo-hæmatin by MacMunn, or hæmoglobin by Hoppe-Seyler and others. The latter compound can, by means of the staining fluid of Shakespeare and Norris, be clearly shown to be strictly confined to the dim bands, which are given a grass-green colour distinctive of hæmoglobin, while the light bands and nuclei are coloured red.[1]

[1] I have pointed out the value of the reagent in this respect in my paper entitled "Studies on the Blood of Amphibia," 'Transactions of the Canadian Institute,' vol. ii, 1893.

A similar conversion of a compound in which the iron is easily attacked by ammonium hydrogen sulphide and by acid alcohols into one from which the liberation of the iron is more difficult, obtains in the dim bands of muscle-fibre in Invertebrates (Oniscus, Chironomus, Musca), but in this case the transformation does not proceed as far as the production of hæmoglobin or myo-hæmatin, if one may judge from the absence of pigment and from the fact that the liberation of the iron, though difficult, is possible, while in the case of hæmoglobin the use of acid alcohols and of ammonium hydrogen sulphide is ineffective for that purpose.[1]

In the development of the blood-corpuscles in the larvæ of Amblystoma there is, as I have pointed out,[2] a conversion of the chromatin of the hæmatoblast into hæmoglobin, a change that is analogous to that described above as occurring in muscle-fibre. In hæmatoblasts, however, the chromatin so transformed is not directly derived from that of the yolk-spherules, as is the case in muscle-fibre, but from that compound after it is transformed into nuclear chromatin. This is very distinctly seen in sections through the aortic arches of the larvæ, which have been treated with acid alcohol to liberate the iron. In the concave side of the arches are seen hæmatoblasts in all stages of division, and in these one may, by the iron reaction, differentiate between hæmatoblasts in which there is no cytoplasmic chromatin, and those in which the cytoplasm between the chromatin loops of the mitotic figure contains dissolved chromatin to an extent varying with the example of hæmatoblast noted. This cytoplasmic chromatin does not act in the same way as ordinary nuclear chromatin does towards staining reagents, as, for example, hæmatoxylin, eosin, and safranin,

[1] The fact that ammonium hydrogen sulphide will liberate the iron from hæmatin in solution, while it does not attack the iron in the compound called myo-hæmatin by MacMunn, indicates that the latter cannot belong to the hæmatin class. Its property in this respect shows that it is related to hæmoglobin. The name given to it by MacMunn certainly appears to be a misleading one.

[2] Loc. cit.

although it has an affinity for them, and it persists with this character for a long time after the stage of the hæmatoblast is passed. I have found that in a large number of the fully-formed red cells in the spleen of the larva of 35 mm. length the disc contains a quantity of the modified chromatin, and from this the iron is readily liberated, but in later stages both the number of such corpuscles and the amount of iron in the disc which may be liberated by acid alcohols gradually diminish and disappear, the hæmoglobin of the disc not yielding its iron on the employment of such methods. The nuclear chromatin, however, of all stages of the corpuscle, readily gives up its iron, even when none can be set free in the disc.

It thus appears that the hæmoglobin of the red corpuscles and the analogous compound in muscle-fibre are formed in the same way, the only difference obtaining between them existing in the fact that the pigment of muscle-fibre does not, in its evolution in the developing ovum, comprehend a stage of nuclear chromatin. The process by which they are formed is a gradual one, and the position of the iron in the molecule is apparently changed. The latter result may be partly accounted for if we consider the composition of chromatin and of hæmoglobin. Chromatin is an iron-holding nucleo-albumin in which the iron is attached to the nuclein, while in hæmoglobin the iron is held in the hæmatin molecule, and in the transformations which result in the formation of hæmatin out of nuclein, it is but natural to expect that the relations of the iron to the molecule should change also.

In secreting cells, as, for example, those of the parotid, Lieberkünian, and pancreatic glands, a certain portion of the cytoplasm gives evidence of the possession of "masked" iron. When the cells of the pancreas of an adult Amblystoma are, after hardening in alcohol, subjected to the action of the glycerine and sulphide mixture for six or seven days at a temperature of 60° C., in addition to the reaction for iron obtained in the nucleus, one is found in the cytoplasm of the so-called "outer zone," in some cases almost as marked as in the nuclear chromatin. The extent of the cytoplasm involved in the reac-

tion in all the specimens which I examined varied considerably, whether according to the stage of secretory activity could not be determined after the use of ammonium hydrogen sulphide, for this reagent, in a day or two at an elevated temperature, causes the zymogen granules to disappear; but in sections of the pancreas from the same animal, after these had been acted on by sulphuric acid alcohol, then with the acid ferrocyanide solution and eosin, the iron-holding area in each cell was demonstrated by the resulting Prussian blue, while the zymogen granules were given an intense red stain, and in this case it was found that, apart from the granular zone, the cytoplasm was uniformly blue. In other conditions of activity the iron-holding area was increased or decreased in correspondence with the decrease or increase in the extent of the granular zone. In the exhausted condition of the gland-cell, that is, in which there were but few granules, arranged in the "border" fashion near the lumen of the tubule, the whole of the cytoplasm exhibited the blue reaction, but the latter was less marked than when it was confined to a narrow zone in the neighbourhood of the nucleus. The relations of the extent of the iron-holding area to the stage of secretory activity were less easy to determine in the Lieberkühnian and parotid glands, for it is not possible to demonstrate the mucigen in the former or the zymogen in the latter as prominently as the zymogen granules may be in the pancreas, but in these the iron-holding area appeared in all cases to correspond, in the main, with the "protoplasmic" or "outer" zone. In the "mucous" cells of the submaxillary gland of the cat and dog only a narrow zone of cytoplasm about the shrunken nucleus contains iron, but in the large crescents of Gianuzzi in the cat the whole of the cytoplasm is iron-holding. In the peptic tubules of Amblystoma the cytoplasm in the outer half of each cell contains iron, and this is also true of the chief cells in the cardiac portion of the stomach in the dog and cat. In the parietal cells in these animals the cytoplasm is absolutely free from iron. The iron-holding zone in each chief cell appears to vary in extent with the stage of secretion, but I am unable to speak

as definitely upon this as upon the relations, in this respect, observed in the pancreatic cells of Amblystoma, for I have not been successful in my efforts to obtain, from examples of the latter animal, preparations of the gastric glands illustrating marked variations in the stages of secretory activity, and have had to rely upon those made from the cat and dog, in which the chief cells are comparatively small and less favorable for observation on this point.

It is only in the mucous glands of the skin of Amphibia, and in the renal tubules of Vertebrates generally, that I find exceptions to the rule that glandular secretion is associated with the presence of an iron-holding cytoplasm. I have not found any exceptions in Invertebrates to this generalisation, but my observations have not been comprehensive enough on this point, and I must speak with some reserve in regard to it. In the Protozoa, as I will show further on, the presence of assimilated iron in the cytoplasm seems to be a constant feature, the iron not being confined to any part of the cell, but uniformly distributed through it, and there is a probability that this cytoplasmic iron-holding compound is also associated with the secretion of ferments functioning in the digestion of the ingested food. In the glands named above, which are mentioned as exceptional instances, the absence of assimilated iron from the cytoplasm may be explained on the ground that the secretory process of a renal cell is widely different from that of a pancreatic cell, the cytoplasm in the latter, but not in the former, elaborating a portion of its own constituents to furnish the secretion, whereas in the renal cell the process is largely one of transference only. If the explanation should hold in all possible cases of exception, then it would follow that the iron-holding compound is an important element in the elaboration of the zymogens. I have elsewhere [1] pointed out the relations that obtain between the chromatin of the nucleus and of the cytoplasm of the pancreatic cell, on the one hand, and the formative process resulting in the production of zymogen on

[1] "Contributions to the Morphology and Physiology of the Cell," 'Trans. Canadian Institute,' vol. i, part 2, p. 247, 1891.

the other; and so intimate did these relations appear that I was led to apply the term prozymogen to the chromatin. I have found, as a result of experiments on the active pancreas of Amblystoma, that the zymogen granules under certain conditions give an iron reaction. When the organ, hardened in alcohol, is put in a quantity of Bunge's fluid, and the preparation kept at the temperature of the room (20° C.) for a week, or when it is kept for two days in a quantity of sulphuric acid alcohol, teased-out portions, after the removal of the acid and on the addition of ammonium hydrogen sulphide, give preparations of which that represented in fig. 38 is an illustration. The zymogen granules give a greenish reaction, the colour making them more prominent than the other elements in the cells. The cytoplasm of the "outer zone" gives but a feeble iron reaction, and this appears only to a minor extent in the nuclear elements, both results being caused by the lessened action and feeble extractive capacity of the acid alcohols when used on the tissue in mass. When the reagents are used for longer periods than those specified the iron disappears from the zymogen granules, while it becomes more strongly marked in the nuclear elements and in the cytoplasm of the "outer zone." Owing to the effect that ammonium hydrogen sulphide exercises on the granules, causing them to dissolve or disintegrate, an effect already referred to above, it is not possible to control the results obtained with the acid alcohols by experiments with this reagent, and one may, therefore, not regard the presence of iron in the zymogen granules as conclusively demonstrated, since it may be urged that the iron reaction which they gave was due to the iron which diffused into them from that liberated in the other cellular elements. When one remembers, however, the fact that the zymogen is elaborated in a cytoplasm which is iron-holding and at its expense, the occurrence of a faint reaction for iron in the granules after the use of acid alcohols is best explained by the view that the zymogen of the pancreas contains iron, and that its antecedent, the prozymogen, is the iron-holding constituent in the cytoplasm of the "outer zone."

In the rods and cones of the retina in Amblystoma and Necturus an iron reaction was frequently obtained like that represented in fig. 37. It was always feeble and confined to the trabeculæ, which stretched across the long axis of the rod, or which formed the network in the cones. In some cases (as in fig. 37, *a*) pigment-granules were observed attached to the rods, probably derived from the cells of the tapetum nigrum, and as the pigment probably contains iron, it is uncertain whether the iron demonstrated in the rods and cones was not derived by diffusion of some iron-holding substance from this source.

The eleidin granules in the stratum granulosum in the human skin give, after treatment of sections of the epidermis with sulphuric acid alcohol, a dark green reaction with ammonium sulphide. I have not succeeded in obtaining a reaction for iron in them when the containing cells, hardened in alcohol, were simply subjected to the prolonged application of the glycerine and sulphide mixture in the warm oven. Since the chromatin of the nuclei in the underlying stratum mucosum is, as elsewhere, iron-holding, while the nuclei in the stratum granulosum are poor in chromatin, it is not improbable that the iron, at least of that part of the latter which disappears from the nuclei, is the source of the iron shown in the eleidin granules. The homogeneous substance constituting the stratum lucidum also gives a reaction for iron, which is diffuse and less marked than in the granules of the underlying layer.

In my observations on preparations of the human thyroid and of that of the dog, although it was easy to demonstrate the presence of iron in the nuclear chromatin, and to a certain extent in the cytoplasm of the cells lining the alveoli, I did not succeed in finding any of it in the "colloid" matter. Under certain conditions this substance absorbs staining matters, and it also gives[1] the molybdate-pyrogallol reaction of Lilienfeld and Monti.[2] These facts suggest that the colloid

[1] F. Gourlay, "The Proteids of the Thyroid and the Spleen," 'Journal of Physiology,' vol. xvi, p. 23, 1894.

[2] "Die mikro-chemische Lokalization des Phosphors in den Geweben," 'Zeit. für Physiol. Chemie,' vol. xvii, p. 410, 1893.

substance is allied to nuclein, and, according to Gourlay, the nucleo-albumin which he isolated from the thyroid was derived in large measure from the colloid matter which he, relying on the reaction of Lilienfeld and Monti, found to contain phosphorus. If colloid matter is therefore a nucleo-albumin, its freedom from iron renders it, in contrast with the chromatins, a subject of special interest.

Assimilated iron is rarely found in the cytoplasm of the cells of the higher vegetable organisms, and amongst the examples illustrating its presence may be mentioned the cells of the nucellus in the ovules of Erythronium americanum, and those of the gluten layer in the wheat-grain. The cytoplasm of the cells of the nucellus, when fertilisation has taken place, and even before this occurs, gives, after treatment with sulphuric acid alcohol, a distinct reaction for iron, which, however, in respect to intensity, is not to be compared with that manifested in the nuclei of the same cells. The iron in the cytoplasm in this case is not due to diffusion from the nuclei during the course of treatment with the liberating reagent, for it is also demonstrated in this situation in the glycerine and sulphide preparations. As the nuclei of the nucellus are much richer in assimilated iron than those of other parts of the ovule, except the embryo sac, it is possible that the cytoplasmic iron compound is intra vitam diffused from the nuclei, and, further, as the cytoplasm of the embryo-sac of this stage sometimes gives a diffuse reaction for iron after it has been treated with acid alcohols, its presence here may be due to a similar diffusion from the cells of the nucellus. I have observed in certain preparations in which the nuclei of the embryo-sac were in the stage of division, a large number of iron-containing granules interspersed amongst the fibrils of the achromatic spindles, and as in other preparations similar granules were stained with hæmatoxylin, like the chromatin loops, it would appear as if the granules were formed of chromatin. The cytoplasm holding these granules gave no reaction for iron.

The cytoplasm of the cells of the gluten, or so-called aleu-

rone layer (Kleberschicht) in the wheat-grain is richly supplied with a "masked" compound of iron. In some cells it is chiefly found in the large granules strewn through the cytoplasm; in others, again, apparently it is wholly contained in the latter; while in certain instances, further, it was demonstrated only in the extreme peripheral portions of the large granules. This is most clearly shown in sections of the grain after they have been treated with sulphuric acid alcohol for twenty-four hours at a slightly raised temperature. When the individual cells of other sections are treated with the glycerine and sulphide mixture for several days the reaction for iron is readily obtained in their cytoplasm, but its localisation, as observed after the use of the other method, is thus less readily determined. The "masked" compound apparently belongs to the class of chromatins, for when sections are treated with the ordinary staining reagents the cytoplasm stains deeply, especially with safranin and hæmatoxylin, and the parts which are specially affected are those which correspond with the iron-holding structures in preparations treated with acid alcohols.

Haberlandt[1] has made experiments upon the question of the site of origin of the diastase in the germinating rye-grain, and these appear to show that the ferment is elaborated in the cells of the gluten layer only. It is possible that the iron-containing compound in the cytoplasm of this layer is the zymogen or prozymogen of the ferment.

IV.—On the Occurrence of Assimilated Iron Compounds in Special Forms.

What I have said in the foregoing pages with regard to the presence of iron in the chromatin of higher forms of animal and vegetable life is true also in regard to the types of lower organisation in both kingdoms. In the investigation of the less highly organised animal and vegetable forms, however,

[1] "Die Kleberschicht des Grasendosperms als Diastase ausscheidendes Drusengewebe," 'Berichte der deutschen botan. Gesellsch.,' 1890, p. 40. Abstract in 'Botan. Centralbl.,' vol. xliii, p. 39.

some important variations were found in the disposition of the iron-holding substance, and it was further determined that in non-nucleated organisms the exceptional distribution of the chromophilous substance is co-extensive with that of the assimilated iron compounds observed. Such facts are worthy of an extended description, and I now propose to detail these and the more important observations allied to them.

Ascaris.—In the species A. mystax the spermatozoids and ova, both before and after fertilisation, manifest special features in the distribution of the iron-containing substance. When they are hardened in alcohol, the spermatozoids are comparatively easily affected by the ammonium hydrogen sulphide, the reagent, mixed with glycerine, giving in a couple of days, under the usual conditions, a reaction for iron, which usually is confined to the "nucleus," a dense homogeneous body (fig. 31); but in several instances the "membrane" also contained iron. The reaction in the latter varied in intensity, and when most marked it revealed a structure in the "membrane" like that represented in fig. 32. The iron compound observed in such a case obtained only in the rodlets constituting the "membrane." What the occurrence of assimilated iron in this situation signifies I am unable to say, except that it possibly represents an abnormal phase of a condition normal to the spermatozoid after it has penetrated the ovum. When the spermatozoid begins to penetrate the latter, its membrane frequently manifests a weak reaction for iron (fig. 29), while its cytoplasm does not give any evidence of the presence of that element; but in the changes it undergoes after reaching the interior, the "nucleus" becomes in part dissolved, and the chromatin, as shown by the iron reaction, diffuses into the cytoplasm and into the membrane, from which some of it passes into the cytoplasm of the ovum immediately adjacent to the spermatozoid. The membrane in this way becomes the most prominent part of the spermatozoid. As the transformation proceeds, the membrane also dissolves, and the iron which it contains appears to pass back again into the cytoplasm of

the spermatozoid, but what is held in the cytoplasm of the ovum apparently is retained by the latter.

These observations on the diffusion of the iron-holding substance from the "nucleus" of the spermatozoid into its cytoplasm coincide with those of van Beneden upon the changes which take place in the spermatozoid of Ascaris megalocephala after it penetrates the ovum. He found that the protoplasm of the free spermatozoids[1] manifests no affinity for staining compounds, while its capacity for absorbing and retaining all colouring matters becomes remarkable immediately after it enters the ovum. As the "nucleus" at the same time loses in part its affinity for stains, he came to the conclusion that a part of the chromatic substance (chromatin) of the "nucleus" becomes dissolved in the cellular substance (cytoplasm). O. Zacharias[2] has also pointed out that the protoplasm of the free spermatozoid, apart from its "nucleus," is absolutely unstainable, but after it penetrates the ovum it at once manifests an affinity for colouring matters. Kultschitzky,[3] referring to the reactions with staining fluids, suggests that possibly the "nucleus" gives off to the cytoplasm of the spermatozoid a portion of its chromatin, or that, in other words, not all of the chromatin of the "nucleus" is employed in the construction of the male pronucleus. I have found in my preparations that the cytoplasm and "membrane" of the spermatozoid which has penetrated the ovum, and, frequently also, that portion of the cytoplasm of the ovum in the immediate vicinity of the spermatozoid, have a slightly greater affinity for colouring matters than the cytoplasm of the free spermatozoid or of the unimpregnated ovum.

In many of his illustrations van Beneden represents that part of the spermatozoid which I have called the "membrane"

[1] "Recherches sur a maturation de l'œuf et la fécondation," 'Archives de Biologie,' vol. iv, p. 265, 1883.

[2] "Neue Untersuchungen über die Copulation der Geschlechtsprodukte und den Befruchtungsvorgänge bei Ascaris megalocephala," 'Arch. für Mikr. Anat.,' vol. xxx, p. 111, 1887.

[3] "Die Befruchtungsvorgänge bei Ascaris megalocephala," 'Arch. für Mikr. Anat.,' vol. xxxi, p. 567, 1888.

as deeply stained, and in these one finds the existence of rodlets indicated, such as those to which I have referred above; but these (les stries transversales de la queue) are more apparent in the penetrating than in the free spermatozoid. I observed only faint traces of such structures in the spermatozoid in the interior of the ovum, the rodlets apparently commencing to disappear immediately impregnation is accomplished.

The chromatin of the nucleus of the ovum gives a deep reaction for iron in whatever stage the nucleus may be found (figs. 29 and 30). The chromatin also of the "polar globules" contains iron, and I made efforts to determine the ultimate fate of this, but these were unsuccessful. It would appear, however, as if the chromatin of the extruded elements were dissolved eventually in the cytoplasm, for it is impossible to find any traces of it after a time.

Chironomus.—Balbiani,[1] who was the first to call the attention of cytologists to the structure of the nuclear elements in the "salivary" glands of the larva of Chironomus, described the nuclear filament as made up of a series of dim discs or bands, each placed transversely, and separated from its neighbour on either side by a band of clear substance, the filament possessing, however, at certain points an annular swelling, and terminating at its ends either in the polymorphous nucleolus or by an attachment to the nuclear membrane. Leydig,[2] the next observer, found each dim stria to be made up of a series of elements whose separation from each other gives a composite character to the stria. The fine lines separating the elements are, according to his observation, continued from one dim disc, through the light disc on either side of it, to the adjacent dim disc. In this way a series of exceedingly delicate longitudinal lines, in addition to the coarse transverse ones described by Balbiani, make their appearance. Leydig also believes that the substance forming the dim band

[1] "Sur la structure du noyau des cellules salivaires chez les larves de Chironomus," 'Zool. Anzeiger,' 1881, pp. 637 and 662.

[2] 'Untersuchungen zur Anatomie und Histologie der Thiere,' Bonn, 1883, p. 90.

is situated immediately under the membrane. Korschelt's views on the structure of the filament are directly opposed to those of Leydig and Balbiani. He regards the transverse striation of the filament as due to a folding of the surface membrane only, and explains the longitudinal striation observed by Leydig as caused by the action of the reagents used. In his opinion, also, the apparent differentiation of the filament is due to the differences in the reflected light.

So far as I know, no one has hitherto observed an arrangement in the nuclear filament of Chironomus similar to that described by Leydig, although Carnoy has found in the salivary gland of a Nemocere larva that the dim disc is formed of a series of longitudinally disposed rodlets, but he attributed the delicate lines observed in the clear discs to folds in the membrane of the filament.[2] The larvæ of the species of Chironomus accessible to me offer preparations less favourable for study than do those of the species C. plumosus studied by Balbiani and Leydig, yet I have been able to determine, with my methods for demonstrating the presence of assimilated iron, the correctness of Leydig's observations so far as they go. The dim discs are of different thicknesses, the thickest appearing to be five or six times the diameter of the narrowest. When the salivary gland, after being hardened in alcohol, is kept for several days in sulphuric acid alcohol, treatment with an acid ferrocyanide solution gives all these dim bands a deep blue reaction, the intensity of the reaction coming out very markedly in the thicker bands. Under the highest magnification of service in such a case (apochromatic immersion 1·5 mm. and compensation ocular 8, Zeiss), the bands of medium thickness are resolved into a series of short rodlets disposed parallel with the filament. If the filament has, in the course of preparation, been isolated from the nucleus, one may then determine that the rodlets forming one dim band are connected by excessively delicate fibrils with the rodlets

[1] Ueber die eigenthümlichen Bildung in den Zellkernen der Speicheldrüsen von Chironomus plumosus," 'Zool. Anz.,' vol. vii, pp. 189, 221, 241, 1884.

[2] 'Biologie Cellulaire,' p. 232.

forming the two adjacent bands. The fibrils, or what are in appearance such structures, have so little iron in them that frequently in a large part of an isolated filament their blue reaction may not be sufficiently deep to betray their presence, but the chances of observing them may be increased by staining such preparations carefully with safranin. Probably the expression fibril is not a correct one to apply to these appearances, for they may be the optical sections of the partition walls of compartments, the extreme ends of which would in that case be formed by the dim bands. What appears to support the latter view is the fact that in some of the thickest dim bands the Prussian blue reaction reveals the presence of a single row of vesicles extending from one end of the band to the other, the vesicles sometimes having an elongated form parallel with the filament. It seemed to me that these were the initial stages in the division of one dim band into two, that the thinner bands represent those most recently formed, and that, therefore, the vesicular mode of formation would result in the production of a series of compartments the thin walls of which, in the clear bands, would appear as fibrils. The structures observed are, however, so exceedingly minute that it is impossible to determine definitely anything on this point.

The iron-holding substance in the filament is, therefore, disposed in the rodlets of the dim band and in the fibril-like elements connecting the rodlets of one dim disc with those of its neighbours. The only exception to this statement may be made in regard to the structure of the swellings which are sometimes found on the course of a filament (fig. 50). In this case the dim discs are replaced by an iron-holding reticulum disposed in the interior of the swollen portion of the filament. A comparison of this portion with the adjacent portions of the filament appears to indicate how the reticulum has arisen and what its relations are. The iron-holding bands on either side are less regular in their disposition than elsewhere, and the fibril-like structures arising from them appear to be directly connected with the iron-holding substance of the reticulum referred to. The swollen portion of the filament

varies in its size and shape, but most frequently it has the appearance represented in the figure.

I have never observed the annular swellings described by Balbiani as present in the filament in C. plumosus, but I take it that the swollen portions here described are the representatives of such structures. Nor have I ever determined that the filament ends by attachment to the nuclear membrane, or to the amœbiform nucleolus, through which it may pass several times in its course. The nucleolus varies not only in form and size but also in composition. It may be homogeneous, but more frequently the central portion contains vacuoles and granules and stains more deeply with eosin or safranin, while the peripheral non-granular portion may possess no staining capacity whatever. In many preparations made from alcohol material and stained with eosin, the nucleolar body alone is stained, and this is particularly the case when the preparation has been treated with acid alcohol and the acid ferrocyanide mixture to demonstrate the iron present. The nucleolar substance, apart from its granules, contains iron, but the iron present is very small in amount compared with that observed in the filament, for, when the latter gives an intensely deep blue reaction, the colour given the nucleolus is a very pale blue, and when the nuclei are kept for a week mounted in the glycerine and sulphide mixture in the warm oven, the isolated nucleoli develop only a greenish colour, portions of the filaments, on the other hand, giving in the same preparations a marked dark-green reaction. Unlike the differences in staining exhibited after treatment with eosin, the faint or light blue reaction is uniform throughout the nucleolar substance.

The dim bands with the excessively fine fibrils in the filament are formed of chromatin, as shown by treatment with the staining reagents, when the preparations have been properly hardened. There is, however, a difference between this chromatin and that of the ordinary animal cell in that while acid methyl-green colours the former it leaves unaffected the nucleoli and the swollen portions of the filament, which stain deeply with hæmatoxylin and carmine.

Balbiani[1] concluded from such results that chromatin (substance chromatique) is present, not only in the dim discs, but also in the annular swellings and the nucleoli. According to Flemming,[2] safranin colours all these elements, but stains the nucleoli very strongly. Flemming's observation is correct only for preparations made with the chrom-osmio-acetic reagent; but when the nuclei have been fixed with alcohol, or with corrosive sublimate, treatment with acid alcohol for two or three days affects the filament in such a way that its discs and their excessively fine fibrils absorb and retain the safranin to a very marked extent, while the nucleolus remains unstained, and the swollen portions of the filament are faintly coloured. It is possible to obtain in such preparations both the safranin and the Prussian blue reactions, and then, with the exception of the faint blue in the nucleoli, both effects are co-extensive and of equal intensity. The marked difference between the substance of the discs and that of the nucleoli is thus shown, but it may be brought out in a more brilliant way by staining Prussian blue preparations with eosin, which then affects the nucleolus only.

The nucleolus thus resembles the similarly named structure obtaining in the nuclei of Vertebrates, but it differs from this in that it is amœboid in form, and does not possess, in any case, a chromatin envelope. The presence of granules and vacuoles, moreover, appears to indicate that it is physically active, which cannot be postulated of the vast majority of the nucleoli of Vertebrate cells.

Whatever effects may be obtained by treating the nuclei with various staining reagents, but one results in the cytoplasm of the secreting portions of the salivary gland in Chironomus. Acid methyl-green in the fresh preparations, and hæmatoxylin and safranin in the hardened glands, demonstrate very clearly that there is a stainable substance, in many respects like chromatin, uniformly distributed through the cytoplasm; that it is chromatin would appear from the fact that the cytoplasm

[1] Loc. cit.

[2] 'Zellsubstanz, Kern- und Zelltheilung,' pp. 112, 113.

holds an assimilated iron compound, for if small fragments of cells, hardened in alcohol, be subjected to the action of the warm glycerine and sulphide mixture for a week or more, they will manifest a dark-green reaction which, when the mixture is washed away and replaced by an acid ferrocyanide solution, is converted into that of Prussian blue. One may more readily obtain the demonstration of the iron in these cells by allowing sulphuric acid alcohol to act on the hardened gland for two days, when the cytoplasm of the secreting cells and the substance of the thread (silk?) in the lumen give evidence of the presence of this element. Whether the iron thus demonstrated in the substance of the thread belongs to the latter, or is derived by diffusion from the cytoplasm of the secreting cells during treatment with the acid alcohol, I am unable to say, since my experiments made to determine this question, by the use of the glycerine and sulphide mixture on isolated bits of the threads, turned out to be failures.[1] The substance forming the threads manifests a strong affinity for dyes, and should it eventually be ascertained that the iron demonstrated in it, after treatment with acid alcohol, is part of a "masked" compound contained in it, the facts will then all indicate that the iron-containing substance in the cytoplasm is the antecedent of at least a portion of the substance of the thread in the lumen, and one will have then also a parallel of what was pointed out as obtaining in the pancreas and other ferment-secreting cells in Vertebrates.

Protozoa.—I have selected the genera Stentor, Epistylis, Vorticella, and Paramœcium for specially illustrating the distribution of the assimilated iron in unicellular animals. A very large number of other forms were used to confirm the results which a study of the named organisms gave, but owing

[1] Gilson (loc. cit.) has referred to the fact "that the silk of certain insects seems to possess a stronger affinity for this metal (iron) than nuclein itself." I have observed this peculiarity, but the iron absorbed is at once demonstrated on the application of any form of ammonium sulphide, a fact which shows that the iron so revealed does not enter into a "masked" condition, and ought not to be confused with that of "masked" compounds.

to the difficulty experienced in getting examples of such forms in the numbers required, it was impossible to make a fully satisfactory, systematic investigation of their iron-holding character. On the other hand, examples of the genera named could be obtained at all times in abundance, and I regard the opportunities thus presented as compensating in some measure for the limited range of genera studied.

One of the difficulties encountered in attempting to study the distribution of iron-compounds in Protoza is the fact that many of the motile forms, and some also of those which are sessile or attached, have in their cytoplasm inorganic compounds of iron, in great part, if not wholly, derived from the food matters ingested, and when such organisms, after being hardened in alcohol, are treated with the glycerine and sulphide mixture, they give at once a deep reaction for iron which, in many cases, obscures other details in the cytoplasm and nucleus. When, moreover, attempts are made with acid alcohols, and especially Bunge's fluid, to remove the inorganic iron, the conditions under which the experiments are made enable the reagent to liberate the "masked" iron at the same time, in which case the liberated portion becomes indistinguishable from that present previously in an inorganic form. To avoid such difficulties it is necessary to select forms in which the amount of inorganic iron is small or infinitesimal, and by determining the amount of the reaction obtained during the first ten minutes after the application of the glycerine and sulphide mixture, one may thus prevent confusion arising from the study of results obtained by the more prolonged application of the reagent. Such forms may be found in the genera above named, and one may, by attention to the character of the medium of the organisms, without any difficulty secure such examples as offer the most favourable conditions for investigating the distribution in them of the assimilated iron. The specimens of Epistylis, for example, which were used by me for this purpose, were obtained from a colonial form attached to the sides and limbs of the common crayfish, and their cytoplasm gave no immediate reaction for iron. Examples of

Stentor and Paramœcium, in sufficiently large numbers, and all but completely free from inorganic iron compounds, were readily obtained. The cytoplasm in Vorticella, on the other hand, usually contains such compounds, but these are very often in the form of granules situated in vacuoles, or at the periphery of the same, a disposition of the compounds which gives every facility for studying the distribution of the assimilated iron.

In the examples of Epistylis there were, as stated, no inorganic compounds of iron, at least none were demonstrable in the glycerine and sulphide mixture within the first hour after the application of the reagent, but on the third and fourth day both cytoplasm and nucleus gave a marked reaction for iron. The latter was, of course, most prominent in the nucleus, in which was revealed, by the dark-green colour, in some examples a granular structure, in others a fibrillar arrangement. The reaction of the cytoplasm was a diffuse one, with here and there large granules in which it had developed more markedly. The membrane and stalk were, in these cases, free from iron. All these points were more readily observed in preparations treated with sulphuric acid alcohol or with Bunge's fluid for twenty-four hours (fig. 28).

In Vorticella a similar distribution of the assimilated iron was observed in both cytoplasm and nucleus, and a diffuse reaction for iron was also obtained in the central or axial portion of the stalk, after the preparation had been kept in the warm glycerine and sulphide mixture for several days. The reactions are represented in fig. 27, drawn from a preparation which contained inorganic iron compounds disposed in vacuoles. In this the central portion of the stalk is shown to be continued into a funnel-shaped organ at the base, which also contains "masked" iron. I was unable to determine how this organ was connected with the cytoplasm. I found no difficulty in obtaining the complete reaction in all the parts at the end of a five days' application of the warm glycerine and sulphide reagent.

Examples of Stentor polymorphus, free from inorganic

iron compounds, were, after being hardened in alcohol and after treatment with ammonium sulphide, isolated from those more or less impregnated with iron salts, the large size of the organisms enabling one to do this readily. One of such, after treatment for fourteen days with the warm glycerine and sulphide mixture, is represented in fig. 25. In this no distinct reaction was obtained during the first two days, definitely showing that no inorganic iron was present. In the interior of the spherical elements constituting the nucleus there appeared eventually a diffuse iron reaction, as well as one localised in granules, and the cytoplasm gave a diffuse reaction like that given by the cytoplasm in Epistylis and Vorticella. I do not think that in this case the reaction had developed to the fullest extent of which it was capable, for I found other examples in which the nuclear and cytoplasmic elements gave a more intense one; but it is usually difficult in such large cells to obtain the best effects of the reagent, since in two weeks' time it is apt to undergo decomposition, when the development of the iron reaction ceases. In order to ascertain how abundant the assimilated iron is, I employed acid alcohols to liberate it, and, after the removal of the acid, treated the preparation with ammonium sulphide. Sulphuric acid alcohol is the best reagent for the purpose, since with it there is less iron diffused from the parts in which it is liberated; but, in order to get the most exact results, the examples of Stentor used should be free from inorganic iron compounds, a point of which one may be certain by putting the hardened examples in ammonium sulphide for a few minutes, when, if they pass this test, they may be washed in alcohol to remove all traces of the reagent and placed in the acid alcohol for one or two days. I have represented in fig. 26 an example of S. polymorphus, in the wall of the funnel-shaped œsophagus of which was found the only inorganic iron compound present, and in this, after it had been treated as described, the ribbon-like nucleus appeared intensely greenish-black, while the cytoplasm gave a deeper reaction than was obtained in any specimen simply by prolonged treatment of it with the warm

glycerine and sulphide mixture. In examples absolutely free from inorganic iron compounds the reaction in the cytoplasm and nucleus was as marked as that represented in the figure. The method is, of course, open to the objection that it may permit a diffusion of the liberated iron from the nucleus to the cytoplasm, but that the latter contains assimilated iron is shown by prolonged treatment with the warm glycerine and sulphide reagent.

In examples of different species of Paramœcium, the cytoplasm, which gave no reaction for inorganic iron, manifested with the warm glycerine and sulphide reagent after ten days a reaction as distinct as that obtained under similar conditions in the cytoplasm of Stentor, Vorticella, and Epistylis. These organisms were the only ones in which the micro-nucleus was revealed by the iron reaction, and the latter appeared to me to develop more slowly than that in the macro-nucleus; but the explanation for this may be that the large quantity of chromatin in the latter renders a reaction of any degree of intensity obtaining in it much more prominent than a reaction of a similar intensity would appear in the micro-nucleus. In both the reaction was almost wholly confined to the granules and fibrillar elements.

All the forms of Protozoa studied illustrated the fact so prominently indicated in the organisms referred to above, that an assimilated compound of iron is a constant element in their cytoplasm. It is probable that this compound belongs to the chromatin class, for the cytoplasm in Protozoan organisms generally stains much more readily, and holds the dyes more tenaciously, than the cytoplasm in higher organisms does. In support of this may be urged other facts. I pointed out, when dealing with the relations of assimilated iron compounds to the ferment-forming cells in Vertebrates, that the substance which elaborates the ferment, or out of which it is prepared, contains iron and acts towards staining reagents like chromatin. Digestion in Protozoa is, in all probability, effected by ferments derived, as in higher forms, from the cytoplasm, and it is only reasonable to suppose that

the antecedent of the ferments is, in this class also, an iron-holding chromatin.[1]

Euglena viridis is a form whose position, whether as a vegetable or as an animal organism, has not by any means been definitely determined, but the distribution of assimilated iron in its interior appears to indicate that if it does not belong to the animal kingdom, its physiological processes possibly resemble those of the Protozoan cell, and it is for this reason that I deal with it in this place. Examples of this organism free from inorganic compounds of iron may be obtained readily, and when hardened in alcohol, they may be subjected to the action of the glycerine and sulphide mixture for twenty-four hours, without manifesting a reaction for iron, but when the application is extended for three days or longer, a reaction for iron is obtained in the nucleus and cytoplasm. The chromatin network is usually so affected by the reagent that its nodal points only manifest the reaction, while the nucleolus exhibits a less intense dark-green colour. The cytoplasmic trabeculæ separating the "amylaceous" corpuscles from each other develop a dark-green reaction, which is found to be most intense at the nodal points. All these features are more clearly seen in specimens which have been hardened in alcohol, then treated for two days with sulphuric acid alcohol, and finally, after being acted on with the acid ferrocyanide mixture to produce the Prussian blue reaction, mounted in balsam (fig. 49). In these preparations the iron revealed in the cytoplasm is most abundant in its nodal points, which, with the reticulum of the nucleus, are thereby rendered most prominent. The nucleolus, separated from the other elements by a clear zone, in which the light blue observed is derived from the nuclear elements above and below the focal plane, gives a less intense reaction than one of the much smaller nodal points of the nuclear network. If the preparation has also been stained with eosin the nucleolus alone appears to be

[1] The ferment or ferments, according to M. Greenwood ('Journal of Physiology,' vol. viii, 1887, p. 263), pass into the fluid surrounding the ingested matter.

markedly affected by it, exhibiting an ochre-red colour so characteristic of the nucleoli in the hepatic cells of Necturus after similar treatment. Safranin leaves the nucleolus unaffected, but colours deeply the chromatin network and the iron-holding portions of the cytoplasm. When, however, the organism has been hardened in picric acid, the nucleolus exhibits no affinity for eosin, while it colours as deeply as the chromatin network does with hæmatoxylin and picro-carmine. From this it would appear as if the nucleolus were intermediate in composition between the nucleolus of higher animal cells and the chromatin of the nuclear reticulum.

The occurrence of assimilated iron in the cytoplasm of Euglena viridis, if it is not chemically associated with the chlorophyll present, appears to indicate that the organism is closely related to the Protozoa, in common with which it has other characters.[1] If the view, that the assimilated iron in the cytoplasm of Protozoa is part of the antecedents of the zymogenic compounds of these organisms, is correct, it would explain the phenomenon in Euglena in which the presence of a short digestive "tract" also postulates, to a certain extent, the occurrence of processes of nutrition belonging to the animal type.

Fungi.—The presence of nuclei has not yet been demonstrated in a large number of the Fungi, nor has the occurrence of a substance similar to the chromatin of other organisms been determined with any degree of certainty, except in a few forms; and, therefore, the question of the occurrence and distribution of assimilated compounds of iron in the cells of this class is not quite as easy of solution as that dealing with the

[1] G. Klebs, who has given special attention to the Euglenaceæ ("Organization einiger Flagellaten-Gruppen und ihre Beziehungen zu Algen und Infusorien," 'Untersuch. aus dem Bot. Inst. zu Tübingen,' 1881–85), is of the opinion that this group should be classed amongst the Protozoa. Khawkine ("Recherches biologiques sur l'Astasia ocellata, n.s., et l'Euglena viridis. Seconde Partie, L'Euglena viridis." 'Ann. des Sciences Nat., Zoologie,' Serie 7, vol. i, 1886, p. 319) came to the conclusion, as a result of experiments, that Euglena takes in organic compounds in the dark, but in daylight assimilates only inorganic compounds.

presence of these compounds in higher organisms. I have, however, endeavoured to solve it by the investigation of a few widely different forms, and the results now to be described show the presence of "masked" iron compounds similar to those found in all the higher organisms. These forms comprise: Saccharomyces cerevisiæ, S. Ludwigii, Hyphelia terrestris Fries, a leucosporous Agaricine, Cystopus candidus, and Aspergillus glaucus.

The question of the occurrence of a nucleus in Saccharomyces bears upon that relating to the presence of iron-containing chromatin-like substances in this genus; and, consequently, it is necessary to give an account of the various observations that have been made on this subject.

The earlier botanists, Nägeli[1] and Schleiden,[2] claimed that they had found a nucleus in the yeast-cell, and the later observers, Schmitz,[3] Strasburger,[4] Zalewski,[5] and Zacharias,[6] have maintained that it exists, while Zimmerman[7] speaks reservedly on the question. Raum[8] found in yeast-cells which had been fixed on the cover-glass by heat or by solutions of corrosive sublimate, and stained, first with warm methylene-blue and then with bismarck brown, black spherical granules, varying in number from one to fifteen, in a more or less brown-tinted protoplasm. These were usually arranged in the

[1] 'Zeit. für wiss. Botanik,' vol. i, p. 45. Reference in Raum's paper.

[2] 'Grundzüge der wiss. Botanik,' 1849, p. 207. Referred to by Raum.

[3] "Untersuchungen über den Zellkern der Thallophyten," 'Sitzungsber. der Niederrhein, Gesell. für Natur- und Heilkunde zu Bonn,' Sitzung. am 4 Aug., 1879.

[4] 'Das Botanische Practicum,' p. 339, 1887.

[5] "On Spore Formation in Yeast Cells," 'Transactions of the Scientific Academy of Cracow' (Polish), 1886. Abstract in 'Bot. Centralbl.,' vol. xxv, p. 1.

[6] "Beiträge zur Kenntniss des Zellkerns und der Sexualzellen," 'Bot. Zeitung,' 1887, Nos. 18–24.

[7] "Die Morphologie und Physiologie der Pflanzenzelle," Breslau, 1887, p. 25. I have not had access to this publication, and my attention was first called to it by a reference made by Raum.

[8] "Zur Morphologie und Physiologie der Sprosspilze," 'Zeit. für Hygiene,' 1891, vol. x, p. 1.

form of a circle or of a segment of a circle at either pole of the oval cell, and there was no relation between their size and that of the cell containing them, although they appeared to have some connection with the budding process, since he observed them undergoing transference to the protoplasm of the bud. What the nature of these granules is Raum does not say, but the results of his experiments would seem to indicate that they are not formed of nuclein, for on submitting the yeast-cells to digestion with an artificial gastric fluid at a temperature of 40° C. for one or two days, and afterwards on washing with ether and alcohol, every trace of the granules had vanished. Nuclein is undoubtedly present in yeast-cells, and Raum prepared some of it from this source, which he mounted in egg-albumen on a cover-glass, and stained, first with methylene blue and afterwards with bismarck brown, when he found that the nuclein particles took a brownish stain while the albumen appeared light yellow, a reaction in marked contrast with that obtained in the granules of the hardened yeast-cells after the employment of the same staining methods. Raum appears to be doubtful concerning the existence of anything resembling a nucleus in the yeast-cell.

The more recent observers who claim to have found a nucleus in the yeast-cell are Möller and Janssens. The former[1] found in older yeast-cells a spherical corpuscle which he regards as a nucleus, but without a membrane or nucleolus. This changes its shape readily, and therefore its position in the cell varies. Owing to this property, it is capable of assuming a thread-like form when budding occurs, a portion of it being thus enabled to pass into the protoplasm of the bud through the narrow tube which connects the mother and daughter elements. The part in the latter eventually breaks off, and both portions become spherical. Janssens,[2] who used

[1] "Ueber den Zellkern und die Sporen der Hefe," 'Centralbl. für Bakt. und Parasitenkunde,' vol. xii, 1892, p. 537; also "Weitere Mittheilungen über den Zellkern und die Sprosse der Hefe," ibid., 1893, vol. xiv, p. 358.

[2] "Beiträge zu der Frage über den Kern der Hefezelle," 'Centralbl. für Bakt. und Parasitenkunde,' vol. xiii, 1893, p. 639.

in his investigations the species S. cerevisiæ, S. Ludwigii, and S. Pastorianus, states that he found in the two former a nucleus provided with a membrane and a nucleolus, the latter spherical and homogeneous and of a diameter one third that of the nucleus. The remaining portion of each cell is occupied by a cytoplasmic network with fine meshes, whose nodal points readily absorb colouring matters, and, in the opinion of Janssens, constitute the granules of Raum. He claims to have observed mitotic stages of the nucleus, which obtain when budding commences and when spore formation occurs.

Two observers only, Brücke[1] and Krasser,[2] have denied the existence of a nucleus in the yeast-cell. Krasser in his later publication asserts that the body described by Möller as a nucleus is not such an organ, and he found, after employing Möller's methods on beer yeast-cells, that the latter possessed no body like the one described by that observer. He further observed that the bodies described by Möller as nuclei, after being submitted to digestion with artificial gastric juice, gave no evidence of the presence of nuclein. The occurrence of the latter substance in yeast-cells, which is readily demonstrable in a macro-chemical way, Krasser attempted to show micro-chemically, and, after many failures, succeeded in finding it in a few specimens in the form of granules at the side of the body regarded by Möller as a nucleus.

I have followed the methods of hardening and staining adopted by Möller, for the purpose of ascertaining the nature of the body considered by him to be a nucleus, and have compared the results thus obtained with those found in yeast-cells after hardening the latter in saturated solutions of corrosive sublimate and staining them with hæmatoxylin and eosin. I have also used Flemming's fluid for hardening, and stained preparations so made with safranin. Möller's methods certainly do reveal, now and then, a structure like that which

[1] "Die Elementarorganismen," 'Sitzungsber. der K. Akad. d. Wiss. zu Wien, Math.-Nat. Classe,' 1861, vol. xliv, Abth. 2.

[2] "Ueber das angebliche Vorkommen eines Zellkerns in den Hefezellen," 'Oesterreich. Bot. Zeits.,' 1885, No. 11; also "Ueber den Zellkern der Hefe," ibid., 1893, p. 14.

he took to be a nucleus, but this body, when hardened with corrosive sublimate, stains with eosin but not with hæmatoxylin, while after fixation with Flemming's fluid it appears to have no particular affinity for any dye. On the other hand, in S. Ludwigii, as it usually develops in the sap of the iron-wood tree (Ostrya virginica), there is in the great majority of cells a corpuscle which corresponds with the "nucleus" of Möller. This structure is round, homogeneous, and in diameter sometimes more, sometimes less, than half the length of the shorter axis of the cell, in the centre of which it is usually placed, and after being hardened with corrosive sublimate it exhibits a special affinity for eosin, but none for hæmatoxylin, while it acts like the cytoplasm towards safranin. In preparations made with Flemming's fluid the results were practically the same, and therefore not indicating on the part of the body in question the possession of a substance in all points like chromatin.

A substance like chromatin appears to be distributed through the cytoplasm. In S. cerevisiæ, after being hardened with corrosive sublimate, the cytoplasm takes, when treated with hæmatoxylin (Delafield's and Ehrlich's), a blue-violet tinge. With favourable illumination and apochromatic objectives, the stain is found to be localised in the trabeculæ of the cytoplasmic network, and, where the vesicular character of the cytoplasm appears pronounced, all the cytoplasm, except the contents of the vesicles, is coloured. In some of the cells granules were observed with a stain slightly deeper than that of the cytoplasm, and similar elements were found in cells hardened with Flemming's fluid and stained with safranin. These, possibly, are those described by Raum. In S. Ludwigii the cell is usually very much larger, and the structure and staining reactions are, therefore, much more distinct. In this form, when hardened with corrosive sublimate and stained with hæmatoxylin, the vesicular structure of the cytoplasm comes out quite markedly through its blue-violet stain, which also is found now and then to characterise prominently granules in the cytoplasm between the vesicles. The granules of

Raum are, however, much more common elements than these, and are to all appearances quite different structures, as is apparent in ordinary cover-glass preparations made after Raum's methods. The larger examples of the granules of Raum seem to be less abundant in corrosive sublimate preparations stained with hæmatoxylin and eosin.

From these results I am inclined to regard the existence of a nucleus in the yeast-cell, in its usual condition, as extremely doubtful, and, on the other hand, to support Krasser's contention that nuclein is disseminated through the cytoplasm. Whether, in other stages, as, for example, those in which spore formation occurs, there is a nucleus I cannot say, but there appears in the ordinary stages of the organism to be nothing which may be looked upon as a specialised chromatin-holding structure.

These conclusions are, on the whole, confirmed by the results of experiments made to determine the distribution of assimilated compounds of iron in these organisms. When specimens of S. cerevisiæ, hardened in alcohol, are subjected to the action of the glycerine and sulphide mixture at a temperature of 60° C. for several days, their cytoplasm acquires a greenish tint. Sometimes, however, the latter reaction may not appear except in a few granules scattered through the cytoplasm (fig. 4). On account of the small size of the cells and of the alteration produced in them by the reagent, one cannot definitely determine whether the granules correspond to those described by Raum. When the cells have been subjected to the action of sulphuric acid alcohol, the subsequent application of an acid ferrocyanide solution gives their cytoplasm a faint blue colour, which is more distinct and deeper when the light transmitted passes through several cells in succession. Blue granules are sometimes observed in such preparations.

It is in specimens of S. Ludwigii that one obtains the clearest evidence of the occurrence of an assimilated iron compound. In these, after being hardened in alcohol, the glycerine and sulphide mixture eventually gives results like

those represented in fig. 5. The differences observed appear to depend on the cytoplasmic structure in the specimen examined. When there are a few large vesicles in the cell, the iron-holding substance seems to be, in great part, at their peripheries. This disposition also obtains in the buds. The remaining portion of the cytoplasm in each element is very slightly coloured greenish, but whether that is due to ferrous sulphide is uncertain. When, on the other hand, the cells are markedly vesiculated, the glycerine and sulphide mixture gives the cytoplasm between the vesicles a distinct reaction for iron. In the majority of such cells there are one or more large spherical elements, which, in the glycerine and sulphide mixture, after the third or fourth day appear dark green, much more so than does the surrounding cytoplasm. They are homogeneous, manifesting a uniform reaction throughout their substance, and their position is, if not in the centre of the cell, at least in that neighbourhood; but smaller granules of the same character may be more remotely situated. From their position, size, and shape, they would appear to be the bodies which, in preparations made with corrosive sublimate, hæmatoxylin, and eosin, stain exclusively with the latter reagent. In cells which are treated with acid alcohol, then with an acid ferrocyanide solution, and finally, after being stained with eosin, mounted in balsam, similar bodies are given a violet tint, while the cytoplasm is coloured bluish, the violet being undoubtedly due to a combination of the Prussian blue colour with the eosin stain. As the granules of Raum are not specially selected by eosin, it would appear that the iron-containing body observed does not belong to that class.

It is thus seen that in S. cerevisiæ the assimilated iron is, like the substance which absorbs hæmatoxylin, distributed through the cytoplasm and sometimes also in the latter in the form of granules, but in S. Ludwigii it may be chiefly found at the periphery of each large vesicle when only a few vesicles are present, while in those cells in which the whole of the cytoplasm is vesiculated, the latter gives a uniform reaction for iron corresponding in its depth with that given by hæma-

toxylin. Further, there is a substance which constitutes corpuscles of a nucleolar character in cells of this form, which stains with eosin and gives a marked reaction for iron, but differing from the chromatin substance in remaining unstained after treatment with hæmatoxylin. There is no nucleus, although such an organ may occur in other stages, especially in S. Ludwigii.[1]

When the mycelial threads and hyphæ of Hyphelia terrestris, Fries, are hardened in alcohol and stained with hæmatoxylin, the cytoplasm generally is coloured, but it is specially affected by the stain in the terminal portions of the hyphæ on which the elements of fructification are developing. One can find also, in such preparations, deeply-stained granules scattered in the cytoplasm of the hyphæ, and at times also a vesicular cavity and a membrane enclosing these granules, which then simulate nucleoli. Sometimes such structures strongly resemble nuclei, and mitotic conditions are suggested by the presence of pairs of rows of deeply-coloured granules placed opposite, and at a very short distance from, each other. In the fully-formed fructification these vesicular cavities and their deeply-stained granules may be most readily seen. Whether such structures are nuclei in the proper sense of the term it is difficult to say, but if they are, they contain only a small portion of what may be considered as the chromatin, which is diffused in the cytoplasm of the mycelial threads in the younger stages, but appears to be transferred to the hyphæ when the fructification of the latter commences. When the latter stage is fully attained the mycelia and lower portions of the hyphæ are found to have little or no cytoplasm and to stain very feebly, a result quite different from that obtained in the fructification.

The distribution of the "masked" iron in this form is found to coincide very closely with the distribution of the stainable substance. In the simplest form of the hypha, the glycerine

[1] Ludwig ('Lehrbuch der niederen Kryptogamen,' 1892, p. 201) appears to regard S. Ludwigii as merely a stage in the development of Endomyces Magnusii.

and sulphide mixture gives in twenty-four hours a reaction like that represented in fig. 13 *a*, while in the slightly more developed structure the reaction is deeper with large dark-green granules (fig. 13 *b*). A similar result is obtained in the hyphæ which terminate in two, three, or more pear-shaped outgrowths (fig. 12). In the hyphæ below the fructification the cytoplasm is of a vesicular character, the walls of the vesicles being formed of an iron-holding substance, and as the terminal element develops, the vesicular character becomes less marked and the iron reaction less distinct, so that, finally, no iron may be found in this part of the filament. At the same time the granules in the fructification become more numerous, larger, and manifest a deep reaction for iron (fig 11). These granules are then found to be situated in small vesicles very much like the vesicles which, in hæmatoxylin preparations, resemble nuclei. The granules revealed by the iron reaction are the same as those indicated by the hæmatoxylin stain. This is also true of the granules in the younger hyphæ. The cytoplasm of the mycelial threads is, at this stage, free from "masked" compounds of iron, but in the earliest stages the mycelial threads give at once, on the application of the glycerine and sulphide mixture, a slight reaction for iron, which, however, becomes deeper at the end of twenty-four hours if heat be applied, this indicating the presence of "masked" iron. Granules in the cytoplasm along the course of the threads give a marked reaction for the metal like that manifested in the hyphæ. It is probable that the absence of iron in the later stages of the threads may be due to the transference of the iron-holding compound to the hyphæ.

The question concerning the occurrence of nuclei in the Hymenomycetes has been dealt with by Strasburger,[1] Rosenvinge,[2] and Wager.[3] The two former describe them as obtain-

[1] 'Das Botanische Practicum,' pp. 301 and 433, 1887.

[2] "Sur les noyaux des hyménomycètes," 'Annales des Sciences Nat., Bot.,' 1886, Serie 7, vol. iii, p. 75.

[3] "On the Nuclei of the Hymenomycetes," 'Annals of Botany,' 1892, vol. vi, p. 146.

ing in the hyphæ, in the basidia, and in the spores of the various species, in the form of small elements which are brought into view only when alcoholic material is acted on by very dilute solutions of hæmatoxylin. Their number in a hypha varies, but in each basidium there is at first only one, which, when the sterigmata are being formed, divides, the daughter nuclei undergoing division also, sometimes a second time, each of the four or eight thus resulting passing through the tubes of the sterigmata into the spores at the end of the latter. When the spores are mature they thus contain, according to the species, one or two very minute nuclei, while the basidia at this stage contain none. Wager also found nuclei in the basidia, but maintains that the spores do not contain any until after the formation of the thick spore-membrane.

It is an easy matter to demonstrate in the hyphæ and sometimes in the basidia and in the mature spores of leucosporous Hymenomycetes,[1] the structures regarded by Strasburger and Rosenvinge as nuclei, but, as was the case in Hyphelia terrestris, such elements contain only a small portion of the chromophilous substance, for when preparations are made, as recommended by Strasburger, with very dilute solutions of hæmatoxylin, the cytoplasm also stains though not quite so deeply as the minute nuclei, especially in young hyphæ. This and other staining reactions indicate that chromatin is dissolved in the cytoplasm, a conclusion borne out by the results of experiments with the glycerine and sulphide mixture and with acid alcohols, in which case the hyphal elements of a very young stage of growth give a reaction for iron diffused throughout the cytoplasm, but when the spores are formed the hyphal cells and their shrunken nuclei rarely give a reaction for iron. At this stage also, in sections of the lamellæ, a reaction for iron is obtained in the hymenium and in the spores, while the hyphal elements of the "trama" appear free from the metal. If the spores and the basidia are teased out and mounted in the

[1] The pigment in the spores of the other divisions of the Hymenomycetes greatly obscure the reaction obtained with the glycerine and sulphide mixture.

glycerine and sulphide mixture, the application of heat to the preparation for a week will bring out appearances in the isolated elements like those represented in fig. 10. The most prominent feature in these is that the cytoplasm in both classes of structures contains "masked" iron. When the bodies regarded by Strasburger and Rosenvinge as nuclei were observed, they manifested a slightly deeper reaction for iron than the cytoplasm generally, but no structure was detected in them and they appeared as large granules rather than nuclei. The most marked reaction for iron was obtained in the spores in which a cytoplasmic reticulum was thus demonstrated. When, however, the spores are provided with a thick membrane, a reaction with the glycerine and sulphide mixture does not appear, but is obtained after the use of acid alcohols. As a rule, the reaction is uniform throughout the cytoplasm of the basidia. There are, however, constituents of the hymenium occasionally observed in which no iron was found. They possessed no sterigmata or spores, and from their association with the basidia I was inclined to regard them as paraphyses, but from the comparative scarcity of such elements free from, or poor in iron, they can scarcely be looked upon as belonging necessarily to that class, which in stained preparations is abundantly represented. The subhymenial cells also give a faint reaction for iron.

It thus appears that in the leucosporous Hymenomycetes the cytoplasm of the hyphæ in the early stages of the fungus contains iron, which is also present in the minute "nuclei," and that in later stages this cytoplasm gives a faint reaction or none at all for iron, while the cytoplasm of the basidia and spores contains enough "masked" iron to give a marked reaction. This distribution of the iron corresponds with the distribution of the stainable substance, and it may, therefore, be fairly concluded that the chromatin is here also iron-holding.

In my earlier communication reference was made to the occurrence of an iron-containing substance in the gonidia of Cystopus candidus, and I stated that the iron compound

was found to be localised in spherical elements of 1·6μ diameter, corresponding to the nuclei of the zoogonidia. I have, since that date, investigated the cytological character of this organism, and have found that though there are, as Fisch,[1] Wager,[2] and others have observed, nuclei in the mycelia and in the gonidia, the whole of the protoplasm, except in the mature gonidia, is chromophilous, that is, it contains chromatin. The nuclei are, indeed, of the more regular form in the mature gonidia, but in the mycelia amongst the cells of the host (Capsella bursa-pastoris) they are chiefly, if not wholly, small masses of chromatin, like those forming the "nucleoli" in the abjointing gonidia. I have not succeeded in finding the mitotic phase either in the mycelia or in the developing gonidia, although I have carefully looked for such in a large number of preparations.

The disposition of the assimilated iron corresponds closely with the distribution of the chromophilous substance in this form. The cytoplasm of the haustoria and of the mycelia gave a marked reaction for iron in all the methods of demonstration.[3] The mycelial membrane gave no evidence of the presence of the element. The small masses of chromatin were found to be rich in organic iron. In the terminal enlarged, sometimes club-shaped, sometimes truncated, portion of each hypha the iron was found to be in a localised as well as in a diffuse form. The "nucleoli" gave abundant evidence of its presence, these structures thus appearing in marked contrast with the remaining portions of the nucleus, which contain relatively less iron than the surrounding cytoplasm in this stage. In the subsequent development of the abjointed gonidia, the nuclei appear to take up from the cytoplasm all, or nearly

[1] "Ueber das Verhalten der Zellkernein fusionirenden Pilzzellen," 'Versammlung deutscher Naturforscher und Aerzte in Strassburg,' 1885. This paper I have not seen, and the only references to it that I can find are those made by Wager and Dangeard ('Comptes Rendus,' cxi, 1890, p. 382).

[2] "Observations on the Structure of Cystopus candidus," 'Rep. Brit. Ass. for the Adv. of Science,' 1892, p. 777.

[3] The material was hardened in alcohol, which was renewed until every trace of chlorophyll was removed from the tissues.

all, of the iron-holding substance, and with this the character of the nuclei seems to change. The "nucleoli," first of all, are converted into fine granules distributed through the nuclear cavity, and, finally, in the mature gonidia the nuclei appear, in the glycerine and sulphide preparations, to be simply more or less homogeneous masses of iron-holding substance, while the cytoplasm does not contain a trace of the metal (fig. 6 *f*).

In Aspergillus glaucus the cytoplasm of the young mycelia and the gonidiophores, especially their globular ends, absorbs staining matters readily, but it contains also, scattered through it, granules of a nucleolar character, which, in very dilute solutions of hæmatoxylin, applied for twenty-four hours or more, stain deeply. The cytoplasm of the sterigmata and of the immature gonidia is similarly affected. In the mature gonidia hæmatoxylin selects large granules which are distributed through the cytoplasm. In what appear to be old mycelial threads, the cytoplasm is stained with difficulty, while the membrane may be deeply coloured. These results correspond in the main with those obtained in regard to the "masked" iron present. When the warm glycerine and sulphide mixture is applied for about a week, the cytoplasm of young mycelia gives a diffuse reaction for iron, while a deeper one appears in the large granules referred to as affected by hæmatoxylin. In the cytoplasm and granules of the gonidiophores a relatively deeper reaction makes its appearance, and a marked one is obtained in the sterigmata. In the immature gonidia the reaction is diffuse, a special one at the same time obtaining in granules collected or scattered in the cytoplasm. In mature gonidia the granules are larger, and give a deeper reaction for iron, the cytoplasm otherwise showing no trace of its presence (fig. 7). The same results are obtained, but more readily, when sulphuric acid alcohol has been employed to liberate the iron present.

Bacteria.—The question of the occurrence in bacteria of a substance like the chromatin of more highly developed organisms has been investigated to a certain extent by

Ernst,[1] Babes,[2] Wahrlich,[3] Bütschli,[4] Trambusti and Galeotti.[5] Ernst found in a large number of species of bacteria granules which stain with hæmatoxylin and other dyes, while the surrounding protoplasm is coloured faintly or not at all. These, which on account of their direct transformation into spores he termed sporogenous, undergo in their earlier stages solution in artificial gastric juice, but in the more advanced condition resist digestion. From Babes' observations, which agree in the main with those of Ernst, it would appear that the granules which absorb and retain colouring matters and take part in spore formation, also stand in some relation to the division of the bacterial cell. According to Wahrlich, the protoplasm is formed of two constituents at least, a ground substance of reticular structure resembling linin, and one forming granules distributed in this reticulum, and, owing to its capacity for absorbing and retaining dyes, regarded by him as chromatin. In Bacillus pseudoanthracis the small granules which appear before the spores are formed are constituted of chromatin, and from them is derived the main portion of each spore, while the plastin serves apparently for the construction of the spore membrane. Bütschli found in species of Beggiatoa, Chromatium, in Spirochæte serpens, Spirillum undula, Bacterium lineola, and in some Cyanophyceæ, a faintly stainable peripheral portion, and a central body, readily stainable, in which a honey-comb structure (Wabenbau) was distinctly

[1] "Ueber den Bacillus xerosis und seine Sporenbildung," 'Zeit. für Hygiene,' vol. iv, p. 25, 1888; also "Ueber Kern- und Sporenbildung in Bacterien," ibid., vol. v, p. 428, 1889.

[2] "Ueber isolirte, färbbare Antheile von Bakterien," ibid., vol. v, p. 173, 1889.

[3] "Bacteriological Studies." Reprinted from 'Scripta Botanica,' vol. iii, St. Petersburg, 1890–91. I have not seen this work, and the representation of Wahrlich's observations and views is taken from 'Bot. Central.,' vol. xlix, 1892.

[4] 'Ueber den Bau der Bakterien und verwandter Organismen,' Leipzig, 1890.

[5] "Neuer Beitrag zum Studium der inneren Struktur der Bakterien," 'Centralbl. für Bakt. und Parasitenkunde,' vol. xi, p. 717, 1892.

seen. The central body is, in Bütschli's opinion, a nucleus. In or on this organ were observed granules which became red after treatment with hæmatoxylin, and were identified with the granules described by Ernst. Trambusti and Galeotti found in one stage of a very large bacillus isolated from drinking water, that the whole of the protoplasm stained uniformly and deeply with safranin, while in a later stage of the same the stainable substance was converted into granules, disposed at the periphery and arranged in the form of a garland of oval outline. The granules eventually fused to form a homogeneous garland out of which arose from three to four elliptical rings, at first connected by their ends, but afterwards independent of each other, and in this condition became free. These changes the observers regard as analogous to those of mitosis in the cells of more highly specialised organisms.

Schottelius[1] and Ilkewicz[2] have described structures in the bacterial cell which they regard as nuclei, and Sjöbring[3] claims to have found many of the phenomena of mitosis, as it obtains in the cells of higher organisms, exemplified in bacteria. The results of these observers appear to me to have been due to defective methods of technique.

I find that in Bacillus subtilis, B. anthracis, B. megatherium, B. tuberculosis, and in the root bacillus, there are granules like those described by Ernst and Babes, and which stain with hæmatoxylin, and in B. pseudosubtilis (?), in which there is only one granule to each rodlet, each granule is developed into a spore, the remaining protoplasm at the same time losing all its affinity for colouring matters. The structures observed are the same whether alcohol, corrosive sublimate, or heat has been employed for their fixation.

Ernst found, as already stated, that the granules, except in the later stages, undergo solution in artificial gastric juice.

[1] "Beobachtung Kernartiger Körper im Innern von Spaltpilzen," 'Centralbl. für Bakt. und Parasitenkunde,' vol. iv, 1888, p. 705.

[2] "Ueber die Kerne der Milzbrandsporen," ibid., vol. xv, p. 261, 1894.

[3] "Ueber Kerne und Theilungen bei den Bakterien," ibid., vol. xi, p. 65, 1893.

This would seem to indicate that they are not constituted of typical chromatin.[1] I have endeavoured to determine whether they contain iron in a "masked" form; but the results of my experiments, except in the case of B. megatherium, have not been decided enough to permit a general conclusion on this point. The organisms are very small, and their size would postulate the occurrence of a very small amount of iron in them, and even in the larger spores. When, therefore, a cover-glass preparation of B. megatherium is treated with sulphuric acid alcohol for twenty-four hours, it is not surprising that the subsequent treatment with an acid ferrocyanide solution should give but a very faint blue reaction. When the granules referred to were under observation they manifested themselves by a blue colour slightly deeper than that apparent in the rest of the protoplasm of the organism. In B. subtilis the granules are the only parts of the bacillus which appear to contain iron, the reaction for which is very faint. I have in none of these forms obtained a reaction with the glycerine and sulphide mixture distinct enough to permit certainty of opinion in regard to this. Sulphate of iron, when present in very minute quantities in preparations, appears less distinct than the same amount of iron when revealed by the Prussian blue reaction, and on this account the apparent absence of the sulphide reaction determines nothing. In some preparations of B. pseudosubtilis the largest granules and the spores gave, after treatment with acid alcohols, a blue reaction with the acid ferrocyanide mixture. The root bacillus gave frequently a diffuse and faint blue reaction under the same conditions.

It is obvious that these organisms are too minute to furnish results which would allow the question, whether they contain "masked" iron, and how it is distributed, to be definitely and decisively answered, and I had to employ other forms, of such a size that no difficulty would be experienced in this respect.

[1] Vandevelde ("Studien zum Chemie des Bacillus subtilis," 'Zeit. für Physiol. Chemie,' vol. viii, p. 367, 1884) states that he has isolated nuclein from B. subtilis.

The most readily accessible form was Beggiatoa alba. This organism, as is well known, manifests itself in five different conditions: long threads composed of cells of varying lengths, shorter filaments also formed of cells usually free and motile, spirillum-like elements, comma-shaped, two-celled, swarming bodies, and simple "cocci." Cover-glass preparations of all these forms, fixed first with heat and subsequently with alcohol, were subjected for about two weeks to the action of the glycerine and sulphide mixture at 60° C., while like preparations were treated with sulphuric acid alcohol for about two hours at a temperature of 30° C. The results of both methods agreed. In the long threads the abundance of sulphur granules causes the cytoplasm to have a reticular, or more properly speaking, a vesicular appearance, brought out very prominently when the glycerine and sulphide mixture has dissolved out the sulphur and at the same time given the cytoplasm a greenish colour, developing into a faint blue on treatment with an acid ferrocyanide mixture. At times the greenish or the blue reaction appears most prominently in some of the nodal points of the "network," but this is doubtless due to the fact that more of the cytoplasm is condensed at such points. The shorter free, motile filaments, which contain, as a rule, very many fewer sulphur granules, have a more homogeneous cytoplasm, and in these the reaction for "masked" iron obtained was a diffuse one. A similar result was obtained in the examples of the spirillum form. In the comma-shaped forms the reaction obtained was, as a rule, slightly deeper, and it frequently appeared most markedly in the central portions of each of the two cells. In some examples a granule in this central mass gave a marked reaction for iron. I did not succeed in determining the relations of the iron in the "cocci."

So far as these results go they correspond with those obtained when cover-glass preparations of Beggiatoa alba are stained with hæmatoxylin, which colours diffusely the cytoplasm in all the forms, but rarely reveals the existence of special chromatin elements. I have been unable to determine, except in a few comma-shaped elements, the occurrence of the denser

central portion described by Bütschli, and I am inclined to regard the structure observed in the exceptional cases as due to shrinkage caused by the method of preparation. In some of the comma-shaped elements the hæmatoxylin stain demonstrates granules like those which were observed to manifest an iron reaction in the glycerine and sulphide preparations. The use of Löffler's solution of methylene blue, followed by that of a saturated solution of bismarck brown, as recommended by Ernst, stains similar granules in the "comma" elements, and in a few of the spirilla only; but it is doubtful if these may be classed with the "sporogenous" granules of other bacteria revealed in the same way. I have not found that there are any granules in the spirilla which contain "masked" iron, although there is the possibility that spirilla, containing granules, were not present in the preparations made with the glycerine and sulphide mixture or with acid alcohol.

The diffusion of the "masked" iron throughout the cytoplasm of Beggiatoa corresponds, on the whole, with what was observed in the other bacteria, but the interpretation of the results in the latter has an element of obscurity in it. It is evident that the iron-holding compound is not, as a rule, localised in granules or in special structures; and although the distribution of this compound, in Beggiatoa alba at least, corresponds with that of the substance which stains with hæmatoxylin and other dyes, it is uncertain whether the two compounds are identical. It is possible that experiments with some of the larger forms, as, e. g., Beggiatoa mirabilis or Crenothrix Kühniana, may result in determining a solution of the question. Unfortunately I had no opportunity of studying the distribution of iron in such large forms.

I did, however, obtain a few preparations of a form which is possibly allied to Crenothrix, and whose size ($2{\cdot}8$—$3{\cdot}2\,\mu \times 6{\cdot}4$—$8\,\mu$) rendered it favourable for such observations as I had an opportunity of making. This organism grew on the surface of some sewage water collected in the fall of 1893, in which also myriads of examples of Euglena viridis throve. It multiplied by fission. Some of them exhibited rounded

ends, while others had an oval shape, but the majority were cylindrical with flat end-surfaces. Several cover-glass preparations of the organisms, fixed by heat and subsequently placed in alcohol, were made, but no cultivations were attempted, since before its value for the purpose of this investigation was ascertained, the original culture fluid had been thrown away.

One of the cover preparations was subjected to the prolonged action of the glycerine and sulphide mixture, but, as sometimes happened in other cases, no result was obtained. The other two were placed in sulphuric acid alcohol for about eight hours at a temperature of about 25° C., and then treated in the usual way with the acid ferrocyanide mixture. One of the preparations was also stained with eosin, and both were, after being washed in water, dehydrated with alcohol and mounted in balsam. Examples of the organism exhibiting the Prussian blue reaction and the eosin stain are represented in fig. 53. The eosin reveals a large central body, sometimes of irregular shape, and always lying free in a cavity in the markedly reticular cytoplasm. The body in question contains no iron, but in other respects resembles the large body present in Saccharomyces Ludwigii. The iron demonstrated appears to be in a granular form distributed in the trabeculæ of the cytoplasm, though sometimes a very large granule, richly supplied with iron, was found adjacent to, or in contact with, the large central body destitute of iron.

As inorganic iron is a constituent of the sheath and other parts in species of Crenothrix and allied forms (C. Kühniana, Leptothrix ochracea and Cladothrix dichotoma), it is possible that all of the iron observed in the form described, and whose relationship to Crenothrix has been suggested, was not derived from a "masked" compound. The amount of inorganic iron must, however, have been very little, for, in the cover preparations subjected to the prolonged action of the glycerine and sulphide mixture, but a few of the forms gave an immediate reaction for iron. The chief difficulty lies in the fact that through the failure of the last-mentioned

method of liberating the "masked" iron in this organism, it is uncertain whether the iron demonstrated after the use of sulphuric acid alcohol had the distribution it obtained in the living organism, or in the cytoplasm before it was treated with acid alcohol. Apart from these matters it seems to me quite certain that the results indicate the presence of iron in a "masked" form in this organism.

Cyanophyceæ.—These organisms, which are generally regarded as closely related to bacteria, offer, on account of their much larger size, fewer and less formidable difficulties to an investigation of the morphological and micro-chemical characters of their cells, and I have, therefore, endeavoured to give a careful attention to the question of the presence of assimilated iron in them. The determination of the relations of the iron compounds in these organisms has entailed also an investigation of the morphology of their cells, and I have, in consequence, obtained a very large number of results, the description of which is beyond the scope of the present paper. These, and a fuller account of the literature of the subject, I propose to detail on a future occasion, and I now deal with the ascertained facts relating to the iron compounds and, in so far as morphological characters are associated with these, with the structure of the cells themselves.

The literature on the subject of the Cyanophyceæ has grown considerably in the last ten years, but as it is only within the last six that improved technical methods have been employed in the investigation of their structure, a short sketch of the more important publications, which have appeared in the latter period, will suffice for present purposes.

Zacharias found that the cell is constituted of a coloured peripheral part, and an uncoloured central portion of a reticulated or granular structure. In the central portion he observed two substances, one exhibiting the characters of a plastin, the other, which he termed the "central substance" (Centralsubstanz), varying in amount in the different cells, and resembling nuclein in its chemical reactions. In the central portion he found granules destitute of nuclein, and related in

many of their characters to the nucleoli of highly developed vegetable organisms.[1] He considers that the central portion differs very greatly from a nucleus, but whether it performs the functions of the latter he is not prepared to say.

Bütschli,[2] whose observations on the structure of bacteria have been already referred to, found one type of structure prevail in both these and the Cyanophyceæ. The cytoplasm is, according to his view, formed of a faintly stainable peripheral zone, and of a denser, deeply stainable central portion which, in the living Cyanophyceæ, is always uncoloured. Both parts are vesiculated. He found that hæmatoxylin colours the cytoplasm blue, while it gives a red stain to granules situated in the central portion, and, in the nodal points of the vesiculated structures, more especially of those of the peripheral zone. These disappear after subjecting the cells to the action of artificial gastric juice, but he nevertheless regards them as chromatin elements, and he looks upon the central portion as a nucleus. Besides these granules, he found in certain Oscillariæ, in the extreme peripheral portions of the cell, and especially adjacent to the transverse cell walls, others which did not stain with hæmatoxylin, but which exhibited a strong affinity for eosin.

Deinega[3] could formulate no conclusion in regard to the presence or absence of a nucleus in these organisms, and also in regard to the nature of the granules, although he is disposed to regard the latter as formed of an isomer of starch. These, of which he found but one species, stain specially with picrocarmine, and dissolve in weak hydrochloric solutions (0·3 per cent.).

Passing over the observations of Zukal,[4] who appears to

[1] "Ueber die Zellen der Cyanophyceen," 'Botanische Zeitung,' 1890, Nos. 1—5.

[2] Op. cit.

[3] "Der gegenwärtige Zustand unserer Kenntnisse über den Zellinhalt der Phycochromaceen," 'Bulletin de la Soc. impér. des Naturalistes de Moscow,' année 1891, p. 431.

[4] "Ueber den Zellinhalt der Schizophyten," 'Sitzungsber. der K. Acad. der Wiss. Wien,' 1892, Math.-Nat. Classe, vol. ci, p. 301.

regard all the granules as nuclei, the next investigator of this subject is Hieronymus,[1] who found in these cells a thin hyaline membrane externally, a chromatophore, and a central body consisting of a single much-wound fibril, comprehending in its turns all the granules in the cell. The granules he looks upon as crystals belonging to the regular system, and composed of a substance "cyanophycin," which, though not identical with nuclein in its reactions, he regards as related to the chromatin and pyrenin of highly specialised vegetable cells. The central body is, in his opinion, an "open nucleus."

According to Palla[2] the cells in the Cyanophyceæ consist of a chromatophore with a vesiculated structure, of a central homogeneous body, and of granules of different composition always outside the latter. The central body is affected, like a nucleus, by staining reagents. In preparations fixed with corrosive sublimate and stained with Böhmer's hæmatoxylin the granules adjacent to, or in contact with, the central body are stained reddish-violet, while those scattered in the chromatophore are coloured blue. He finds that those which thus become blue dissolve in dilute solutions of hydrochloric acid (0·3 per cent.) and do not stain intra vitam when treated with solutions of methylene blue. The substance constituting these, and which he calls "cyanophycin," he regards as the first assimilation product of the activity of the chromatophore. Those which stain reddish-violet with hæmatoxylin are composed of a viscid substance, are not soluble in dilute acids, and in the living cell manifest a strong affinity for methylene blue. To such structures he has applied the name "mucous spherules," first given them by Schmitz. They correspond with the granules which, in Bütschli's preparations, stained red with hæmatoxylin, but, in opposition to the views of that observer, Palla regards it as extremely doubtful if they contain any compound comparable to chromatin.

[1] "Beiträge zur Morphologie und Biologie der Algen," Cohn's 'Beiträge zur Biologie der Pflanzen,' vol. v, 1893, p. 461.

[2] "Beitrag zur Kenntniss des Baues des Cyanophyceen-Protoplasts," Pringsheim's 'Jahrbücher für wiss. Bot.,' 1893, vol. xxv, p. 511.

From all this it may be gathered that nuclei, in the strict sense of the term, are not present in the cells of the Cyanophyceæ, and that if any structure performs the functions of such an organ, it must be the colourless central body. In regard, however, to the composition, the position, and the number of varieties of the granules, there is less of concordance. All the observations quoted would appear to indicate that a typical chromatin substance is absent. If a "masked" iron compound is present in these organisms, with what part of the cell is it associated?

The forms which I used, in endeavouring to determine an answer to this question, were: Oscillaria Froelichii, Oscillaria princeps, Oscillaria sp., Tolypothrix sp., Scytonema sp., Microcoleus terrestris, Cylindrospermum majus, Anabæna (Spherozyga) oscillarioides, and Nostoc commune. The fixative reagents used were alcohol, corrosive sublimate, the stronger Flemming's fluid, and saturated solutions of picric acid; while the staining fluids employed were hæmatoxylin (Ehrlich's and Delafield's), alum cochineal, picro-carmine, safranin, and eosin. In determining the presence of iron compounds, material hardened by alcohol only was used.

The results of my experiments, so far as they affect the question of the relations of iron to the cytoplasm of these cells, may be summarised as follows:

1. The cytoplasm consists of a dense central portion and of a vesiculated peripheral zone, the former staining with hæmatoxylin, alum cochineal, and safranin more deeply than the latter when it is free from granules or vesicles, but when vesicles are present they stain deeply, while the remainder of the central portion acquires a faint colour only slightly more marked than that of the peripheral portion. The size of these vesicles of the central portion varies from that, in which they appear as scarcely larger than granules, to that observed in Tolypothrix sp., in which they measured in diameter a third of that of the cell. The stainable substance of these forms a thick membrane enclosing an apparently inert sub-

stance, and when subjected to the action of artificial gastric juice for two or three days it lessens slightly in volume, but its presence is quite as readily demonstrable then as it was previously. In this case the central portion of the cell also diminishes in volume slightly, the diminution entailing a shrinkage of the peripheral portion away from the original limits of the cell. Digestion does not affect the capacity, on the part of the central substance or of the membrane of the vesicles referred to, of absorbing staining matters, but on subsequently treating such preparations with a solution of potassium hydrate of 0·1 per cent. strength for twenty-four hours, the vesicles disappear and the central body, now somewhat swollen, has lost its capacity for fixing colouring matters in itself. Evidently there is here a substance which has the characters of nuclein. This is confirmed by the results of experiments to determine the presence of "masked" iron. The central body always gives, with the glycerine and sulphide mixture, in an interval of from two or three days to two weeks in length, depending apparently on the size of the cell, a diffuse greenish reaction which is changed to light blue on the addition of a drop of an acid ferrocyanide solution. When granules and vesicles stainable with hæmatoxylin are present, they also give a reaction for iron, but it does not always manifest the same intensity. The iron in them is most readily demonstrated after they have been treated with sulphuric acid alcohol (fig. 51).

2. In the peripheral portions of the cytoplasm, in well-nourished forms only, are granules not so readily stainable with hæmatoxylin, but which are intensely coloured with picrocarmine. These are dissolved out of the fresh cell with dilute hydrochloric ac id, and even in preparations thoroughly hardened in alcohol they are but slightly less soluble in the same reagent. In Oscillariæ they are placed in a row at each end of the cell and adjacent to the transverse walls, but in Microcoleus terrestris and Cylindrospermum majus they are disposed in all the peripheral portions of the cytoplasm. In the spores of the latter some of them appear as if embedded

in the central body. These are the "cyanophycin" granules of Palla and such as Bütschli found in Oscillariæ to be unaffected by hæmatoxylin but markedly stained by eosin. They may give a reaction for iron, but not always one of the same intensity, for in Oscillariæ it was very slight, and in one preparation of Microcoleus terrestris none was obtained, while in preparations from the same specimen of fresh material made a few days later than the other, the reaction was quite distinct. In two preparations of Scytonema sp. the granules gave no reaction, a result which I attribute to a deterioration of the solution of the sulphide reagent then used. In Cylindrospermum majus these granules give an intense reaction for iron (fig. 8). The iron is not less firmly combined in the substance of these granules than it is in the chromatin, for in the last mentioned species the glycerine and sulphide mixture brought out its complete reaction only after an application of ten days or more. Within twenty-four hours after the addition of the mixture, they gave, in all the species in which they were iron-holding, a slight greenish reaction. I have not succeeded in demonstrating the presence of iron in them after the use of sulphuric acid alcohol, and the explanation for this is that the latter reagent liberates, but at the same time wholly extracts the iron in these granules, the substance of which, unlike chromatin, is incapable of retaining it.

3. Beyond the fact that the "cyanophycin" granules may contain iron, there is nothing to show a relationship, chemical or physiological, between them and the vesicles. From their situation the "cyanophycin" granules would, as Palla suggested, appear to be the assimilation product of the activity of the chromatophore, while the chromatin vesicles and granules might be regarded as due to processes of elaboration on the part of the central body. In Cylindrospermum majus, which grows on soft mud, the former are usually extremely abundant, but in twenty-four hours after placing the thallus in water, the granules diminish very much in number, and on the third day they may be wholly absent in very many of the filaments. Central vesicles, on the other hand, are in this form extremely

few in number, and the conditions which greatly influence the number of the "cyanophycin" granules have apparently no effect upon them. In Oscillaria Froelichii a filament may contain large numbers of both elements, another may contain "cyanophycin" granules only, while a third may be free from the latter but contain a large number of vesicles, and all in the same preparation. In Cylindrospermum majus the "cyanophycin" granules of the spore diminish somewhat in number and volume during the formation of the episporium, and in the spore which is undergoing its initial division their number is very greatly reduced, the central body appearing at the same time increased in volume.

4. In the heterocysts of Nostoc commune, Cylindrospermum majus, and Scytonema sp., picro-carmine demonstrates the presence of "cyanophycin" substance in a button-shaped body at one or both ends of the cell, according as the heterocyst is terminal or intercalary. A strand of "cyanophycin" connects this body with the contents of the neighbouring cell.[1] In the heterocyst the "cyanophycin" body is quite unconnected with the homogeneous cytoplasm which occupies the remainder of the cavity, and stains but faintly with hæmatoxylin and not at all with picro-carmine. When subjected to the prolonged action of the glycerine and sulphide mixture the "cyanophycin," both of the button and of the strand, gave a deep reaction for iron, and a feebler reaction was obtained in the cytoplasm (fig. 8).

It thus appears that in the Cyanophyceæ there is a substance, containing "masked" iron, in many respects like the chromatin of more highly organized cells, and that the "cyanophycin," a compound of undetermined nature, may, in some forms at least, also give evidence of the presence of the element in a firmly combined condition.

[1] This connection has already been described by Hansgirg, 'Physiologische und Alyologische Studien,' Prague, 1887, pp. 125, 126. The description is quoted in full by Deinega.

General Remarks.

The facts described in the preceding pages appear to indicate that a substance, in which iron is firmly held, is a constant constituent of the nucleus, animal and vegetable, of the cytoplasm of non-nucleated organisms and those possessed of apparently rudimentary nuclei, and that, further, a similar iron-containing substance obtains in the cytoplasm of ferment-forming cells. This substance, to which cytologists apply the term chromatin, cannot, on theoretical grounds, be regarded as constant in its molecular structure, even in the same organism, and its most marked characteristic, apart from the iron in its composition, is the occurrence in it of nuclein or nucleinic acid.

Beyond the fact that the iron is firmly held, it is difficult to say how it is disposed in the molecular structure of the nuclein or nucleinic acid. It is, possibly, united directly to the carbon of the latter. The acid alcohols liberate it as a ferric salt, but this fact cannot be held to indicate that it is combined in the nuclein or nucleinic acid in a ferric state, since from solutions of potassium ferrocyanide, in which the iron is contained in a ferrous state, acids liberate the iron in a ferric condition,[1] as evidenced by the formation of ferric ferrocyanide or Prussian blue.

It is also difficult to say whether there is, in the way in which the iron is held in the animal cell, anything different from that obtaining in the vegetable organism. I have, as a rule, found it easier, in the case of the vegetable cell than in that of the animal cell, to liberate the iron with ammonium hydrogen sulphide; but upon this no conclusion may be founded, since the same reagent liberates the iron of free hæmatin readily, while it does not affect the iron of hæmatin in hæmoglobin, and it is possible that in the animal cell the

[1] The iron immediately on liberation may be in the ferrous state, but it quickly assumes the ferric form. Similarly, the iron liberated in the chromatin may at first be a ferrous compound which, with the continued action of the liberating reagent and under the conditions obtaining in the hardened tissues, may further undergo a conversion into a ferric salt.

proteid molecules attached to the iron-containing nuclein or nucleinic acid may more greatly affect the activity of the reagent than those of the vegetable cell are capable of doing. Since, on the other hand, hæmoglobin, which, as I have pointed out, is derived, in Amblystoma, from chromatin, occurs in a large number of animal forms, but is present in no vegetable organism, it would appear to follow that the iron is combined in animal chromatin in a way unlike that in which it is held in the vegetable cell.[1]

The apparently universal occurrence of such iron compounds renders intelligible the fate of the iron salts absorbed by plants from the soil, and of the iron compounds found by Raulin[2] and Molisch[3] to be necessary for the growth of Aspergillus niger. Chromatin, to the formation of which the iron absorbed contributes, is, as the results of cytological investigations show, a substance of primary importance to the cell, and a diminution in, or a cessation of, the supply of iron to the vegetable organism, which produces the condition known as chlorosis, instead of affecting only the formation of its chlorophyll, as generally supposed, strikes at its very life.

The conditions known as anæmia and chlorosis in the higher Vertebrates have been hitherto explained as caused by a diminished production of hæmoglobin directly from organic or inorganic iron compounds absorbed by the intestine from the food matters; but they must now be referred to a deficient supply of the primary iron-containing com-

[1] Compounds which appear to resemble, somewhat remotely, the hæmatins of animal organisms have been found in Palmella cruenta (Phipson, "Sur la matière colorante du Palmella cruenta," 'Comptes Rendus,' vol. lxxxix, p. 316, 1879), and in Aspergillus niger (Linossier, "Sur une hématine végétale; l'aspergilline, pigment des spores de l'Aspergillus niger," 'Comptes Rendus,' vol. cxii, p. 489, 1891). The colouring matter of the latter is, as I have found, held in the membrane, but not in cryptoplasm of the spore, and it would, therefore, appear to be simply a degeneration product.

[2] "Études chimiques sur la végétation," 'Annales des Sc. Nat.,' Bot., Série 5, vol. xi, 1869, p. 93.

[3] Op. cit., pp. 97—117.

pound, chromatin, not only in the hæmatoblasts, but in all the cells of the body. The consequently lessened proliferation of cell and tissue would explain the hypoplasia of the imperfectly developed vascular system observed by Virchow[1] in chlorotic human subjects.

Accepting this explanation of the nature of chlorosis, one may infer that this condition is not limited to animal organisms in which hæmoglobin is found, although its occurrence in others may be difficult to detect because of the total absence of this pigment. From this point of view animal chlorosis is fundamentally similar to the chlorosis of the vegetable kingdom.

The oxygen-carrying property of hæmoglobin and of hæmatin is generally attributed to the iron present in these, because when hæmatin is deprived of its iron, the resulting compound, whether hæmatoporphyrin or bilirubin, manifests no affinity for oxygen. The proof may not be quite conclusive, for we cannot be certain that either compound represents the unchanged remainder of the hæmatin less its iron, but assuming that it is correct, it follows, as I have pointed out in my previous communication, that the antecedent of hæmoglobin, chromatin, has the capacity of absorbing and retaining oxygen, and that one may attribute the processes grouped under the term "vital," to an alternation of the conditions of oxidation and reduction in the iron-holding nuclear constituent. This hypothesis, reasonable as it now appears to me to be, I do not regard as free from difficulties, since in vegetable cells the two processes of respiration and assimilation, involving two activities of different natures, so far as the oxygen is concerned, appear to postulate the existence of two different iron compounds in the same nucleus.[2] There are no facts to indicate

[1] "Ueber die Chlorose und die damit zusammenhängenden Anomalien im Gefässapparate, insbesondere über Endocarditis puerperalis," 'Vortrag.,' Berlin, 1872.

[2] On the relations of the vegetable nucleus to the processes of assimilation, see Strasburger, 'Ueber Kern- und Zelltheilung im Pflanzenreiche,' 1888, pp. 194—204.

the occurrence of such, and it is scarcely possible to explain away the objection without advancing some hypotheses regarding the action of the sulphur and the phosphorus in the nuclein. I propose to detail these on another occasion.

EXPLANATION OF PLATES 10—12,

Illustrating Dr. A. B. Macallum's paper "On the Distribution of Assimilated Iron Compounds, other than Hæmoglobin and Hæmatins, in Animal and Vegetable Cells."

Explanation of Figures.

Note.—In the preparation of all the figures Abbé's camera lucida was employed when the size of the objects represented permitted its use, and all except 25, 26, 35, and 36 are illustrated as they were seen with an apochromatic immersion objective (Zeiss 3 mm., 2 mm., or 1·5 mm.). The exceptions are represented as they appeared under a Zeiss D. Figs. 1—40 show the distribution of assimilated iron as it was demonstrated by the dark green colour of ferrous sulphide, but in Figs. 41—53 the disposition of iron compounds of this kind is indicated by the colour of the Prussian blue reaction.

Fig. 1.—A nucleus and a cell from the testicle of Necturus lateralis. Alcohol, the glycerine and sulphide mixture eleven days. × 620. This and the two succeeding illustrations were drawn from the very first preparations made with this reagent.

Fig. 2.—Testicular elements of another example of N. lateralis. Alcohol, the glycerine and sulphide mixture eleven days. × 620.

Fig. 3.—*a*, a leucocyte, *b*, a red corpuscle, of N. lateralis. Alcohol, the glycerine and sulphide mixture six days. × 500.

Fig. 4.—Two yeast-cells, Saccharomyces cerevisiæ. Alcohol, the glycerine and sulphide mixture ten days. × 1500.

Fig. 5.—Four yeast-cells, Saccharomyces Ludwigii. Alcohol, the glycerine and sulphide mixture four days. × 1640.

Fig. 6.—The developing and fully-formed spores of Cystopus candidus. *a*, *b*, *c*, *d*, *e*, alcohol, sulphuric acid alcohol two days, ammonium hydrogen sulphide in glycerine. × 750. *f*, alcohol, the glycerine and sulphide mixture ten days. × 680.

Fig. 7.—Spores of Aspergillus glaucus, *a*, in the unripe, *b*, in the ripe condition. Alcohol, the glycerine and sulphide mixture three days. × 1640.

Fig. 8.—Cells, heterocyst (*h.*), and spore (*sp.*) of Cylindrospermum majus. Alcohol, the glycerine and sulphide mixture fourteen days. × 1640.

Fig. 9.—Three cells of a filament of Microcoleus terrestris. Alcohol, the glycerine and sulphide mixture four days. × 2000.

Fig. 10.—*a*. Spores (immature), *b*, *c*, and *d*, basidia of a leucosporous Hymenomycete. *d*. Basidium with sterigmata and one attached spore. Sterile (?) element in *b*. Alcohol, the glycerine and sulphide mixture eight days. × 820.

Figs. 11—13.—Portions of hyphæ of Hyphelia terrestris Fries, illustrating the development of the fructification, 13 *a* and *b* representing the simplest form. Alcohol, the glycerine and sulphide mixture three days. × 820.

Figs. 14—18.—From the ovary of a specimen of Erythronium americanum hardened in alcohol. Fig. 14 illustrates the effect produced by diammonium sulphide and glycerine in two days; Figs. 15, 17, and 18 represent that produced by ammonium hydrogen sulphide and glycerine in the same time; and in Fig. 16 is shown how intense the reaction appeared after treatment for four days with the same reagent. × 1240.

Figs. 19—22.—From the ovary of a specimen of Erythronium americanum hardened in alcohol. Sections treated for thirty hours with sulphuric acid alcohol, and mounted in a mixture of glycerine and ammonium hydrogen sulphide. × 1240.

Fig. 23.—Four hepatic cells from a specimen of Necturus lateralis. Alcohol, the glycerine and sulphide mixture eight days. × 620.

Fig. 24.—Two hepatic cells from the same animal, illustrating the distribution of the iron and the nuclear structure after they were treated with sulphuric acid alcohol for twenty-four hours, and mounted in a mixture of glycerine and ammonium hydrogen sulphide. × 620.

Fig. 25.—An example of Stentor polymorphus. Alcohol, the glycerine and sulphide mixture two weeks. × 305.

Fig. 26.—An example of Stentor polymorphus. Alcohol, Bunge's fluid thirty-seven hours, ammonium hydrogen sulphide and glycerine. × 305.

Fig. 27.—Examples of Vorticella sp. Alcohol, the glycerine and sulphide mixture seven days. × 600.

Fig. 28.—An example of Epistylis sp. Alcohol, Bunge's fluid twenty-four hours, glycerine and ammonium hydrogen sulphide. × 600.

Fig. 29.—An ovum of Ascaris mystax, fixed during impregnation. Only a portion of the ovum is represented. Alcohol, the glycerine and sulphide mixture eight days. × 820.

FIG. 30.—An impregnated ovum of Ascaris mystax, showing the division of its nucleus (*n.*) and the condition of the spermatozoid (*sp.*). Alcohol, the glycerine and sulphide mixture ten days. × 750.

FIGS. 31 and 32.—Spermatozoids of Ascaris mystax. Alcohol, the glycerine and sulphide mixture nine days. 31, × 820; 32, × 1640.

FIGS. 33—36.—Ovarian ova of the lake-lizard, Necturus lateralis, illustrating differences in the distribution of the "masked" iron. In 35 is shown the iron-containing peripheral nucleoli, and *a* represents a more highly magnified (= × 1240) portion of the nuclear structure. In 36 is seen an earlier stage with *a*, a portion of its nuclear network, more highly magnified (× 1240). Alcohol, sulphuric acid alcohol thirty-six hours, glycerine and ammonium hydrogen sulphide. × 305.

FIG. 37.—Retinal rods and cones from a larva of Amblystoma. Alcohol, whole of retina in Bunge's fluid two days, glycerine and ammonium hydrogen sulphide. × 620.

FIG. 38.—Cells from the pancreas of a larva of Amblystoma. Alcohol, Bunge's fluid (on the whole of the organ) two days, glycerine and ammonium hydrogen sulphide. × 620.

FIG. 39.—A portion of a section of the human epidermis, illustrating the occurrence of "masked" (?) iron in the granules (eleidin) of the stratum granulosum and in the stratum lucidum. Alcohol, sulphuric acid alcohol two days, glycerine and ammonium hydrogen sulphide. × 620.

FIG. 40.—Strands of fibrils from the muscle fibre of a larva of Amblystoma. Alcohol, sulphuric acid alcohol two days, glycerine and ammonium hydrogen sulphide. × 750.

FIGS. 41 *a* and *b*.—From the ovary of a specimen of Erythronium americanum; *b* represents an isolated nucleus. Alcohol, sulphuric acid alcohol thirty hours, acid ferrocyanide mixture, balsam. × 1240.

FIG. 42.—A cell from a section of the ovary of the same specimen, with the iron demonstrated as in last case, but the preparation, before being mounted in balsam, was stained with eosin. × 1240.

FIGS. 43 and 44 *a* and *b*.—Nuclei of the embryo sac of a specimen of E. americanum. Alcohol, sulphuric acid alcohol thirty-six hours, acid ferrocyanide mixture, balsam. × 620.

FIGS. 45 *a* and *b*.—Nuclei from the liver of a specimen of Necturus lateralis. *n.* Nucleoli. Alcohol, sulphuric acid alcohol thirty-six hours, acid ferrocyanide mixture, balsam. × 1240.

FIGS. 46 *a*—*d*.—Hepatic nuclei treated as in foregoing case, also stained with safranin to illustrate the differences between the chromatin network and the nucleoli in regard to the effect of this reagent. × 1240.

FIG. 47.—Two hepatic nuclei treated as in the preparation illustrated by

Fig. 45, but also stained with eosin, which deeply colours the nucleoli and non-iron-containing constituents (see text). × 1640.

Fig. 48.—Nuclei of the epithelial cells of the intestinal mucosa of Necturus lateralis. Alcohol, sulphuric acid alcohol thirty-six hours, acid ferrocyanide mixture, balsam. The preparation from which *b* was drawn was, before being mounted in balsam, stained with safranin. × 1240.

Figs. 49 *a* and *b*.—Two examples of Euglena viridis. Alcohol, sulphuric acid alcohol thirty-six hours, acid ferrocyanide mixture, balsam. × 820.

Fig. 50.—A portion of a nuclear filament from the "salivary" gland of a larva of Chironomus. Alcohol, sulphuric acid alcohol thirty-six hours, acid ferrocyanide mixture, balsam. × 1640.

Figs. 51 *a*, *b*, *c*, *d*.—Cells and portions of filaments of Oscillaria Froelichii; *b* and *d* represent the isolated cells as seen through their transverse walls. The "cyanophycin" granules are coloured red. Alcohol, sulphuric acid alcohol three hours, acid ferrocyanide mixture, picro-carmine, balsam. × 1640.

Fig. 52.—A portion of a filament of Microcoleus terrestris. Alcohol, sulphuric acid alcohol three hours, acid ferrocyanide mixture, balsam. × 1500.

Fig. 53.—Examples of an organism obtained from sewage water (see text). Alcohol, sulphuric acid alcohol eight hours, acid ferrocyanide mixture, balsam. × 2000.

On the Structural Changes in the Reproductive Cells during the Spermatogenesis of Elasmobranchs.

(From the Huxley Research Laboratory, R. Coll. Sci. Lond.)

By

J. E. S. Moore, A.R.C.S.

With Plates 13—16, and figs. in text.

"Plagiostomorum spermatosomata, quæ magnitudine et peculiari quadam forma in primis ad evolutionis studium apta sunt, hac ratione iam plurium observationes in se contraxerunt."—Lavalette St. George.

1. During the development of a metazoan embryo, after the differentiation of the generative cells from those of the general somatic "anlage," the reproductive elements pursue a course of evolution peculiar to themselves. Instead of attaining to the high specialisation, decay, and final dissolution, characteristic of somatic tissues, their variation is of much less amplitude, but cyclical, and returns at length to the production of elements similar to those in which both series started.

In animals, the course of such a reproductive cycle appears at first sight to be differentiated into two distinct periods of activity, the one extending from the earliest embryonic development of the generative elements to the commencement of the proper spermato- or ovo-genesis, the other beginning with the spermato- or ovo-genesis and ending with the formation of the mature reproductive cells.

In reality, however, the transition from the first of these periods to the second is much more expressive of changes

incident to the surrounding parts, than any alteration in the structure of the generative cells themselves.

In fact, no definite change occurs in them till late in the second period, when the advent of the so-called "reduction process" produces a sharp and definite alteration in the morphological value of all the elements affected. So characteristic is this change, that the time of its commencement can be used as a point of reckoning in all those reproductive cycles in which it is apparent.

For the sake of clearness, that part of the reproductive cycle in Elasmobranchs which comes before the proper spermato- or ovo-genesis will be called the primary or embryonic period, while the cellular generations of spermato- or ovo-genesis before and after the numerical reduction of the chromosomes will be distinguished as those of the first and second spermato- or ovo-genetic series.

It is my purpose in the present paper to give a detailed exposition of the changes witnessed in the generative cells during the reproductive cycle of Elasmobranchs, after the completion of the embryonic period in the male, i. e. during the proper spermatogenesis in the fish, and then to draw such conclusions as may seem legitimate from a comparison with other forms.

I. Technical.

2. The materials with which the present investigation was carried out were obtained while I occupied the British Association Table in the Naples Laboratory during the months of October, 1893, to July of the following year. They consist of a large number of Elasmobranch testes, including those of Scyllium canicula, Scyllium catulus, Pristiurus, Torpedo, Raja macrorhynchus, and Raja maculata. The testes were cut up into small pieces about the size of half a cubic centimetre, and fixed in various ways. I obtained the most successful preparations after the use of Flemming's strong solution, Hermann's fluid, osmic acid in various strengths, and corrosive sublimate, both with and without acetic acid. Valuable comparative material was also obtained by treating the

testes with glacial acetic acid, and washing quickly in water, by tearing up the fresh material in acetic carmine, by fixing in a 2 or 3 per cent. solution of formic aldehyde, by the use of Carnoy's fluid, and last but not least, by a formic acid method which I hit on quite by accident. This consists in placing small fragments of the living testis in a 50 per cent. solution of formic acid for a few seconds, and then transferring directly to 50 per cent. alcohol, after which they are treated for sectioning in paraffin, in the usual way. By this means the chromosomes were in some cases rendered admirably distinct, but the fixation, so far as I have yet tried it, renders the material difficult to stain.

Although the cells of these different kinds of fish examined necessarily differ somewhat inter se, the differences do not appear to be of any morphological importance, and the following description, although taken more especially from those of Scyllium canicula, is, so far as I am aware, applicable to them all.

II. The Resting Cells in the First Spermatogenetic Period.

3. The successive generations of the smaller and smaller cells which gradually fill up the testicular ampullæ during the first spermatogenetic period are all alike, and I find no essential difference between their structure and that of the single embryonic elements (fig. 1) budded off into the stroma from which they gradually arise.

The chromatin is dispersed as a coarse reticulum throughout the interior of the nucleus, and is not in the least specially related to its surface; in fact it is really denser towards the interior than at the periphery, and contains a large oval or flask-shaped nucleolus, generally attached to the nuclear wall (figs. 2, 11, 12, *n.*).

The chromatic threadwork itself is composed of innumerable staining granules, embedded in a scaffolding of clearer substance (linin) (fig. 12), and the unoccupied nuclear space is filled up with a thin nuclear sap.

4. The cytoplasm presents the usual fine reticulation in its substance (figs. 1, 11), and the nucleus is placed excentrically within its mass, so that there is more cell body on the one side than on the other.

The whole reticulation of the cytoplasm is disposed radially towards a point just outside that part of the nuclear wall which faces the larger mass of the cytoplasm (fig. 2, *r.*), and the point itself is occupied by two small centrosomes (*c.*), which can be stained bright red by treatment with fuchsin and orange G.

There is hardly any archoplasmic substance round the centrosomes, and they, together with their cytoplasmic radiation, which extends quite out to the periphery of the cell,[1] constitute a good example of what I have elsewhere called[2] a simple sphere.

There are one or two small chromatic bodies in the cytoplasm (fig. 2, *b. c.*).

III. The Divisions of the Cells of the First Spermatogenetic Period.

5. Just as the successive generations of resting cells in the first spermatogenetic period are all alike, so also are the divisional metamorphoses by which they are produced.

At the commencement of mitosis the nuclei become swollen, their smooth round contours appearing as if turgid with an excess of intra-nuclear sap (fig. 13), and at the same time the chromatic framework shortens up into a lesser number of stouter threads. But I have not seen any indication of early

[1] Dr. Heidenhain, in his paper published in the 'Arch. für Mikr. Anat.,' Bd. xliii, p. 496, 1894, is anxious to claim priority over me in the discovery of radii extending from the sphere in leucocytes to the periphery of the cell. The words I used in a former paper were these: "a delicate radiation spreads from the whole sphere to the periphery of the cell" ('Quart. Journ. Mikr. Anat. Sci.,' vol. xxxiv, p. 188); and my meaning would have been equally well expressed had I used the word "towards" instead of "to" the periphery. I have therefore no claim at all in the matter, and it seems to me to have about as much importance as the conclusions which Dr. Heidenhain has drawn from it.

[2] "On the Morphological Value of the Attraction-sphere," 'Science Progress,' vol. ii, No. 10.

splitting in this threadwork or the granules which compose it, like that given by Brauer[1] in the corresponding stage of the spermatogenesis of Ascaris. The individual granules (microsomes) become completely massed together into axial cores, from which delicate filamentous radii of linin (figs. 12, 13, *l.*) spread in all directions, and the continuation of this metamorphosis in the chromatin results eventually in the formation of one or two long chromatin threads coiled round the inner surface of the nuclear membrane (figs. 14, 15), the nucleolus lying a little more within.

These threads, as soon as they are found, break up apparently simultaneously into twenty-four bent rods, which form the twenty-four chromatic elements characteristic of the divisions of the first spermatogenetic period ; and at the same time the nucleolus, becoming smaller and smaller, breaks up and disappears.

6. Concomitantly with the above intra-nuclear changes, the centrosomes, originally occupying the focus of the cytoplasmic radiations, separate from one another and pass successively through the positions represented in *c.*, figs. 11, 12, 13, 14.

Owing to the absence of the archoplasmic constituent of the sphere, there is in these cells no real archoplasmic spindle formation (as in the spermatocytes of Salamandra described by Hermann[2]). Each centrosome, with its crown of radiations, simply travels away from the other, until, at the period of the nuclear evolution reached in the last paragraph, they lie on opposite sides of the nucleus, with almost the whole diameter of the cell between them (cf. *c.*, figs. 12, 13, 14).

7. As soon as the cytoplasmic conditions just described have been attained, the nuclear wall becomes irregular and disappears, while the chromosomes, collecting under the contraction of their connecting linin filaments, form a long oval mass stretched across the nuclear sap between the centrosomes (figs. 15, 16, 17).

[1] "Zur Kenntniss der Spermatogenese von Ascaris megalocephala," 'Arch. für mikr. Anat.,' Bd. xlii, pp. 153—208, figs. 3—5, Taf. xi.

[2] 'Arch. für. mikr. Anat.,' Bd. xxxvii.

At first the chromosomes are attached to the centrosomes by a few faint protoplasmic strands, which are apparently of cytoplasmic origin; but as time goes on the chromatin assumes a more and more equatorial position, and the linin filaments, being left stretched towards the centrosomes, help to form the central portion of an achromatic spindle figure, the equatorial moiety of which is nuclear, while its extreme ends appear to be cytoplasmic (figs. 17, 18, 19).

8. A portion of the astral radiation round the centrosomes becomes connected with the outer ends of the chromatic rods, clothing the inner achromatic spindle with a sheath of cytoplasmic fibres (*hm.*, fig. 19), structurally equivalent to Hermann's "outer mantle."

9. The chromosomes to which these fibres are attached assume the form of short bent rods, and lie (fig. 19) at all angles on the equatorial plane, being by no means specially related to the surface of the spindle figure, and in surface view they consequently present the appearance of a somewhat irregular chromatic disc (fig. 21).

10. The achromatic spindle would thus appear to have a dual origin, its superficial portion and extreme ends originating in the cytoplasm, while its greater internal and equatorial mass arises from the nucleus—a state of things approximately coinciding with Flemming's [1] views respecting its complex origin in Amphibia, as opposed to the general acceptation of its wholly cytoplasmic nature among plants.

11. When the equatorial plate is fully formed, the chromosomes, after becoming extremely broad and flat, split longitudinally down the middle, each into two daughter-threads, which gradually separate from one another towards the spindle-poles (figs. 19, 20, 22, 23). During their transit those daughter-elements, which were at first internal, work outwards to the surface of the spindle in such a way that by the time they are halfway from the equator to the poles, the chromosomes of each daughter-nucleus have assumed the

[1] "Neue Beiträge zur Kenntniss der Zelle," ii Theil., 'Arch. für mikr. Anat.,' Bd. xxix, p. 389.

well-known open ring-form of the diastral figure represented in fig. 24.

12. In consequence of this outward motion of the inner chromosomes, the spindle (now intra-zonal) fibres with which they are connected become drawn out from their original axial position, and form a central fibrous tube (fig. 24, *i. s.*) enclosed by (i) the remains of the nuclear sap, (ii) the external spindle (intra-zonal) fibres (*o. s.*), and (iii) some linin filaments left stretched between the separate chromosomes, which last, in my opinion, represent the true[1] "Verbindungsfäden." The "Verbindungsfäden" and the intra-zonal fibres now become indistinguishably fused, while the differentiation of the achromatic spindle into an inner and an outer sheath, becoming more distinct, gives to that structure the appearance of two concentric tubes, one of which (*o. s.*, fig. 24) is stretched directly between the outer edge of the chromatic rings, the other (*i. s.*, fig. 24), passing internally through them, to its termination in the centrosomes. The unstained fluid which separates the outer from the inner of these sheaths is that which previously filled the interspaces between the younger spindle-fibres, and it was once the parental nuclear sap. It contains a few irregular chromatic particles (fig. 24, *b. c.*), which appear to have been left as débris of the previous chromosome formation, and which sooner or later pass (fig. 26, *b. c.*) into the cytoplasm of the cell.

While the above changes are in progress the centrosomes become gradually surrounded by a dusky zone (figs. 25, 26, 27), which is caused by the shortening up and coalescence of the cytoplasmic fibres between them and the chromosomes, *i. e.* by those described above (§ 8) as structurally equivalent to Hermann's mantle.

13. The chromosomes in the two daughter-rings (fig. 24) are at first quite distinct from one another, although lying closely side by side, but as time goes on they fuse together,

[1] See Ishikawa, "Studies of Reproductive Elements; Noctiluca," 'Journ. Sci. Col. Imp. Univers. Tokio,' vol. vi, p. 322.

until the chromatin eventually forms two solid chromatic rings, one in each daughter-cell (figs. 25, 26).

14. About this time the outer spindle-fibres begin to spread so widely in the equatorial plane (fig. 24) that they actually come in contact with the membrane of the cell, and at each of these rather angular connections there appear slight thickenings of the fibres (*bi.*), which stain, and thus constitute an interesting stepping-stone between the true cell-plate and Flemming's[1] intermediate bodies.

The chromatic rings now gradually lose their original connection with the outer spindle-fibres, which begin to bulge out, and pass round them to the poles (fig. 27′); the chromatic rings are thus left in a surrounding vacuole, but the core of fibres (*o. s., i. s.*, fig. 27 *a*) still passes through them to the poles. The bulging out of the spindle-fibres round the nucleus increases, and is accompanied by a corresponding collapse of the same in the division plane of the daughter-cells (figs. 25, 26, 28). This brings the outer and the inner spindle-tube together in the division plane (figs. 26, 27, 28), and the whole spindle figure at last acquires the appearance of a sharply differentiated fusiform body between the daughter-cells (fig. 27 *a*). The terminal portions of this body (the remains of the spindle-fibres) as they pass round the daughter-nuclei (fig. 27 *a*, *n.g.*), are at first distinctly seen, but they become shortly indistinguishable from the surface of the vacuole and are consequently lost; but the conical extremities of the fusiform equatorial portion which remains are still prolonged as delicate protoplasmic filaments, which extend towards the nuclei in each daughter-cell (figs. 29, 30). These thread-like prolongations are the remains of that inner core of spindle-fibres above described (§ 12) as passing through the daughter-rings. In a polar view (fig. 27, *l.*) they perforate the daughter-nuclei very much to one side, and the little orifice (*o.*) is all that is left of the originally wide passage through the chromatin.

15. The closing up of the chromatic rings commences on the equatorial side, and is produced by the formation of a

[1] Flemming, loc. cit.

thin chromatic floor. In consequence of this, the spheres (fig. 27, *a. b.*) appear to occupy the hollow of a little nuclear cup, and by the continuance of this filling-up process the remnant of the inner spindle-core is pushed gradually to one side, and eventually out of the nucleus altogether, but it continues to pass round the nucleus (in a more or less deep furrow) (fig. 27 *a, n.g.*) towards the spheres.

16. The course of the terminal spindle-filaments becomes generally coincident with the surface of the vacuole about each nucleus, and they consequently take a curved course from each end of the fusiform remains of the original spindle-figure (between the cells) to the spheres in the polar faces of the nuclei. So that the whole arrangement of the daughter-nuclei and spindle-fibres at this time bears (see fig. 27 *a*) a curious resemblance to the figures seen in the divisions of the micronucleus[1] during the conjugation of many infusoria.

17. The chromatin in the daughter-nuclei now blows up once more into a foam, and eventually completely fills the vacuoles originally surrounding them (figs. 28, 29, 30) while a nucleolus appears in the reticulum of each, generally at the base of the shallow depression (*ng.*, fig. 29), which persists as the remains of the nuclear cup described above (§ 15). This depression, together with the spheres, is gradually rotated somewhat to the equatorial side (as in fig. 29), and the chromatic granules existing in the cytoplasm, becoming fewer in number and larger in size, assume the characters of the chromatic bodies described by Hermann in the spermatogenesis of Mammalia (fig. 30, *b. c.*).

18. The cells are now practically at rest once more, but the fusiform spindle remnant, with its equatorial band of intermediate bodies (fig. 30, *b. i.*), continues long after the daughter-cells have come to rest, and eventually degenerates and vanishes in the equatorial plane.

Mitoses of the above description are carried out with hardly any variation in their details, through all the cellular divisions

[1] See Maupas, "Le Rajeunissement Karyogamique chez les Ciliés," 'Arch. de Zool.,' exp. tm. vii, 1889 (pl. ix, figs. 14—20).

of the first spermatogenetic period, and in the course of this the features which appear to be of primary comparative importance may be summarised as follows :

I. The existence in the resting cells of a large round nucleolus lying near the nuclear periphery.

II. The evolution during the prophasis of division, of twenty-four bent chromosomes, which shorten up, and split longitudinally in half to form the same number of chromosomes, twenty-four, in each daughter-cell.

III. The existence of an extra-nuclear attraction sphere, which, during this period of the spermatogenesis, is practically destitute of archoplasm, being surrounded by a simple cytoplasmic radiation like that observed in many forms of tissue cells.

IV. The consequent non-formation of an archoplasmic spindle figure, and the dual origin of this latter structure, partly from the simple cytoplasmic radiation, partly from the intra-nuclear substance.

V. The differentiation of the spindle during the dyastral figure into an outer and an inner fibrous sheath, which, after the escape of the parental nuclear sap, collapse and coalesce, forming a delicate connecting thread between the attraction spheres of both daughter-cells.

VI. The formation of extra-nuclear chromatic bodies from the débris of the nuclear chromatin.

IV. The Rest of Transformation (Synaptic Phase) between the First and Second Spermatogenetic Periods.

19. As I have already pointed out, the transition from the first into the second spermatogenetic period is completed in the cells during the rest which follows the last division of the first, and when the elements in the ampullæ are seven or eight rows deep (fig. 34). Such cells, although at first retaining the characteristics of those of previous generations, gradually acquire new ones, but so gradually that it is some time before we realise the profound nature of the changes wrought, and that, while yet

apparently at rest, the cells have passed completely over from the first into the second spermatogenetic period. The commencement of this metamorphosis is marked by an increasing fineness of the reticulum in the nuclei, which continues to increase until cells with nuclear elements like that represented in fig. 35 are seen, and about the same time there appears a curious secondary nucleolus surrounded by a vacuole (fig. 31 *n'*), which, so far as I can ascertain, is in these fishes diagnostic of the change. After a while the nuclear threadwork again grows coarser and thicker, displaying at the same time a peculiar tendency to contract to one side of the nucleus, leaving a great clear space (fig. 39) across which stretch numerous linin filaments. The contraction is not so marked when the cells have been preserved with osmic acid, nor on the outside of sections which have been preserved with Flemming's fluid, where the osmium has acted directly upon the cells. I have, however, seen it in elements of Torpedo which were simply immersed in dilute glycerine; and whether it exists in nature or not, the cells display at this period, and at no other, a remarkable tendency to have their chromatin contracted, in consequence of some internal change which renders these nuclear figures diagnostic of the particular period in question. Similar figures have been obtained at corresponding periods in the spermatogenesis of Amphibia, Mammals, Nematodes,[1] and various Arthropods,[2] and I do not think it probable that the contraction in many of these cases has anything to do with the reagents used.

20. In the cytoplasm the conversion from the first to the second spermatogenetic period is marked by a gradual increase in the small dark zone about the centrosomes, until it eventually attains the dimensions of a veritable spermatic "Nebenkern" or archoplasm (figs. 35, 36, 37), and from what has been said (§ 12) it follows that this body is here of an entirely cytoplasmic origin. The archoplasm, with its contained cen-

[1] Brauer, loc. cit. (pl. ix, figs. 12—18).

[2] See Toyama, "On the Spermatogenesis of the Silkworm," 'Bull. Agric. Coll. Imp. University Tokio,' vol. ii, No. 3, 1894, pl. iv, figs. 25, 26.

trosomes, is at first closely applied to that part of the nuclear wall within which the curious lopsided condensation of the chromatin goes on.

21. The fine-meshed, tightly-coiled condition of the chromatin persists some time, but it gradually resolves itself into a coarse chromatic network on the nuclear periphery (figs. 37, 38). The strands of this network are sharply polarised towards the position occupied by the archoplasm and the centrosomes. The large oval nucleolus present in the resting cells of the first spermatogenetic period becomes now somewhat modified, both in position and character. Instead of being disposed casually along the nuclear circumference, it takes a position, generally, but not always, in line with the long axis of the archoplasm (fig. 37, *n.*). Along this line there is still to be seen the secondary nucleolus (fig. 36, 37, $n.^1$) surrounded with a vacuole, which I described in § 19.

These two peculiar forms of nucleoli are always to be found after the transition from the first into the second spermatogenetic period, and throughout all the generations of the latter.

22. The archoplasm, which at first lies closely applied to the nuclear wall, during the early stages of the conversion of the first into the second spermatogenetic period, migrates away, quite into the cell body, while the two centrosomes which it contains, moving faster in the same direction, appear shortly on its exterior surface just beneath the membrane of the cell (fig. 37).

V. The Divisions of the Second Spermatogenetic Period.

23. The advent of the first division in the second spermatogenetic period is characterised by the strong polarisation of the chromatin, represented in fig. 37. The chromatic strands are seen on close examination to be composed of a thick core of innumerable microsomes, which, collecting together into groups, give to the strands their curious monilated appearance,

also described by Hermann[1] in the prophasis of the great heterotype division of the spermatogenesis in Salamandra. These monilations in Elasmobranchs, however, do not consist of one large microsome, as Hermann's figure would lead one to expect, but are each formed by a group of numerous chromatic granules, and these are embedded in a scaffolding of linin. Delicate connecting filaments of this substance spread from the monilations on the threads in all directions. The polarised threadwork is disposed throughout the nucleus in long parallel loops (figs. 37, 38), the free ends of which, if they exist, are difficult to discern. After a time the threads begin to show longitudinal splitting (figs. 38, 40), and the double ropes thus formed, dividing into equal segments, eventually give rise to twelve thick loops which (fig. 42) form the twelve ring chromosomes (fig. 43) typical of the divisions of the second spermatogenetic period.

24. There are thus, after the rest of transformation, only half as many chromosomes, i. e. separate chromatic masses, as there were before, and the halving of their number, being brought about while the nuclei are still at rest, is to that extent comparable to what is now known to go forward during the maturation of the reproductive elements of plants. I therefore propose the term Synaptic phase[2] to denote the period at which this most important change appears in the morphological character of reproductive cells.

25. Concomitantly with the formation of the twelve ring chromosomes, the centrosomes (figs. 40, 41, *c.*) begin to separate, and their greatly enlarged archoplasmic envelope (Nebenkern) (fig. 41, *a.*) is drawn out between them into a little archoplasmic spindle (fig. 42), strictly comparable to that described by Hermann during the division of the spermatocytes of Salamandra. As in the divisions of the previous spermatogenetic period, the separation of the centrosomes occurs with great rapidity, the archoplasm being drawn asunder into two parts (figs. 43 and 44), although it sometimes presents the appearance of a

[1] Loc. cit.

[2] Gr. *συνἄπτω*, to fuse together.

fine achromatic line stretched round the nuclear membrane. My preparations indicate both these methods of procedure.

The protoplasmic contents of the cell become radially disposed, not directly to the centrosomes, as in the divisions of the previous spermatogenetic period, but towards the outer surface of the daughter-archoplasms (fig. 43, *r*.), and it consequently follows that the sphere of the first period is structurally less complex than that of the second. In cells which possess an archoplasm, any radiation in the cytoplasm external to this structure has not generally been considered a part of the attraction sphere ; nevertheless, such external radiations are obviously similar to those directly related to the centrosomes in the cells of the first spermatogenetic period, where they would certainly be regarded as a portion of the sphere. To save confusion, therefore, I shall speak here, as I have done elsewhere, of spheres which possess an archoplasm, as compound, and those which do not, as simple, and thus avoid the necessity of determining whether any particular set of radiations should or should not be regarded as constituents of the sphere.

26. The ring chromosomes, which, when fully formed, become dispersed over the nuclear periphery, like those of the first spermatogenetic period, are in like manner connected to one another by numerous filamentous strands of linin (fig. 43, *l*.).

The nuclear membrane eventually becomes irregular, and, giving way at various points, leaves the chromosomes to collect, by the contraction of these connecting filaments, into a long oval mass stretched across the nuclear sap between the centrosomes (fig. 44). The nuclear sap is traversed from the first by numerous fine strands, putting the chromosomes into connection with the outer cytoplasmic network, and which are in all probability part of the latter, dragged inwards from without after the disruption of the nuclear wall. The central chromatic mass is somewhat stretched, and more firmly attached to the old nuclear surface in the direction of the spheres, appearing as if slung between the centrosomes (fig. 44).

As time goes on, the chromosomes assume a more and more equatorial position; but their linin filaments remain stretched out towards the centrosomes, and form the greater portion of an achromatic spindle, the equatorial part of which is consequently nuclear.

27. The astral radiations which surround the centrosomes become connected with the chromosomes in such a way as to clothe the achromatic spindle with a fibrous sheath, structurally equivalent to that described by Hermann (ante, § 8), while even at this early period in the formation of the spindle figure, the centrosomes are sometimes divided at the poles (figs. 45, 46, *c.*).

28. The exact form of the chromosomes, when they appear in the monaster of this first heterotype of the second spermatogenetic series, varies a good deal from cell to cell; but in the majority of cases the loops are at first bent up upon themselves, in the manner represented in figs. 45, 45′. The rod-like bodies thus produced at first stand stiffly out from the surface of the spindle (fig. 45′), but after a time they flatten down in the manner represented in fig. 45. In consequence of this, the two limbs of the loop appear in profile to have the form of two Greek Ω's place, side by side, and the outer surface of the bends being greatly thickened, the original opening of the loop is reduced between them to the merest slit (fig. 45, *s.*). These thickenings on the outer curves of the Ω's would appear to correspond with the thickenings on one side of the heterotype loop of Salamandra, but in Elasmobranchs they developed equally on both limbs. I was consequently interested to find that in the great heterotype division of the spermatogenesis of newts, these thickenings sometimes occur on one, sometimes on both limbs of the elongated loops. From the drawings given by Hermann, Flemming, and vom Rath, who deal with this form of chromosome in the salamander, it would appear that the loops are often intentionally represented with the plane of their openings at right angles to the surface of the spindle, that is, with one limb on and the other off the spindle. However this may be, it is certainly rarely if

ever the case in either Elasmobranchs or newts, in both of which the loops lie flat, with both limbs on the spindle surface.

As the loops lengthen out towards the poles, the outward bends are gradually reduced, but they never disappear, and at the time the chromosomes divide (fig. 47) they separate from one another in such a way that the original openings of the loops are clearly seen. The separation into daughter-elements is effected by a transverse splitting of the loop across the central thickening, at right angles to the then long axis of each chromosome (fig. 47), as is usual in Heterotype metores.

After their separation, the daughter-chromosomes form superficial chromatic rings (fig. 48—51), as did those in the divisions of the first spermatogenetic period (see § 13), and the spindle-fibres in like manner become differentiated into two concentric tubes, separated from one another by the nuclear sap. This differentiation of the spindle into fibrous tubes is carried further than in the divisions of the first spermatogenetic period, the whole structure appearing to be composed of two completely closed cylinders of fibres, one (fig. 49, *o. s.*) stretched directly between the outer edge of the chromatic rings, and the other (*i. s.*) passing internally through them to the centrosomes. The unstained fluid which separates the outer from the inner of these sheaths, is that which previously filled the interspaces between the fibres of the younger spindle, and it was once the parental nuclear sap. It contains irregular chromatic granules (*b. c.*, fig. 50) which appear to have been left as débris of the chromosome formation, and which sooner or later pass into the cytoplasm of the cell (*b. c.*, fig. 54).

29. While the above changes are in progress, the centrosomes become surrounded by the dusky zone, created by the shortening up and coalescence of the cytoplasmic fibres between them and the chromosomes (figs. 48, 49, 50 *a*).

30. The chromosomes of each daughter-nucleus are at this time quite separate and distinct, although lying closely side by side; but as time goes on they begin to fuse together, so that the chromatin eventually forms two solid chromatic rings, one in each daughter-cell (figs. 51, 52, 53).

31. About this time the intra-zonal fibrils spread out, until those from either pole meet at the circumference of the cell (fig. 49), and at these somewhat angular connections there appear beaded thickenings in the threads, which (fig. 50, *b. i.*) stain and thus form an interesting stepping-stone between Flemming's intermediate bodies and a true cell-plate.

32. At the same time the chromatic rings gradually lose their original connection with the outer spindle-fibres (*o. s.*, fig. 52), which begin to bulge out and to pass round the nuclei towards the poles (fig. 53). The chromatic rings are thus cast loose in a surrounding fluid vacuole, but the inner core of fibres (fig. 52, *i. s.*) continues to pass through them.

The bulging out of the intra-zonal fibres round the nuclei is marked by a collapse at the point of their original distension at the equator (figs. 51, 52, 53), which brings the outer and the inner sheath together in the median plane (figs. 52, 53). Thus the central portion of the residual spindle figure presents the appearance of a sharply differentiated fusiform body between the cells (figs. 53, 54, 55). Across its middle there is a chromatic band, produced by the fusion of the intermediate bodies (figs. 53, 54, 55, 56, 57, *b. i.*).

While contemplating the changes I have just described, it is impossible to avoid the impression that the rupture of the outer spindle-sheath and the consequent outflowing of the enclosed fluid to form the nuclear vacuoles (figs. 54, 55, 56, *n. v.*), are the primary causes by which the expanded equatorial spindle-figure is made to collapse, and that it may also have a direct mechanical connection with the formation of the primary constriction between the daughter-cells.

33. As in the divisions of the first spermatic period, the spheres (fig. 37, *a*) begin now to travel over the surface of the nucleus, generally along a groove (*n. g.*, fig. 54) like that described in § 15, towards its equatorial face.[1]

34. The expanded central portion of the spindle remnant now lies between the daughter-cells (which are otherwise quite

[1] 'Arch. für mikr. Anat.,' Bd. xliii, p. 423; cf. M. Haidenhain, op. cit., Taf. xxv (figs. 14, 21, 22, 23).

separate from one another), and inserts a conical termination into both (figs. 53, 54); but the delicate filaments into which these terminations are prolonged, after the translocation of the spheres from the polar to the equatorial surface of the reconstructed nuclei, disappear, the last function of the remains of the outer and inner spindle-tube being to form an open connection with an equatorial chromatic band (the intermediate bodies) between the daughter-cells (*b. i.*, figs. 56, 57, 58, 59).

35. The spheres, during their passage from the polar to the equatorial nuclear faces, pass in a more pronounced manner through a similar metamorphosis to that described in § 22, and which, when rightly understood, appears to be of the most profoundly interesting nature. When the archoplasm (*a*, figs. 58, 59) has reached some point halfway between the pole and the equatorial nuclear side, it begins to move away from the nucleus, while the centrosomes, travelling faster in the same direction, pass from the centre to the surface of its mass (figs. 58, 59, *c.*). From this point (*c.*) there grows out a fine protoplasmic thread (fig. 59, *f.*), extending to the cell periphery. The cell membrane is indented slightly where the thread approaches it (fig. 59), but the thread itself is prolonged beyond it as a fine protoplasmic process, comparable to a short flagellum (figs. 59—64, *f.*). By the time this structure has been formed the archoplasms of each daughter-cell are more or less facing each other, with the tubular remains of the spindle stretched between (figs. 59 and 61).

36. When the cells come perfectly to rest, there appears on each side of the archoplasm, or in its immediate vicinity, a marked condensation of the cytoplasmic substance (fig. 62, *x.*), which, in the absence of the attraction sphere, might readily be taken for an enlarged representative of that body; and as this mass is of some importance in understanding the process of conversion of the next generation of cells into the spermatozoa, I shall speak of it as the Nebenkern.

37. Before the prophasis of the next division, the remains of the spindle become no longer visible between the cells, and

the rudimentary flagellum is withdrawn, but the centrosomes remain immediately beneath the membrane of the cell.

VI. The Last or Second Heterotype Phase of the Second Spermatogenetic Period.

38. The last division in the second spermatogenetic period is a heterotype, like the first, but the elements are scarcely more than half the size. The number of the chromosomes is again twelve, and, like those of the earlier divisions, they become eventually grouped together as a globular mass in the centre of the nuclear sap (figs. 66, 67, and 68).

When the spindle has been formed, the ring chromosomes are not altogether on its surface, and, owing to their small size, a polar view of the monastral figure often presents the curious appearance represented in fig. 70, on account of the upper and lower edges being in focus at the same time. The chromatic loops eventually split transversely, like those of the previous division, the daughter V's travelling to the pole in the manner represented in figs. 69, 71, and 72.

Thus the last division in the spermatogenesis of these fishes is, as I pointed out so early as in 1893,[1] a perfectly normal affair, each mother-chromosome splitting into two daughter-elements, so that the two cells produced contain each the same number (twelve) of residual chromosomes. Consequently, like the last division in Mammalia, it presents nothing in common with the "Reductionstheilung" described by Hertwig, and upon the assumed universality of which so much of Weismann's latest theory of heredity is built.

39. The process of return to rest of the daughter-cells (spermatids) is in all respects essentially the same as that in the previous generation. The distension of the outer spindle-sheath (fig. 73) and the formation of intermediate bodies (*b. i.*) (cell plate) is perhaps more marked, the inner tube (*i. s.*) appearing consequently more isolated and alone. But there is the same detachment of the chromatin from the fibres of the outer sheath

[1] "On the Germinal Blastema of Cartilaginous Fishes," 'Anat. Anz.,' Bd. ix, p. 547.

(figs. 75 and 76), the same formation of a nuclear vacuole in each daughter-cell (fig. 76, *n.v.*), the same gradually diminishing connecting spindle filament between the nuclei (figs. 76 and 77), and lastly, the same formation of nuclear grooves (fig. 77, *n. g.*) along which the spheres travel to the equatorial side. Further, when the spheres have reached some point halfway between the polar and equatorial nuclear faces, the archoplasm leaves the nuclear wall. The centrosomes (fig. 79, *c.*) pass on to the outer archoplasmic surface, and from this there passes a fine protoplasmic strand (fig. 80, *f.*) to the cell periphery, and the cell membrane is indented where this strand perforates it as the whiplash-like spermatozoon tail. It thus becomes evident that the metamorphoses described in § § 22, 35 are nothing more nor less than abortive attempts at tail-formation, and it consequently follows that the synaptic phase in these fishes marks the assumption by the cells, during spermatogenesis, of a flagellate condition.

40. Besides the nucleus, there appears a dusky condensation of the cytoplasm (*x.*, figs. 80—83), which at first sight gives the cells the appearance of possessing more than one attraction sphere, and is obviously similar to the nebenkern of the preceding generation (of § 36). This body when it first appears is closer to the nucleus than the archoplasm (fig. 80), and in the latter there is seen at the point of origin of the flagellum a clear round vesicle (fig. 82, *a. v.*), which enlarges and eventually moves, with its archoplasmic surroundings and the centrosomes, into close apposition with the nucleus (fig. 83, *a. v.*).

41. The nuclear chromatin rises up into a shallow collar round the base of the archoplasmic vesicle, while the rest of the chromatic substance, contracting from the nuclear membrane, becomes condensed into a flask-shaped mass below the collar (figs. 84 and 85). This contraction increases rapidly while the collar, elongating, spreads into the nuclear membrane at the base of the archoplasmic vesicle, to form a small chromatic flange round the neck of a bottle-like structure

(figs. 85, 86, 87), the body of which is filled with nuclear chromatin, and the neck of which is stopped with the archoplasmic vesicle (fig. 85, *a.v.*). Beyond the chromatic flange the nuclear membrane encases the whole, much in the same way as the basket-work used to protect an Italian wine-flask, the nuclear sap between it and the chromatin representing the glass.

42. The base of the intra-cellular part of the flagellum, with the centrosomes, now lies between the archoplasmic vesicle and the chromatic flange, but the point of attachment of the flagellum moves round the surface of the nucleus, the archoplasmic substance penetrating the nuclear membrane, and resting with the base of the flagellum on the chromatin, in a funnel-shaped mass (fig. 85, *a.*). The centrosomes are no longer visible, being either lost or becoming indistinguishable among the rest of the chromatic substance at the base of the tail. The translocation of the point of attachment of the flagellum continues (figs. 86, 87) until it finally comes to rest at the side of the nucleus opposite to the neck and the archoplasmic vesicle where it started (fig. 88). The "nebenkern" is implicated in this motion, and its substance is eventually mixed up with that of the true archoplasm, both structures forming a distinctly differentiated protoplasmic mass extending along the intra-cellular part of the flagellum, from its base in the nuclear chromatin to its exit through the nuclear wall (figs. 85—89, *x. a.*). The whole of this mass (composed of the "nebenkern" and the archoplasm, together with the intra-cellular part of the flagellum) eventually forms the long Mittelstück of the mature spermatozoon (figs. 90, *m.*). The origin of the Mittelstück in these fishes will thus be seen to coincide with what I have related respecting this structure in Mammalia, and probably with Hermann's description of its formation in Salamandra together with what occurs in a number of Invertebrate spermatogeneses.

43. At the opposite end of the nucleus the archoplasmic vesicle (*a. v.*, figs. 88, 89, 90) becomes first flattened, and then elongates out, together with the nuclear chromatin, forming a definite

cephalic point to the spermatazoon head. The nuclear jacket (figs. 88, 89, 90, *n.v.*), formed by the sap separating the nuclear chromatin from the nuclear wall, continues well marked even at maturity, and the swelling on the cell membrane, where the flagellum originally passed out, remains (fig. 90, *mb.*) as the little bead at the hinder end of the Mittelstück.

The spermatogenesis is now practically complete, and the facts of the second spermatogenetic period which appear to be of primary comparative importance are:

I. The transformation of the cells of the first spermatogenetic period into those of the second, which I have termed the synapsis, is accomplished while the cells are in complete repose, and is marked by a peculiar evolution in the chromatin with the formation of peculiar nucleoli (which are repeatedly characteristic of the succeeding cellular generations) and by the formation of an archoplasmic constituent round the centrosomes.

II. The evolution during the prophases of the first and second divisions of the second spermatogenetic period of twelve ring chromosomes, which split transversely to form the same number, twelve, in each daughter-cell.

III. The differentiation of the spindle during the diastral figure into an outer and an inner fibrous sheath, which coalesce, forming a delicate connecting thread between the attraction spheres of both daughter-cells.

IV. The existence during the synapsis of a peculiar evolution among the constituents of the attraction sphere, whereby the centrosomes are brought to its exterior surface, beneath the membrane of the cell.

V. The repetition of the process in a more pronounced manner, after the first heterotype division in the second spermatogenetic period, so that a short flagellum is protruded from the centrosomes through the membrane of the cell.

VI. The origin of the long whiplash tail of the spermatozoon in a similar manner, after a corresponding metamorphosis of the sphere during the formation of the final cellular generation.

44. The whole course of the spermatogenesis may now be diagrammatically represented as follows: In Diagram I the rings under α represent a succession of resting cells in the first spermatogenetic period, while the signs of division n^1 (24), n^2 (24), &c., stand for the successive divisions by which they are produced. The number 24 represents the constant number of chromosomes in each.

The cone under β represents the synaptic change, while under γ are represented (by black dots) the cellular generations of the second spermatogenetic period, up to the formation of the final spermatozoa.

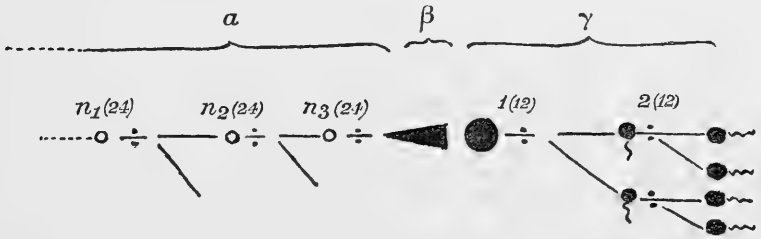


Diagram I, illustrating the course of Elasmobranch spermatogenesis. (α) First spermatogenetic period. (β) Synapsis. (γ) Second spermatogenetic period. n_1 (24), &c., number of chromosomes in each division of first period, where n represents an indefinite number of previous divisions. 1 (12), &c., same in second.

VII. Comparative.

45. As the majority of the operations performed by living protoplasm are inexplicable on any structural arrangement in the parts of cells which has hitherto been observed, it is obvious that the structural relationships which, if known, would render the actions of protoplasm self-explanatory, lie somewhere below the present range of vision, and it consequently follows that the theoretical explanations of this or that property which protoplasm exhibits are, at bottom, nothing more nor less than hypothetical forecasts of the ultimate structure on which this or that manifestation of vitality depends. The probability of any forecast being true is proportionate to the

capacity which its premises exhibit of being logically worked up into harmony with what has been actually observed. Thus, according to Weismann,[1] the phenomena of heredity depend ultimately on the existence of innumerable little unities in the "germplasm," or "ids," and these are in reality the hypothetical doers of everything that is done. They are capable of influencing the protoplasm which surrounds them in different ways, and by coming into action successively during development, they produce the structural differentiation of a complex form. Representatives of all the different kinds of "ids," actual or potential, which exist in any given animal or plant, are continually being locked up for future use in every ovum or spermatozoon formed, and in consequence of the indefinite multiplication of the "ids," which must occur after every act of fertilisation, it appears, according to Weismann, a logical necessity from the premises of his theory, that the reproductive cells, before fertilisation, must each get rid of half their hereditary substance (i. e. "must each get rid of half their nuclear rods"). This is supposed to be accomplished by there being two kinds of division among cells. In the first of these (the ordinary somatic division) the chromosomes split in half, there being consequently the same number in each daughter-cell, and this method of division has consequently been termed "Equationstheilung," to distinguish it from the second or "Reductionstheilung," which is apparently introduced only during the final stages of the development of the reproductive elements, and is brought about by half of the entire number of chromosomes formed during a mitosis passing unsplit into one daughter-cell, and half into the other.

The value of these hypothetical speculations touching the nature of the phenomena immediately antecedent to fertilisation, appeared to be enormously enhanced by O. Hertwig's description of a process answering to the Reductionstheilung in the final stage of the spermatogenesis of Ascaris, because if this process should turn out to be universal, as at one time seemed probable, it would give to the "id" theory an actual

[1] 'The Germplasm,' English trans.

demonstration in fact. Unfortunately, however, for the Reductionstheilung, as well as for the enormous superstructure which Weismann has lately piled upon it, O. Hertwig's observations have been shown by Brauer to be quite erroneous, there being in the spermatogenesis of this animal no such thing as a division in which alternate chromosomes pass unsplit to daughter-cells. So also, during the Elasmobranch spermatogenesis with which we have been dealing (the course of which will be found summarised in these pages at the end of each spermatogenetic period), there is nothing comparable with the "Reductionstheilung" of Hertwig, which is made such an integral part of Weismann's last theory of heredity. It is true that there is a numerical halving of the chromosomes, between the first and second spermatogenetic periods, but this is brought about in the synapsis which separates the one period from the other, and has nothing to do with division at all.

It is so necessary to be quite clear about this, that I have subpended a few lines of Weismann's treatise in which his conceptions of the "Reductionstheilung" are given in full. On page 11 of the English translation of the 'Germplasm,' its author, after speaking of the necessity of a "Reductionstheilung," and as though the universality of its occurrence was an established fact, goes on to say:—"The hypothesis of the Reductionstheilung has been thoroughly substantiated by subsequent observations—in fact, it has even been proved that in many cases this reduction occurs exactly as I had foretold and represented in a diagrammatic figure; that is to say, by the non-occurrence of the longitudinal division of the chromosomes, which occurs in ordinary nuclear division, and by the distribution of these in the daughter-nuclei. This holds good for the ovum as well as for the sperm-cell in animals, and as far as is known, in plants also. The germ-cell must in all cases by division get rid of half its nuclear rods." Again on page 236:—"We now know that this reduction in the number of the ids, by one half, is of general occurrence, and is effected by means of the nuclear divisions which accompany cell divi-

sion. The divisions which result in the formation of the polar bodies perform the function of the Reductionstheilung as regards the ovum, and the final divisions of the sperm mother-cells have this function in the case of the spermatozoa. In both cases the Reductionstheilung does not consist in the idants (chromosomes) becoming split longitudinally, and in their resulting halves being distributed equally amongst the two daughter-nuclei, as in ordinary nuclear division, but in one half of the entire number of rods passing into one daughter-nucleus, and the other half into the other."

46. The absence in the spermatogenesis of Elasmobranchs of any Reductionstheilung is thus of peculiar interest, because the fundamental way in which Weismann has used this conception of a Reductionstheilung as a basis on which to build up his supposed explanation of heredity, renders it evident that any widespread collapse in the alleged universal existence of this process, either among animals or plants, will in the long run bring down the whole speculative superstructure with it.

47. Now with respect to plants, the two highest living authorities, Strasburger[1] and Guignard,[2] have already dissented from Weismann's views; and the former, in his address to the Biological Section of the British Association meeting at Oxford last year, expressed his opinion of the Reductionstheilung as follows:—"There is no such thing among plants as nuclear division resulting in the reduction of one-half of the chromosomes. Such a conception involves the assumption that the entire, not longitudinally-split, chromosomes of the mother-nucleus become separated into two groups, each of which goes to form a daughter-nucleus." So we may take it that the Weismannistic conception of the "Reductionstheilung," "so far as is known in plants," fails.

48. With respect to animals, Boveri,[3] in his "Befructung," as far back as 1890, after postulating the chromatin as the

[1] 'Ans. Bot.,' vol. viii, 1894.

[2] 'Anns. d. Sc. Nat.,' Bot., 1891.

[3] "Ergebnisse der Anat. und Entwicklungsgeschichte," Bd. i, 1891, pp. 458, 459.

hereditary substance, argues, like Weismann, for the necessity of some sort of chromatic reduction, before the maturation of sexual cells; but he comes also to the conclusion that the reduction processes hitherto described are numerical reductions of the chromosomes, and not quantitative with respect to their substance. He draws a sharp distinction between "id" reduction and chromosome reduction, the latter of which he apparently disregards, but he seems to entertain the idea that the former, by the numerical reduction of the chromosomes, may in reality be carried out. He shows further that the numerical reduction of the chromosomes in ovogeneses, like that of Echinus and Pterotrachea for example, represented in the diagram which I have borrowed (Diagram II), and which will become intelligible on reference to my § 44, is not brought about by any of the divisions in the first ovogenetic period, α, and up to the formation of the first ovocyte after the rest β, i. e. the ovum before the extrusion of the polar bodies, but that there are only half as many chromosomes in the first ovocyte when it emerges from the rest, β, in the polar body-spindle as there were before, and this number is retained after the polar bodies are extruded in the ovum. Therefore the numerical reduction is not brought about by any division of the ovogenesis, but occurs during the synaptic rest, β, and before the prophasis of the first "Richtungspindel."

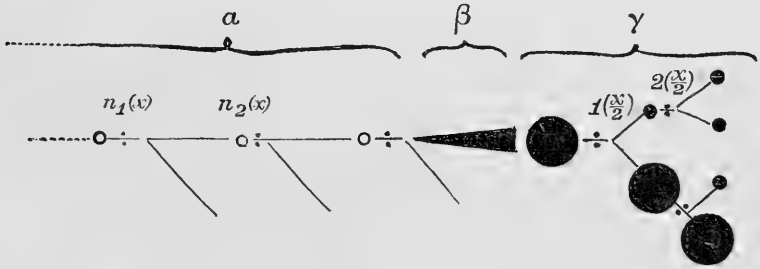


Diagram II, representing course of typical ovogenesis (after Boveri). Reference letters same as in I.

Finally, he shows that the so-called reduction processes hitherto described are irreconcilable among themselves, and

concludes with the following characteristic phrase:—"Durch die vorstehenden Erörterungen, glaube ich gezeigt zu haben dass zwar gewisse Vorgänge bescrieben worden sind, die vielleicht mit der Chromosomenreduction in Zusammenhang stehen, dass uns aber eine wirkliche Einsicht in diesen Vorgang bisjetzt fehlt. Es bleibt weitere Forschung vorbehalten, dieses Dunkel aufzuhellen."

49. In 1893 I published[1] a preliminary account of some investigations concerning the course of the spermatogenesis of mammals, which I summarised in these words: "There is in the Rat (i)[2] a period of indifferent cell formation, terminated by a mitosis with sixteen chromosomes, both in the primary and daughter-nuclei; (ii) a period of growth (or rest) during which the sixteen chromosomes are converted into eight, and terminated by a division in which the daughter-nuclei spermatids still retain the number eight; (iii) a period in which the spermatids are converted into spermatozoa." If

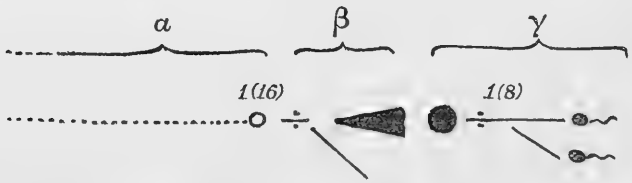


Diagram III, showing course of Mammalian spermatogenesis. Reference letters same as in I.

we now construct a diagram of the first and the second spermatogenetic periods in Mammalia (as in Diagram III), and place it side by side with that of the Elasmobranchs given

[1] "Mammalian Spermatogenesis," 'Anat. Anz.,' Bd. viii, 1893, pp. 683—688.

[2] Dr. Toyama, who apparently writes under the wing of Professor Ischikawa, speaks of my results respecting these phenomena in mammals as being "very improbable," but as he produces no evidence relevant to the subject, I fail to see the force of such a criticism, and have therefore refrained from applying it to several portions of his own treatise, more especially as very little trouble with any native mammal would have enabled him to see whether the "improbable" was true.

in § 44, it will be seen that the former differs from the latter only at the beginning and the end. These differences are produced by the shortening up of the generations of the first spermatogenetic period in mammals, (α) into what is practically a kinetic budding, so that there is only one distinct homotype division with sixteen chromosomes (I, 16) before the synapsis; (β) (equal growing cells) in which the chromatic individuals are reduced or fused together into eight. The process of transformation and the succeeding heterotype correspond exactly with that of the Elasmobranchs. But the daughter-elements produced do not, as in Elasmobranchs, divide again. They are converted directly into spermatozoa, and it thus appears that one of the two generations of ciliated cells, present after the heterotype in Elasmobranchs, in some mammals is unrepresented.

50. Brauer,[1] as I have said, in his admirable account of the spermatogenesis of Ascaris, published in 1891, also denies the existence of the "Reductionstheilung" described by O. Hertwig, both in the uni- and bivalent form of this curious worm. There is a period of cell multiplication, equivalent to the first spermatogenetic period, with two or four chromosomes, as the case may be, and in the divisions of which, as Professor Brauer has recently informed me, the chromosomes split longitudinally, like those in ordinary divisions. Then a period of rest, equivalent to the rest of transformation, in which the number of the chromosomes is halved, followed by divisions of a totally different character, in which there appears to be precocious splitting of the chromatic elements and rapid separation of daughter-cells,[2] without the nuclei

[1] Loc. cit.

[2] It is probable that all the cases of the so-called "Reductionstheilung" are in reality referable to a process of precocious splitting among the chromosomes, whereby the elements for several daughter-cells are produced at once, and are then distributed, either by successive divisions without rest, or by multipolar spindle formation. An admirable example of the latter method is afforded by Farmer's description of the spore formation in Pallavicinia decipiens.

In these plants the number of the chromosomes in the sporophyte generation is always eight, but as soon as the spore mother-cells are formed, the eight

returning into rest. The closeness of the similarity of the spermatic reduction described by Brauer with those detailed above is perhaps best seen when presented in the same schematic form.

51. There are thus several well-established cases of spermatogenesis in which the reduction process described by Weismann is departed from. Besides Boveri's account, it is apparent from Brauer's[1] figures of the ovogenesis of Branchipus, published in 1889, that the twenty-four chromosomes of the cells of the first ovogenetic series are reduced to twelve, during the synapsis, before the commencement of the second, while each of these twelve chromosomes splits twice at the beginning of the first "Richtungspindel." The quadripartite chromosomes thus formed, divide and redivide in the two subsequent mitoses, without any intervening rest, so that there are twelve single chromosomes left finally in the ovum.

52. There are thus several well-established cases of both spermato- and ovo-genesis in which the reduction process described by Weismann is departed from, not only in the absence of the "Reductions"—as opposed to the "Equationstheilung,"—but also in the fact that the halving of the number of the chromosomes takes place in resting nuclei, one or more generations before the formation of the final sexual cells—from all of which it will have become sufficiently apparent that the Reductionstheilung of Weismann is universal neither among animals nor plants, and although an attempt may possibly be made to foist the theoretical burden which it carries on to the "synapsis" instead, there are cogent reasons for believing that the advocates of such a process will simply travel further, and in the end fare worse. It is obvious that the objections

chromosomes have, during the previous rest stage, been numerically reduced to four. These four chromosomes now split up, first into eight, and then into sixteen, and all these residual chromosomes are distributed by a quadripolar spindle figure in groups of four, amongst four spores, and this final number of the chromosomes persists through all the succeeding divisions of the gametophyte generation. ('Annals of Bot.,' vol. viii, pp. 35—51, 1894.)

[1] "Über das Ei von Branchipus Grubii," 'Abhandl. d. preuss. Ak. d. Wiss.,' Berlin, 1892.

which have been raised, by botanists, against the numerical halving of the chromosomes in the resting reproductive cells of plants having anything in common with the Reductionstheilung, can be used with equal weight in the case of the synaptic phenomena in the animals I have just described. And there is yet another and much more formidable obstacle to such a view, namely the possibility, if not probability, of both the synapsis among animals and the analogous processes in plants being interpretable on common and quite different grounds.

53. It will have been seen that throughout the whole course of the evolution by which the halving of the number of the chromosomes in the above animals is produced, there exists at least a superficial similarity to that accompanying the formation of the spore mother-cells and embryo-sacs in plants; and Strasburger,[1] in the address to which I have referred above, has already put forward, in a more or less provisional way, the ingenious suggestion that the halving of the number of the chromosomes in the reproductive cycles of living organisms may be interpretable on phylogenetic and not on physiological grounds. This attempt, however, to bring the whole of the phenomena into line is sadly hampered, thanks to the influence of the "Reductionstheilung" on investigation, by the insufficiency of recorded observation on the zoological side. I am enabled now, however, with these new facts relating to the reproductive cycles of Elasmobranchs, to draw Strasburger's comparison between animals and plants much closer, and to show that the phylogenetic interpretation of the numerical halving of the chromosomes of both is probably true.

54. It will be seen on reference to § 19 of the descriptive part of this paper that the prophasis of the heterotype division following the synapsis in Elasmobranchs is preceded by a peculiar readiness of the chromatin to contract into forms like those represented in fig. 39, which is characteristic of this particular phase in the spermato- and ovo-

[1] Loc. cit.

genesis in a great variety of animal forms. Now, exactly similar figures are obtained before the division of the pollen mother-cells, during the formation of the so-called "paranucleus" in plants; but considerable diversity of opinion exists on the botanical side as to the real or artificial nature of the paranucleus and its associated contraction figure, i. e. whether the whole appearance is not in reality more a "Gerinnung's Erscheinung" than a reality. However this may be, I do not believe that the one-sided nuclear figures seen at a corresponding period in the spermato- and ovo-genesis of animals, and with which most histologists must be quite familiar, are artifacts at all; and whether the contraction really exists in plants or not, it has been generally conceded by the botanists I have interrogated on the subject, that it is especially related to the time in question, while Professor Farmer tells me that such shrunken nuclear figures are practically diagnostic of the synapsis in certain liverworts of Ceylon, and so there can be little doubt that there exists, at any rate at this period, a peculiarly sensitive condition of the chromatin, common to both animal and plants.

55. In Elasmobranchs, Mammals, Amphibia, and probably many other animals, the division which immediately follows the synapsis is, as will be seen from §§ 23—37, different from all those preceding it. The chromosomes as they emerge from the reticulum of rest, being no longer longitudinally-split rods, but closed loops or rings, the divisions thus fall under the category of Fleming's heterotype. In animals the exact form and placing of these closed loops differs a good deal in different forms, but they all agree in this, that the loops split finally in the equatorial plane. In Elasmobranchs, Amphibia, and many other forms, the loops at first become bent up in the equatorial region of the spindle, so that when seen in profile they present the appearance of two Greek omegas placed side by side, the ends of which unite towards the poles (Diagram IV, 1, *b*, *c*). The outer curves and the closed ends of these figures are much thickened, and consequently the space between the two enclosed omegas is reduced to a mere slit.

Viewed from above, such chromosomes present the appearance represented in Diagram IV, 1, *c*, and when the chromosome divides, the polar extremities lengthen out, while a transverse split appears across the equatorial thickening, and the daughter V's, gradually separating, present a curious fourfold appearance represented in *d*, *e*.

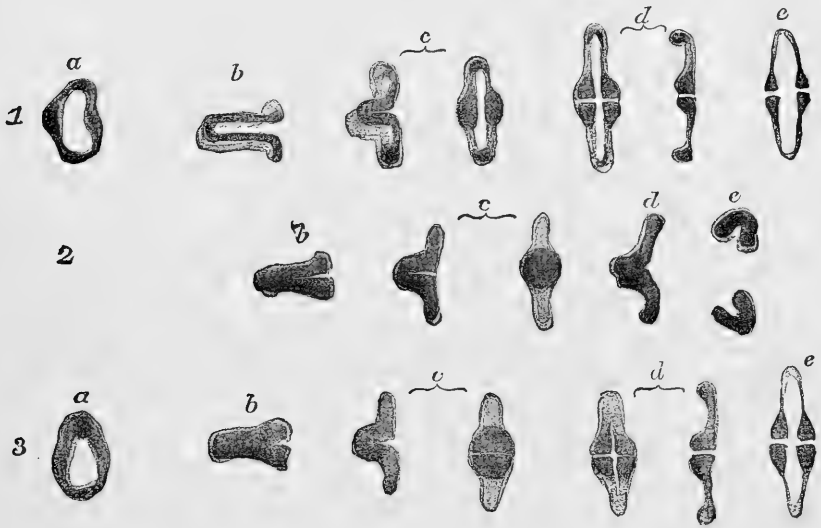


Diagram IV, representing division of heterotype chromosomes. (1) In Elasmobranchs. (2) In Phanerogams (according to Guignard and Strasburger). (3) In Phanerogams (after Farmer). *a*, *b*, *c*, *d*, *e*, corresponding stages in division.

56. In phanerogams the division which succeeds the long rest after the formation of the spore mother-cells, and which in general superficial characters corresponds to the synaptic phase (cf. § 19) among animals, differs, like its zoological counterpart, entirely in the arrangement of the chromatin from all the previous mitoses of the reproductive cycle. But the manner in which the daughter-chromosomes separate and go apart is, according to Strasburger and Guignard, quite different from what obtains in the corresponding animal cells. According to these authors, the chromosomes, after arising as stout, longitudinally-split rods (Diagram IV, 2, *b*) are attached

at one end to the spindle surface, the two halves gradually separating in the manner represented at 2, *d*, *e*. Quite recently, however, Farmer showed[1] that in the division of the pollen mother-cells of Lilium candidum this description of the origin of the daughter-chromosomes by no means fits the facts. After arising as long closed loops of microsomes, the chromosomes assume the rod form previously known; but the apparently longitudinal splitting extends only part of the way towards the outer end. When they have become flattened out on the spindle surface—sometimes before, and always as soon as the transverse fission is apparent (Diagram IV, 3, *d*.)—there is seen another longitudinal split, which converts the chromosomes into a closed loop, exactly comparable to those of the animals I have just described (IV, 1), the four masses into which the equatorial thickenings are divided at the time of separation being very marked (IV, 3, *d*, *e*.).

57. Professor Farmer has kindly given me photographs of these details, which I have copied in Diagram IV, 3. It therefore appears extremely probable that the chromosomes described by Guignard and Strasburger are, in reality, like those of Lilium candidum, closed loops bent upon themselves, but that the great shortening up and thickening they undergo leads here, as it often does in animals, to the internal opening being difficult to see.

58. The outcome of all this is that the reproductive cycles of animals and plants correspond, not only in the number of the chromosomes typical of the somatic cells of any species being halved, but also in the successive and complex phases by which their numerical reduction is brought about, as well as in the type of modification which the post-synaptic cellular generations may undergo.

59. Now, with repect to the nature of these post-synaptic generations, it is made obvious by the fact that in Elasmobranchs there are two, while in mammals there appears to be only one, that their number is of no physiological importance in the

[1] "Ueber Kerntheilung in Lilium-Antheren in Bezug auf die Centrosomen-Frage," 'Flora. Bot. Zeitung,' 1895, Heft 1.

formation of the mature sexual cells. They appear rather to constitute a sort of vanishing quantity, the existence of which becomes intelligible only on the supposition that they represent a phylogenetically decreasing succession of post-synaptic generations.

The flagellum which I found in the first cellular generation after synapsis in Elasmobranchs appears to me to indicate more clearly than anything I know, that the cells, before and after the synaptic phase, are morphologically distinct. If the spermatogeneses of Mammalia were the only ones we knew, the tail developed in the generation following the synapsis might legitimately have been regarded as a purely physiological structure acquired simply to suit the exigencies of the case. But the fact that in Elasmobranchs the complex initial phases of tail formation (cf. § 35) are gone through in the first as well as the second post-synaptic generation, is to me quite unintelligible, unless these flagella represent similar structures once possessed by the representatives of the cells' remote ancestry. This view is greatly strengthened by the complete analogy of structure which exists between the post-synaptic generations of Elasmobranchs and some of the simplest forms of sexually reproductive cells. It is well known that in many Algæ, reproduction can be carried on by means of fusion between two flagellate gametes, and quite recently Strasburger has discovered[1] that the flagella of such gametes arise from the kinoplasm, a structure which there is every reason to believe is the vegetable homologue of the archoplasm. Moreover, among these organisms there exist species which exhibit every gradation between those in which both gametes are alike and flagellate, and others in which there is a true tailed spermatazoon, and a tail-less ovum.

60. It would appear thus that if the foregoing comparisons are just, the existence of cellular generations with vestigial flagella, after the synapsis and before the spermatids have been formed, indicates that the synaptic phase marks a period in the reproductive cycle at which the cells return to a flagel-

[1] 'Histolg. Beitr.,' 1892, Heft iv.

late condition, with only half as many chromosomes as they had before.

61. It is conceivable that this capacity of periodically altering their chromatic valency, which the cells of both animals and plants possess, and which is accompanied in some by incipient tail formation, may have arisen in either of two ways, viz. :

It is conceivable that sexual reproduction may have begun before the periodic alteration in chromatic valency was evolved at all; but that, owing to the constant doubling of the number of the chromosomes after every act of fertilisation, a reduction in their number became physiologically necessary; or it is conceivable that the periodic variation in chromatic valency was evolved first, and that after its introduction, sexual conjugation, with all its attendant advantages, became physiologically possible. I do not know of the existence of any evidence which is decisively in favour of one view or the other. This much, however, is certain: on the one hand, variations in the number of the chromosomes, of a capricious and indeterminate character, are known to exist to-day, as in the case of the cellular structures accessory to the reproduction of many plants; while, on the other hand, variations which are neither capricious nor indeterminate exist, as we have seen, at certain times and places among the elements of complex animal and vegetable forms;—and I think most people will probably be disposed to agree in regarding this orderly variation as the expression of adaptive selection, which has worked towards some physiological end in the past, and the disorderly variation, if I may use the term, as the remains of something approaching a primitive chaos, on which adaptive selection has not yet acted.

I regard the above speculations, however, of relatively small importance beside the long series of structural homologies which I have established before, during, and after the synaptic phase in the reproductive cycles of both animals and plants, because this close correspondence, among a host of complex structural details, renders it improbable in the extreme that the two series of phenomena can have been independently

evolved; and whatever the synapsis may eventually turn out to be, it is evidently a cellular metamorphosis of a profoundly fundamental character, which would appear to have been acquired before the animal and vegetable ancestry went apart, and to have existed ever since.

In conclusion, I would express my sincere thanks to Prof. Howse for much help, and to the Royal College of Science for granting me the Marshall Scholarship for the completion of this investigation.

DESCRIPTION OF PLATES 13—16,

Illustrating Mr. J. E. S. Moore's paper, "On the Structural Changes in the Reproductive Cells during the Spermatogenesis of Elasmobranchs."

Reference Letters.

a. Archoplasm. *b. c.* Chromatic body. *c.* Centrosome. *c.f.* Foot-cells. *ch.* Chromosomes. *c. s.* Seminiferous cells. *b. i.* Flemming's intermediate bodies. *fr.* Fragmented portions of foot-cells. *l.* Linin filaments. *m.* Mittelstück, and portion of flagellum contained in it. *n.* Nucleolus. *n. g.* Nuclear groove. *n. v.* Nuclear sap-spaces. *i. s.* Inner spindle-sheath. *o. s.* Outer spindle-sheath. *r.* Cytoplasmic radiations round the sphere. *f.* Flagellum. *x.* Indeterminate body at the base of the flagellum.

The figures were drawn with Zeiss' hom. immer., 2 mm., 140 ap., and oculars 12, 18, except Figs. 1, 3—10.

Cells of the first spermatogenetic period.

Fig. 1.—Single primitive cells from which the contents of the ampullæ are formed.

Fig. 2.—Single seminiferous cell, of the first spermatogenetic period.

Fig. 3.—Early relative position of seminiferous and foot-cells.

Fig. 4.— Do. do. with foot-cell in division.

Fig. 5.— Do. do. later.

Fig. 6.—Fragmentation of foot-cells.

Fig. 7.—Migration of foot-cells.

Fig. 8.—Arrangement of the contents of an ampulla, before migration of the foot-cells.

Fig. 9.—Arrangement of the contents of an ampulla, with fragmentation products.

Fig. 10.—Division of foot-cells.

Fig. 11.—Early stages of division in the first spermatogenetic period.

Figs. 12—20.—Successively later stages of division in the first spermatogenetic period.

Fig. 21.—Polar view of monastral spindle-figure.

Fig. 22.—Details of the division of the chromosomes.

Figs. 23—26.—Later stages of division.

Fig. 27.—Final details of the spindle, before separation of the daughter-cells.

Fig. 28.—Final details of the spindle, before separation of the daughter-cells.

Fig. 29.—Reconstruction of the nucleus in its surrounding vacuole.

Fig. 30.—Reconstruction of the nucleus in its surrounding vacuole.

The synapsis and the divisions of the first spermatogenetic period.

Fig. 34.—Characters of the ampullæ at the time of the synapsis.

Fig. 35.—Seminiferous cell in stage of transformation.

Figs. 36—41.—Successive stages of chromosome formation.

Fig. 42.—Separation of the centrosomes, and initial spindle formation.

Fig. 43.—Superficial distribution of the chromosomes when fully formed, with further divarication of the centrosomes.

Fig. 44.—First stage of spindle formation.

Figs. 45—52.—Successively later stages of the same.

Fig. 53.—Seminiferous daughter-cells in later stage, with remains of spindle figure between them.

Figs. 54—56.—Successively later stages of the same.

Fig. 57.—First stage in the reconstruction of the daughter-nuclei, with residual spindle filaments attached to sphere.

Fig. 58.—Later stages of the same, showing translation of, and metamorphosis in the spheres.

Fig. 59.—The same.

Final division and structure of the spermatozoa.

Fig. 60.—Daughter-cells of first heterotype division, with vestigial flagellum.

Fig. 61.—Daughter-cells of first heterotype division, with vestigial flagellum.

FIG. 62.—Daughter-cells of first heterotype division, with vestigial flagellum, showing cystoplasmic condensation about the sphere.

FIG. 63.—Daughter-cells of first heterotype division, with vestigial flagellum, showing cytoplasmic condensation about the sphere.

FIG. 64.—Daughter-cells of first heterotype division, with vestigial flagellum, showing cytoplasmic condensation about the sphere.

FIG. 65.—Initial stage of final division.

FIG. 66.—The same later.

FIG. 67.—The same later, showing divarication of the centrosomes at the cell periphery.

FIG. 68.—Complete formation of chromosomes of the final heterotype.

FIG. 69.—Monastral spindle figure.

FIG. 70.—Monastral spindle figure, polar view.

FIGS. 71—78.—Successively later stages of final heterotype division.

FIG. 79.—Daughter-cells of the final heterotype, showing translation of the spheres, and initial stages of tail formation.

FIGS. 80—81.—Successively later stages of the same.

FIG. 82.—Cells showing the origin of the archoplasmic vesicle, and peculiar form of nucleolus.

FIG. 83.—Cells showing the origin of the archoplasmic vesicle, and peculiar form of nucleolus; showing elongation of the intra-cellular part of the flagellum, and the attachment of the archoplasmic vesicle to the nuclear wall.

FIG. 84.—Cells showing the origin of the archoplasmic vesicle and peculiar form of nucleolus; showing elongation of the intra-cellular part of the flagellum, and the attachment of the archoplasmic vesicle to the nuclear wall.

FIG. 85.—Cells showing the origin of the archoplasmic vesicle and peculiar form of nucleolus; showing elongation of the intra-cellular part of the flagellum, and the attachment of the archoplasmic vesicle to the nuclear wall; showing position of the archoplasm, and re-formation of ring chromosomes.

FIGS. 86—87.—Two views of the tail during its passage away from the archoplasmic vesicle.

FIGS. 88—90.—Successive stages in the elongation of the spermatozoids.

FIG. 91.—Optical section of spindle in first heterotype division, showing outer and inner spindle tubes, *o. s.*, *i. s.*

Notes on the Fecundation of the Egg of Sphærechinus granularis, and on the Maturation and Fertilisation of the Egg of Phallusia mammillata.

By

M. D. Hill, B.A.Oxon.,

Assistant to the Professor of Zoology in the Owens College, Manchester.

With Plate 17.

It is somewhat remarkable that since the late Professor Fol's (4) account of the "Quadrille des Centres" in the Echinoderm egg, up till very recently, no further work had been published either to confirm or refute Fol's original statements. It is therefore with all the more satisfaction that zoologists will welcome the conjoint paper of Messrs. Wilson and Matthews (10), being, as it is, a record of so much careful and accurate work. To the student of cytology, however, the theoretical importance of a clear insight into the complicated phenomena accompanying the maturation and fertilisation of ova is so great, that all evidence gained from independent work, with the help of reliable optical instruments and the most fitting reagents, necessarily becomes of some interest. I propose, therefore, to give in this paper an account of a portion of the work done by me whilst holding the British Association's table in the Marine Zoological Station at Naples. I have, however, to regret that circumstances forced me to leave Naples before I had completed my observations, especially on the maturation of the sexual cells in Phallusia mammillata. In all cases where a doubt still exists as to the real facts, I have been careful to state so expressly. My thanks are due to the British

Association for kindly allowing me the use of their table, and to the officials of the station, by whom I was treated with all possible friendliness.

The form I chose to work upon was Sphærechinus granularis, which is exceedingly abundant at Naples, and could always be had in any number. Mr. Wilson studied Toxopneustes variegatus, while Mr. Matthews divided his attention between Asterias Forbesii and Arbacia punctulata. With regard to Wilson's interesting observations on the polarity of the egg and the axial relations of the two pronuclei, I am not in a position either to corroborate or refute his statements, my attention having been almost entirely directed to the behaviour of the centrosomes. I will therefore pass over the first part of his paper, which has to do with the living egg, without further comment, The result of his and Mr. Matthews' study of series of sections of the eggs of the different forms they worked upon are summed up in the following words :—"After the formation of the second polar body the egg archoplasm soon disappears, and no egg centrum, or egg archoplasm ('ovocentre' as opposed to 'spermcentre') can be discovered at any subsequent period. There is nothing like a quadrille to be seen save in doubly fertilised eggs (Toxopneustes). The archoplasm of the first cleavage-amphiaster is developed entirely from, or under the influence of the sperm archoplasm ('spermocentre' of Fol), and this is derived not from the apex of the spermatozoon, but from its base, undoubtedly from the middle piece (Toxopneustes Arbacia). . . . There is no centrum save as an artefact."

With regard to all but the last sentence, my results are practically the same as Wilson's. In as far, therefore, as my work agrees with his, I shall deal very shortly with the facts, dwelling in greater detail on those points where our results differ.

Method.—At once after the fertilisation of a great number of ova certain quantities were preserved, in a mixture of corrosive sublimate and acetic acid, at intervals of about five minutes, until the first cleavage-plane made its appearance. This usually took place about one and a half hours after fertilisation, but the time varied greatly with the temperature of the

surroundings. After being hardened in alcohol, the eggs were embedded in paraffin, cut into sections, and stained with Heidenhain's iron-hæmatoxylin.

Fig. 1 is a drawing of a section of an unfertilised ovum, killed immediately after leaving the parent's body. The polar bodies are given off before the egg leaves the mother, but the nucleus has not yet taken up a central position. At this stage there is no sign of an astrosphere or of a centrosome. The next stage is taken (fig. 2) shortly after the entrance of the spermatozoon, which may be at any point on the surface, and is not affected by the position of the female pronucleus. The tail of the spermatozoon has dropped off, and the head and middle piece have turned completely round, so that the latter is nearest the centre of the egg. That this rotation takes place, as Wilson is the first to point out for Echinoderm eggs, I had convinced myself long before seeing his paper. The process is exactly parallel to that described by Fick (3) as occurring in the egg of the axolotl. For a very short time the middle piece persists as a small faintly-stained body attached to the sperm head. It soon becomes separated, and is converted into the astrosphere. At first the rays are very short, and all start from a central point, but gradually they lengthen out, and a finely granulated central mass makes its appearance, in the midst of which lies a single centrosome. As the astrosphere grows the granular central mass at first becomes reticulate, and in this condition the division of the astrosphere takes place, Fig. 5, but finally the network disappears, leaving a clear homogeneous central mass. The centrosome is of extreme minuteness. Figs. 2, 3, and 4 show the above stages.

The sperm head consists of a mass of chromatin enclosed in a loose membrane. At first cone-shaped, it gradually becomes more irregular in contour, till it appears as a roundish or oval lump, and the membrane so closely approximated as to become indistinguishable. As regards the so-called "fusion" I have nothing to add to Wilson's account. The astrosphere divides into two about the same time as the sperm head comes into contact with the egg-nucleus, which has by this time taken

up a central position. The two products of division both resemble the original astrosphere at the stage drawn in fig. 3. There is a finely reticular central mass in each, but in no single instance was I able to see a centrosome. Although I find a similar absence at this stage in Phallusia, still I believe that further examination is all that is necessary to prove that the centrosome exists at this stage also. The two astrospheres gradually travel to opposite poles of the ovum, as Wilson has already described. Shortly after taking up their positions there, they exhibit each a clear inside space[1] (the granular central mass having disappeared), in the middle of which are two clearly distinct centrosomes (fig. 6). It is probably this stage that Fol interpreted as being the one in which the two halves of the egg- and sperm-centrosomes respectively are about to fuse, one sperm half with one egg half, to form two single composite centrosomes (cf. Fol, fig. 9). From his drawings, however, even when it is taken into consideration that they are but rough woodcuts, one is tempted to doubt whether Fol ever really saw a centrosome at all. It is quite certain, as Wilson points out, that the clear area round the nucleus in which the "quadrille" is drawn as taking place is an artefact. I have obtained like results after using certain reagents, especially with the eggs of sea-urchins which had been kept some time in the tanks previously. With regard to the decrease in the length of the rays (mentioned by Wilson) during the Amphiaster (two-starred) stage, I cannot confirm his results. On the contrary, in some instances they are larger than at any other time, reaching nearly to the periphery of the ovum, and the "stratum corneum" is extremely well marked.

Further than the Amphiaster (two-starred) stage of the first segmentation nucleus I have not investigated. The conclusions which I think may be drawn from these facts I shall reserve till later, in the general summary at the end of the paper.

[1] By the word "space" I do not mean a hollow cavity in which the centrosome is somehow suspended, but a portion of the protoplasm which is so homogeneous as to give the impression, even when looked at under high powers of the microscope, of being an empty space.

Phallusia mammillata.

I studied the maturation and fertilisation of the ova of this Ascidian, partly with a view to comparing the origin of and the rôle played by the centrosomes with what I had found to be the case in the egg of Sphærechinus, but more especially to compare the case of Phallusia with that of Styelopsis described fairly recently by Julin (5). The method employed was precisely similar to the one already given for Sphærechinus. A mixture of 90 to 95 per cent. saturated solution of corrosive sublimate with 10 to 5 per cent. glacial acetic was found in both cases to be the best preservative when followed by Heidenhain's iron hæmatoxylin stain.

Here again I took no account of the changes to be observed in the living egg. These were sufficiently described by Strasburger (7) so long ago as 1875.

The ova were examined by means of sections in the ovary, oviduct, unfertilised after leaving the parent's body, and after fecundation. Unfortunately with regard to the development of the ova I can give no details. I never succeeded in getting satisfactory preparations of the nuclear figures of the ovogones while in the germinative zone. (I use Boveri's (1) nomenclature in his well-known diagram of the sexual cells of Ascaris.)

I can therefore give no such details as described by Julin for Styelopsis. Although I obtained preparations showing karyokinetic division in the ovaries of very young Ascidians, yet the cells themselves were so small that counting the chromosomes was impossible. I found precisely the same difficulty with the testes.

Transverse sections of the ovary show ovogones in various stages in the zone of growth. The nucleus is relatively of enormous size, is vesicular, and contains a large circular "nucleolus" of chromatin (fig. 8). As the egg passes into the branches of the oviduct, the nucleus begins to get smaller, and lessens so quickly in size that it becomes hardly one sixth of its original diameter. The nucleolus disappears, the chromatin is more regularly dispersed in the form of a long

thread, and then is split into eight chromosomes. The details I have not been able to follow (fig. 9).

Formation of the first polar body.—The polar bodies are given off shortly after the ova are shed into the sea water, irrespective of whether they are fertilised or not. The nucleus loses its membrane, a spindle is formed, and the eight chromosomes are arranged in the equatorial plane. As to what follows I do not wish to lay down any absolute facts. The polar spindles are so excessively small, and the chromosomes lie so close to one another, that accurate observation is a matter of extreme difficulty. From the study of a large series of sections, however, I am convinced that the rôle played by the chromosomes is very different from what has been described for Ascaris, and on which so many theoretical speculations have been based. The eight chromosomes of the first polar spindle lengthen out, become dumbbell-shaped, and finally divide in the middle (fig. 10). Eight chromosomes pass into the first polar body, which also divides karyokinetically into two, each having eight chromosomes. (I have never counted more than six or seven chromosomes in the products of division of the first polar body, but I think it may be taken for granted that there must be eight [fig. 11].) The chromosomes left in the first polar spindle again divide in the same manner (?), and about eight—certainly more than four—chromosomes pass into the second polar body (I have counted six distinctly), and eight (?) remain. To exactly determine the number of chromosomes left in the female pronucleus after the formation of the second polar body, and before it passes into the resting condition, is a matter of great difficulty, as the time between the two phases is very short (fig. 12). I have counted four, six, seven, eight, and nine in different instances. This discrepancy is partly due to the great tendency the chromosomes have to lump themselves together into one mass, so that the female pronucleus resembles the nucleus of the ovogone in having a large "nucleolus" of chromatin. This is broken up into a network as soon as the nucleus develops a membrane and passes into the resting condition. It then

withdraws somewhat from the periphery of the egg, though still maintaining an excentric position. It should here be remarked that throughout the whole process of maturation there is no sign of a centrosome or archoplasmic sphere.

As is shown, however, in fig. 9 *c*, at either end of the spindle is a deeply-stained body, which may be called a pseudocentrosome. This is nothing more than the point where the slightly stained spindle-threads meet. Although there is no astrosphere, the protoplasm at the ends of the spindle is distinctly modified, the reticular structure giving way to a more granulated condition.

Fertilisation.—The spermatozoa consist of the usual three pieces—head, middle piece, and tail. When ripe they are very active, piercing through the two layers of test and follicle cells in a very short space of time. The egg puts out a "cone d'attraction," which embraces the head of the spermatozoa. The tail drops off, and the head rotates very rapidly. In fig. 13 the stage is drawn where the head has rotated 90°. At the end of the middle piece is a deeply-staining body, which may be the centrosome. Already the rays of the astrosphere are apparent. The "cone d'attraction" subsides as soon as the sperm-head has made its way into the ovum. The head itself broadens and grows rapidly, until it reaches about twice its original size. It then suddenly splits into two. At first regular, these pieces gradually take on a beaded irregular shape, and subsequently break up into small irregularly-shaped chromosomes, usually about eight or nine in number (figs. 14, 15, and 16). These chromosomes are only transitory structures, at least as far as outward appearances go, for very shortly afterwards the male pronucleus passes completely into the resting condition. Meanwhile the astrosphere has grown considerably, and passed through the same phases of formation as described in the case of Sphærechinus. The rays are remarkable for their length and thickness, the whole structure being much coarser than the astrosphere of Sphærechinus. The centrosome is also very large and distinct, and soon after the centre of the astrosphere has become finely granular, divides into

two, although the corresponding division of the astrosphere itself does not take place till somewhat later. As Boveri (2) has described and figured for Ciona intestinalis, the astrosphere lengthens out,—the rays contracting somewhat,—becomes dumb-bell-shaped, and finally constricted off in the middle into two separate spheres. In Phallusia, however, the pronuclei fuse somewhat differently, as will be seen by comparing fig. 18 with fig. 29 in Boveri's paper (2). The two astrospheres and also the two nuclei are at this stage very difficult to stain, and this may account for the fact that from this until the Amphiaster stage there was no trace of a centrosome to be found. The interior of the astropheres show a reticular structure, which is maintained till the Amphiaster stage, when the centrosomes become visible again, and the network gives place to a clear "space," or at most a slightly reticular central mass (figs. 17 and 18).

In the first cleavage-spindle I have counted from thirteen to sixteen chromosomes, and on theoretical grounds it is necessary to assume that the latter is the correct number, which would give eight derived from the female, and eight from the male pronucleus respectively. I do not wish, however, to lay too much stress on the exactitude of these numbers, though I believe they are approximately correct (fig. 19).

With regard to the conditions of the chromatin in the development of the spermatozoa, it was only possible to substantiate the fact that one spermatocyte I. gives rise to two spermatocytes II., and these again each divide into two, forming in all four spermatids. Further, the two nuclear divisions take place without any intermediate resting phase. Beyond this, however, nothing could be definitely ascertained as to the number of the chromosomes in any stage of development, owing to the extreme minuteness of the cells themselves.

It may be interesting to note that the ova of certain specimens, which had been kept in the aquarium tanks for a long time, were found to be infested with long rod-like bodies seemingly of a bacillic nature. Although these ova were mixed with ripe sperm, no single fertilisation ever took place.

That they were not, however, quite destitute of vitality is shown by the fact that polar-spindles were formed, although of an apparently pathological nature (fig. 20).

For the structures needing high powers of the microscope, Zeiss's apochromatic 2·0 mm., apert. 1·30, homogen. immers. was used with compensating ocular No. 8, and an Abbé condenser.

Summary and Conclusion.

The above results may be shortly summarised as follows:

(1) In Sphærechinus granularis and Phallusia mammillata there is no egg astrosphere or egg centrosome. Both these structures are brought into the ovum by the spermatozoon, and they give rise by division to all the subsequent astrospheres and centrosomes throughout ontogeny. There is, consequently, no such thing as a "quadrille."

(2) In both forms the sperm head rotates through 180°, and the astrophere and centrosome are elaborated out of or under the influence of the middle piece.

(3) In Phallusia the nucleus of the ovocyte I. contains eight chromosomes irregularly dispersed throughout its substance.

(4) In the two succeeding nuclear divisions these eight chromosomes divide into sixteen each time, eight passing out into the first and eight into the second polar body. There is consequently no equalling or "reducing division" at this period.

(5) The sperm head breaks off into eight chromosomes, and sixteen are found in the first segmentation spindle.

The origin and fate both of astrospheres and centrosomes in Sphærechinus and Phallusia certainly tend to support Boveri's generalisation, that the ovum when ready for fertilisation possesses two out of the three essentials for cell division, viz. cytoplasm and nucleoplasm, but is without the third, or centrosome; while, on the other hand, the ripe spermatozoon possess nucleoplasm and centrosome, but little or no cytoplasm. It is therefore only when the union of the male and female cells takes place that the requisite conditions for development are

fulfilled. Where careful observations have been made this supposition has almost always been found to hold good, e.g. in the case of Rhynchelmis (Vejdoffsky, 8), Ascaris (Boveri, 2), Axolotl (Fick, 3), and Styelopsis (Julin, 5); while only in one instance has it been incontestably denied, viz. by Wheeler (9) for Myzostomum. When, however, it is borne in mind that nuclear and cell-division can take place without the presence of an astrosphere or centrosome, the supposed importance of the latter as an organ of division is greatly lessened. It is, moreover, extremely difficult to offer any explanation as to why the first cleavage spindle should have two astrospheres and two or four centrosomes, while the polar spindles (Phallusia, Ascaris, Sagitta, Ciona, &c.) may have none. Again, the relation between centrosome and astrosphere is very obscure; are the rays produced by the action of the centrosome, or vice versâ? The former alternative seems to the most probable in the case of Phallusia, where, as is shown in fig. 13, while the spermatozoon is quite at the surface of the ovum the posterior half of the middle piece has become a centrosome, and already acted on the cytoplasm of the egg to produce a radial appearance. In spermatozoa simply killed and stained there is no sign of a centrosome in the middle piece, which points to a direct metamorphosis of part of the middle piece into a centrosome as soon as the spermatozoon penetrates into the ovum.

Neither in Sphærechinus nor Phallusia is there any evidence to point to the centrosome as being artefact. In the former it appears to be all that remains of the middle-piece, and is brought to light by the latter's disintegration into granules and final disappearance.

Finally, with regard to a more important matter, viz. the relations of the chromatin substance during the maturation of the ovum, Phallusia agrees more with the vertebrates than the invertebrates. I cannot go into the subject, however, in any great detail, because I have been unable to trace the history of the eight chromosomes in the nucleus of ovocyte I. Still, there are one or two points worth noting. In the first

place, Phallusia agrees with all the other forms in the ovocyte I. containing half the number of chromosomes typical for the given species. Rückert (6) puts this first and foremost among the few ascertained facts that we have at present in this complicated subject. He writes:—"Alle genauere Untersuchungen der letzen Jahre stimmen darin ueberein, dass schon vor der ersten Reifungstheilung Chromatin-portionen auftreten deren Zahl die Hälfte beträgt von der Normalzahl der Chromosomen der betreffenden Species. . . . Am klarsten liegt der Reifungsvorgang, wenn sie aus vier deutlich geschiedenen Unterabtheilungen, Stäbchen oder Kugeln, bestehen (Vierergruppen). In diesem typischen Fall geht die Reifung bekanntlich folgendermassen vor sich: Durch zwei ohne Ruhephase auf einander folgende mitotische Reifungstheilungen werden die Vierergruppen in der Weise gevierteilt dass in jede Enkelzelle (Spermatiden, Reifes Ei) von jeder Gruppe ein einziges Stäbchen als Chromosoma gelangt, womit die schon durch die Zahl der Vierergruppen vorbereitete Reduktion definitiv vollzogen wird."

It is obvious that if these "Vierergruppen" are to be looked for in Phallusia, it is not in the nucleus of the ovocyte I. that they are contained, but in the three polar bodies and the female pronucleus. That is to say, there are eight "Vierergruppen," as each of the eight chromosomes divides twice, making thirty-two pieces in all. Julin, on the other hand, looks upon the eight chromosomes in Styelopsis as themselves forming two "Vierergruppen;" and the subsequent formation of the polar bodies, albeit on these he confesses to have made few observations, bears out this view. At the first polar division four chromosomes pass out, and at the second two, leaving two quarters of the two original chromosomes, which had divided twice precociously to give rise to two "Vierergruppen." The spermatozoon brings into the ovum two quarters likewise, so that the first segmentation nucleus possesses four chromosomes, or double the number contained in the nucleus of the ovocyte I. In fact, as regards the number of chromosomes, Styelopsis exactly resembles Ascaris bivalens. In Phallusia each of

the original eight chromosomes is a single complete structure; and if the process were like what occurs in Styelopsis, the nucleus of the ovocyte I. would contain thirty-two chromosomes. Owing to its extremely small size this is a mechanical impossibility, and hence there is no such precocious splitting to form eight "Vierergruppen."

Hence it seems to follow that those who would see in this precocious splitting a process especially brought about in order to ensure a more varied combination of ancestral units, are obliged to recognise that it may take place in one species of the same group and not in another. In Phallusia at no one stage of maturation there are thirty-two chromosomes "to choose from," so to speak, but the division takes place exactly as in normal cell-division, except that the chromosomes divide transversely and not longitudinally (? in second polar body). On the contrary, the two cases of Phallusia and Styelopsis seem to point towards the phenomena of maturation being nothing more than normal cell-division. Whereas in the former case the large number of chromosomes prevents precocious division, in the latter the small number allows it, and it is possible that some good to the organism may be gained thereby. The point to be found out is at what stage does the reduction from sixteen to eight chromosomes in the development of the sexual cells of Phallusia take place, which is the only real "reducing division" of the kind.

The fact that the process of maturation of the egg of Phallusia resembles what has been described for the egg of certain vertebrates, may be an additional point of evidence for a phylogenetic connection between them and the Ascidians.

Naples; March, 1895.

Addendum.

Shortly after the above notes were sent in to await publication an interesting and suggestive paper by Boveri[1] appeared, which it is necessary for me here to briefly notice.

If I understand Boveri aright, his results and mine agree up to the stage I have figured in fig. 3, but after that they differ considerably. In the stage I have drawn in fig. 6 Boveri finds the centrosome, not as one, or two, very minute intensively staining granules, but as a hollow vesicle swollen to such a size as to be separated from the radial striations by a very narrow "heller Hof." He finds no trace of any deeply-staining central body. In going carefully over my preparations for a second time, I cannot discover the slightest reason for Boveri's interpretation. The one, or two, central bodies are so clearly defined that I am surprised Boveri has not found them. Of course it is possible that, as he suggests, this body, or bodies, may be merely the "Centralkörner" of the centrosome; but if this is so I am at a loss to know where the real centrosome is. I must, however, state that in one or two preparations I have distinctly seen a ring round the centrosomes, e.g. fig. 6, which according to Boveri should be the centrosome in an earlier stage of swelling up than he has figured. I consider, however, that this appearance is unimportant and may be an artefact, as in many cases where the centrosomes were very plain,—e.g. in figs. 3, 4, and 11,—there was no trace of it. It is, I think, a matter of some regret that Boveri has as yet only published a single figure illustrating his work. I hope, however, I may not be construed as in any way trying to depreciate the accurate and valuable work of such a distinguished observer as Professor Boveri, who has had such a far wider experience in cellular morphology than myself, but in this particular case I cannot help thinking that his interpretation is erroneous.

[1] "Ueber das Verhalten der Centrosomen bei der Befruchtung des Seeigelries nebst allegemeinen Bemerkungen über Centrosomen und Verwandtes." 'Verhand. der Phys.-med. Gesell. zu Würzburg,' N. F. Band, xxix.

Finally, I may mention that the process I have described, viz. the homogeneous middle-piece of the spermatozoon becoming first the granular, then the reticular, and finally again the homogeneous central mass of the astrosphere (figs. 3, 4, 5, and 6 for Echinus, and figs. 14, 15, 17, 18, and 19 for Phallusia) is almost exactly the same as Vejdoffsky described for Rhynchelmis. From these results he believes that protoplasm is at first quite homogeneous and structureless; that then very small granules appear which group themselves together to form a reticulum, which may become once more homogeneous. I do not pretend, however, to use this word "homogeneous" other than as before explained, in a purely relative sense. With higher powers of the microscope I see no reason to suppose that the middle-piece would not itself present a "Wabenstructur," and that the network is anything more than a coarse protoplasmic reticulum. It is right, moreover, to mention that I was not biased by Vejdoffsky's work when noting the above process, for my attention was not drawn to this particular point until reading his paper again after my own had been finished and sent in for publication.

A point worthy of notice is that, at least in one particular instance (fig. 14), the homogeneity of the central mass has been arrived at before the division of the spermastrosphere.

M. D. Hill.

June, 1895.

List of Works referred to.

1. Boveri, Th.—"Befruchtung," 'Erg. der. Anat. u. Entw.,' Merkel and Bonnet, Band i, 1891.
2. Boveri, Th.—'Zellen Studien,' i—iii, 1887, 1888, 1890, Jena.
3. Fick, R.—"Ueber die Reifung und Befruchtung des Axolotleies," 'Zeit. wiss. Zool.,' Bd. lxv, 1893.
4. Fol, H.—"Le Quadrille des Centres," 'Arch. Sci. Phys. et Nat.,' xxv, 1891.

5. Julin, C.—"Structure et développement des glandes sexuelles; ovogénèse, spermatogénèse et fécondation chez Styelopsis grossularia," 'Bull. Sc. de la France et de la Belgique,' t. xxxv, 1893.

6. Ruckert, J.—"Die Chromatin reduktion bei der Reifung der Sexualzellen," 'Ergeb. der Anat. u. Entw.,' Merkel and Bonnet, Band iii, 1893.

7. Strasburger, E.—'Zellbildung und Zelltheilung.'

8. Vejdoffsky, F.—"Entwickelungsgeschichtliche Untersuchungen," Heft 1, 'Reifung, Befruchtung, und Furchung des Rhynchelmis-Eies,' Prag., 1888.

9. Wheeler, W. M.—'The Behaviour of the Centrosomes in the Fertilised Egg of Myzostoma glabrum," 'Journal of Morphol.,' vol. x, 1.

10. Wilson, E. B., and Matthews, A. P.—"Maturation, Fertilisation, and Polarity in the Echinoderm Egg," 'Journal of Morphology,' vol. x, No. 1.

DESCRIPTION OF PLATE 17,

Illustrating Mr. M. D. Hill's "Notes on the Fecundation of the Egg of Sphærechinus granularis, and on the Maturation and Fertilisation of the Egg of Phallusia mammillata."

cent. Centrosome. *chr.* Chromosomes. *f. pron.* Female pronucleus. *m. pron.* Male pronucleus. *1st p. b.* First polar body. *2nd p. b.* Second polar body. *Sp. ast.* Sperm astrosphere. *Sp. h.* Sperm head. *Sp. cent.* Sperm centrosome. *1st seg. sp.* First segmentation. *1st seg. n.* First segmentation nucleus. *m. p.* Middle piece. *1st p. s.* First polar spindle. *2nd p. s.* Second polar spindle.

Figs. 1—6.—Cross sections of eggs of Sphærechinus granularis.

Fig. 1. Section of unfertilised egg.

Fig. 2. Section of egg ten minutes after fertilisation.

Fig. 3. Section of egg fifteen minutes after fertilisation. Sperm astrosphere has grown in size, and the centrosome is apparent.

Fig. 4. Section of egg twenty minutes after fertilisation. Sperm astrosphere is in contact with female pronucleus.

Fig. 5. Section of egg twenty-five minutes after fecundation. Male pronucleus has "fused" with female, and astrosphere has divided. No trace of sperm centrosome.

Fig. 6. Section of egg forty-five minutes after fertilisation. Centrosome has appeared again and divided into two in each astrosphere. Central mass has become entirely homogeneous.

Figs. 7—20.—Phallusia mammillata.

Fig. 7. Cross-section through ovary of Phallusia mammillata, with ovarian ova.

Fig. 8. Cross-section of an unfertilised ovum directly after leaving oviduct. Nucleus is enormously reduced in size, and chromosomes are eight in number.

Fig. 9. Shows changes in nucleus in forming first polar spindle.

Fig. 10. Shows extension of first polar body.

Fig. 11. The first polar body has divided into two, and the second polar body has also been formed. (Slightly less magnification than Fig. 10.)

Fig. 12. Shows the changes through which remaining chromosomes from second polar spindle pass during formation of female pronucleus.

Fig. 13. Cross-section of egg a few minutes after fertilisation. Sperm head has rotated through 90°.

Fig. 13*a*. Sperm head enlarged.

Fig. 14. Cross-section of egg ten minutes after fertilisation. Sperm head and astrosphere have increased in size, and centrosome is evident in the granular central mass.

Fig. 15. Cross-section of egg fifteen minutes after fertilisation. Sperm head has split into two.

Fig. 16. Cross-section of egg twenty minutes after fertilisation. Sperm head has broken up into eight chromosomes, and central mass has (unusually early) become homogeneous.

Fig. 17. Sperm head has passed into the resting stage of the male pronucleus. The astrosphere is in act of division, and central mass reticular.

Fig. 18. Sperm astrosphere has divided into two, and two pronuclei are in contact.

Fig. 19. First segmentation spindle with sixteen chromosomes. Central masses of atrospheres have become nearly homogeneous, and centrosomes divided into two.

Fig. 20. Cross-section of an unfertilised ovum, taken from oviduct of a specimen living in aquarium.

Fig. 21. Shows transformation of middle piece of spermatozoon into central mass of astrosphere. Slightly diagrammatic.

Further Remarks on the Cell-theory, with a Reply to Mr. Bourne.

By

Adam Sedgwick, F.R.S.

In a paper published last autumn (this Journal, vol. 37), I called attention to the apparent inadequacy of the cell-theory. Recently a criticism upon my article has appeared from the pen of Mr. G. C. Bourne, to which I may be allowed to devote a few words. But before replying to Mr. Bourne, I should like to state my position with regard to the theory a little more fully than I have hitherto done. In my previous communication I used the word "inadequacy" because it seemed to me to express, as nearly as possible, my own views with regard to the theory. A theory to be of any value must explain the whole body of facts with which it deals. If it falls short of this, it must be held to be insufficient or inadequate; and when at the same time it is so masterful as to compel men to look at nature through its eyes, and to twist stubborn and uncomformable facts into accord with its dogmas, then it becomes an instrument of mischief, and deserves condemnation, if only of the mild kind implied by the term inadequate.

The assertion that organisms present a constitution which may be described as cellular is not a theory at at all; it is—having first agreed as to the meaning and use of the word cell—a statement of fact, and no more a theory than is the assertion that sunlight is composed of all the colours of the spectrum. The theory comes in when we try to account for the cellular constitution of organisms; and it isthis theoretical part of the cell-theory, and the point of view it makes many of us assume, that I condemn. It is not the word "cell" which

I am at issue with, for structures most conveniently called cells undoubtedly exist, as the ovum, spermatozoon, lymph-cells, &c.; and I fully agree that the phenomenon called cell-formation is very general in organic life. But at the same time I hold with Sachs and many others that it is not of primary significance, but "merely one of the numerous expressions of the formative forces which reside in all matter." No one who has studied animal tissues could for one moment deny that nuclei have in many cases a relation to the surrounding protoplasm, a relation which is expressed in the arrangement and structure of that protoplasm. They have not always this relation, but it is usually present, and the question is, how are we to interpret it? That we cannot interpret it finally until we know the relative values of nucleus and extra-nuclear protoplasm, and the functional relation between the two, is clear; but we may form and hold provisional theories. The hypothesis or idea which holds the field at the present day is the cell-theory in its modern form. This theory, recognising the cellular structure (while not admiring the phrase, I must use it for want of a better one) asserts that organisms of Metazoa are aggregations or colonies of individuals called cells, and derived from a single primitive individual—the ovum—by successive cell-divisions; that the meaning of this mode of origin is given by the evolution theory, which allows us to suppose that the ancestor of all Metazoa was a unicellular Protozoon, and that the development of the higher animals is a recapitulation of the development of the race. Thus the holoblastic cleavage of the ovum represents the process by which the ancestral Protozoon became multicellular, and the differentiation of the cells into groups the beginning of cellular differentiation. According to this view the order is: unicellular stage—multicellular stage—differentiation of cells into tissue elements; cellular structure preceded cell-differentiation, and to get tissues you must first have cells. And ten years ago it was commonly held that these cells were primitively separate from one another, and that the connections found between them in the fully

formed tissues were secondary. You had your neuro-epithelial cell, and your musculo-epithelial cell, each derived from a distinct cell produced by division of the ovum; and the question was, how do they find each other and become connected?[1] Further, in studying the development of a tissue you had to find a group of cells, each of which became modified into one tissue element. Thus the primitive streak was a proliferating mass of cells which eventually gave rise to a number of mesodermal tissues; the nerve-crest similarly was a mass of cells which gave rise to nervous tissues; a nerve-fibre was one of these cells elongated, and before you would get your nerve-cell and fibre you must have your nerve-crest cell produced by division from the cells of the nerve-cord, and subsequently sending out a process which elongated and travelled to the periphery as a nerve-fibre.

My work on Peripatus first led me to doubt the validity of this view of the origin of the Metazoon body. In the first place I found that in some forms there is no complete division of the ovum, and on examining the facts I discovered that such forms were more numerous than had been supposed. It therefore appeared that in some Metazoa the ovum divided into completely separate cells, while in others it did not so divide. The question then arose, which of these methods is primitive? and the answer naturally was, the complete division, because this fitted in with our ideas as to the supposed evolution of the Metazoa from a colonial Protozoon. But on reflection this difficulty arose: the individuals of colonial Protozoa are in protoplasmic connection, while the cells of the completely segmenting ova are separate; so that the parallel between the ontogeny and the phylogeny breaks down in an important particular. To get over this difficulty it was necessary to suppose that the isolation of the segments of incompletely segmenting ova was apparent and not real, that they were really connected by protoplasmic strands which had

[1] For exposition of this view vide Flemming, 'Zell-Substanz, Kern u. Zell-Theilung,' Leipzig, 1882, p. 74, and Balfour's Address to the Department of Anatomy and Physiology at the British Association in 1880.

escaped observation. But, on the other hand, there was the possibility that the completely segmenting ova were secondary acquisitions of ontogeny, and that the development in such forms as Peripatus, Alcyonaria, &c., was more primitive, and that the passage from a Protozoon to a Metazoon had taken place by way of a form more resembling a multinucleated cilated Infusorian than Volvox. In other words, that the differentiation of the Metazoa had been effected in a continuous multinucleated plasmatic mass, and that the cellular structure had arisen by the special arrangement of the nuclei in reference to the structural changes. This was the stage to which my researches on Peripatus led me. Since then I have paid attention to Vertebrata, and I have found that a number of embryonic processes have been wrongly described, amongst them such important matters as the development of nerves and the origin of the mesoderm; and I thought that I traced the errors referred to to the dominating influence of the cell-theory in its modern form, for the facts seemed so obvious in themselves that it would have been impossible to make any mistake about them had they been examined without the prejudice imparted by a preconceived theory. A theory which led to such obvious errors must, I thought, be wrong, and I denounced it. But my denunciation in no way implies that I fail to recognise the so-called cellular structure of organisms or their origin from the one-celled ovum. On the contrary, I was led to a reconsideration of the question, what is the meaning of the predominance of the structure called cellular, which is characterised by a definite relation of the nuclei to the functional tissues, and of the fact that the organism so often passes through a unicellular stage. With regard to the former I must say that I have arrived at no conclusions which enable me to formulate to myself any satisfactory hypothesis, and, as I stated at the outset, I do not think it is possible to do this until we acquire some more understanding of the relative function of nuclei and protoplasm. But with regard to the latter there are some facts which might well be considered. In the first place, the unicellular origin

is only found in sexual reproduction, not in asexual. The characteristic of the unicellular form is its simplicity of structure, and the essential feature of sexual reproduction is the conjugation of the reproductive cells. Now in the Protozoa, in which the amount of formed tissue is generally slight and the structure of the body simple, conjugation can and does often take place between the ordinary form of the species. But in the Metazoa, in which conjugation is as necessary a phenomenon in the specific cycle as in Protozoa, conjugation is impossible between adult or ordinary individuals of a species from mechanical causes. How is this difficulty got over in nature? My answer is, by the formation of special individuals of extremely simple structure—a structure so simple that conjugation between them is possible. To put the matter in another way, I should regard the ordinary diœcious Metazoon as a tetramorphic species, consisting of male, female, ovum, and spermatozoon, the two latter being individuals which are specially produced to enable conjugation to take place.

Mr. Bourne, in his criticism, begins by complaining that he cannot ascertain from my article my own views on the subject of the cell-theory. Why should he expect or wish to discover them? My remarks were simply directed to show the shortcomings of the theory with regard to certain anatomical facts. As explained above, my own view is that the cell-theory is inadequate to explain the facts, and that it is not possible at present to explain them by any theory. He proceeds to state that I am abusive because I say that certain observers "are constrained by this theory with which their minds are saturated, not only to see things which do not exist, but also to figure them" (I am referring to embryonic mesoderm of vertebrates). He calls this abuse, not argument. I venture to differ with him—it is neither abuse nor argument; it is merely a statement of fact (unless, indeed, it be considered abusive to say that a man accepts and believes in the cell-theory). If you disbelieve it, consult the memoirs of the last twenty years in which this tissue is referred to, and in most of them you

will find the mesenchyme described or figured as consisting of branched, isolated cells.

Mr. Bourne then refers to certain remarkable researches which emphasize the distinction and complete isolation of the cells formed in the segmentation of the egg; with what object is not apparent, for he proceeds on the next page to condemn those who hold that the organism is constituted of independent and isolated units. He even maintains that no reputable biologist holds such a view. However that may be, I do not think that his quotation from Haeckel in support of his contention is a happy one, for it is perfectly clear from the quotation that Haeckel, who indeed goes so far as to call the units individuals, holds the view which Mr. Bourne condemns.

Haeckel even calls them individuals of the first order, and says that in the adults they frequently unite to form colonies; and he particularly implies that the loss of independence caused by their colonial union is secondary. Mr. Bourne has completely failed to grasp Haeckel's meaning, else how can he write as he does on the same page with the quotation from Haeckel—"So that, as a matter of history, while plants used to be considered to be colonies of independent life units, animals were not."

The most remarkable part of Mr. Bourne's criticism is that in which, after strongly animadverting on my statement that it is difficult if not impossible to enunciate the cell-theory in a manner satisfactory to every one,—indeed he quotes from Schwann and Hertwig to show how precisely it can be stated,—he proceeds to devote a dozen or more pages of his paper to a consideration of the various views which are held and which may be held as to what a cell really is! If this amount of discussion is required to arrive at the meaning of the word cell, is it likely that there will be simple agreement as to the theory which is supposed to explain and account for the so-called cellular constitution of organisms?

Again he says, referring to my description of the embryonic mesoderm as a protoplasmic reticulum with nuclei at their

nodes: "Does he accept the logical consequences of this, and say of the epithelial cells of the salamander or of unstriped muscle fibres, that they are protoplasmic reticula with nuclei at their nodes?"

Now, with all due respect to Mr. Bourne's logical faculties, may I ask him where logic comes in here? If I describe London as a network of streets, with public-houses at many of the street corners, am I obliged by logic to give the same description of the Gog-Magog Hills?

However, on the next page Mr. Bourne makes up for all the hard strictures he has passed upon me; for he says that, after all, reflection may induce us to abandon the cell-republic or colonial theory; thus he admits a very important part of my contention, for the assertion that organisms present a constitution which may be described as cellular is not a theory at all, it is a statement of fact (having agreed to the use of the word cellular). The theory comes in when we try and account for the cellular constitution of organisms; and it is this theoretical part of the cell-theory which I condemn, and which Mr. Bourne after a great effort agrees with me in condemning. At the same time it is possible that we might still disagree as to the meaning of the word cellular.

May I call attention to Mr. Bourne's remarkable faith in the rapidity of evolutionary changes? He says (page 169) that Schwann's assertion that "the elementary parts of all tissues are formed of cells, &c.," is even more true to-day than when it was written. Also I should like to know how he reconciles the implication at the top of page 170, that "specialisation is not possible in continuous tracts of protoplasm," with the statement a few lines further on, that "in the Protozoa there is differentiation within the limits of a single corpuscle."

The criticism on page 172 as to my use of the word empty is not quite fair. On reference to the context it will be seen that the word empty clearly means "empty of structural elements."

The Development of Asterina gibbosa.[1]

By

E. W. MacBride,
Fellow of St. John's College; Demonstrator in Animal Morphology in the University of Cambridge.

With Plates 18—29.

The investigations which form the subject of the present memoir were commenced with the object of seeking in Asterids the results which the author (14) had already obtained from the study of Ophiurids, viz. the development of the so-called heart and its accompanying sinuses.

A study of the literature soon led to the conclusion that our knowledge of the development of most organs in the Asterid body was very defective, and that a thorough revision of the whole embryonic and larval history would be most desirable. This work has occupied my attention for the last two years, and I am now in a position to give a fairly complete account of the whole organogeny; an account which will, I hope, place our knowledge of Asterid development on the same level as that to which our acquaintance with Crinoid ontogeny has been raised by the researches of Bury (1) and Seeliger (18); I have to express my warm thanks to Mr. Sedgwick not only for the suggestion of Asterina gibbosa as a proper type to investigate, but also for much assistance and advice in revising the proofs of this paper.

That there was an immense lacuna in our knowledge to be

[1] A preliminary account of the observations recorded in this paper was the subject of the successful essay in the competition for the Walsingham Medal of the University of Cambridge in 1893.

filled up will become evident when I state in the first place, that my researches have made it clear that the Crinoids are only very distantly related to the other classes of Echinoderms, and secondly, that our previous knowledge of the metamorphosis of Asterids and their allies was confined principally to the changes which take place in their external form.

It will be most convenient, I think, to give first a general account of the development, and then to point out how far the results of other workers have been confirmed, as by this means needless repetition will be avoided.

Methods adopted.

My material consisted of a large number of larvæ of all stages including those which had just completed the metamorphosis, and of a considerable number of young adults varying from an age of about three weeks to several months from the metamorphosis. Of these the former, with the exception of two small collections made by myself in Plymouth, 1893, and Jersey, 1894, were collected for me and preserved according to my directions by the authorities of the Naples Zoological Station; the latter were obtained for me and preserved by myself during my stay in the Naples Station in 1892. I have to express my deep sense of my indebtedness to Prof. Dohrn for his kindness in meeting my wishes, and to Cav. Salv. Lo Bianco for the extreme care and attention with which he carried out my directions.

All the stages were preserved in osmic acid, followed by 14—24 hours in Müller's fluid, as this method had yielded me the best results in the case of Ophiurids. It makes the specimens exceedingly brittle, but at the same time gives the most excellent preservation of the minute histology; preserved in this manner the various tissues are differentiated as to their staining capacities, so that the sections look almost like coloured diagrams.

On account of their brittleness, and in order to avoid shrinkage in the tissues, the larvæ were embedded in celloidin, and the celloidin block subsequently embedded in paraffin.

They were then cut into series of sections in most cases $4\frac{1}{2}$ μ thick—in the case of the adults 7 μ; these sections were mounted on hot water on the slide to flatten them, and stained in either Grenacher's hæmatoxylin or Mayer's carmalum. Two points of interest in connection with this process may be mentioned: first, I found that when the slide was transferred from turpentine to absolute alcohol some of the sections were sure to be lost, but that this could be avoided by placing the slide for a minute or so, after taking it out of turpentine, into oil of cloves, and thence into 90 per cent. alcohol; second, that the readiness with which sections, especially when overcharged with osmic acid, will take up either hæmatoxylin or carmalum is greatly increased by immersing them for twenty-four hours in borax-carmine, though they do not acquire a particle of stain from it.

In the youngest stages the osmic acid produces too great impenetrability for either celloidin or paraffin, and accordingly my best results were obtained from some specimens preserved for me by Sig. Lo Bianco in a mixture of three parts concentrated aqueous solution of corrosive sublimate, and one part glacial acetic acid. This method also gives most excellent preservation, though without that fine differentiation of the tissues yielded by osmic acid and Müller's fluid; as during the stages in question however the larvæ consist almost exclusively of epithelial cells, this is not a matter of any importance. This second method was recommended to me by Dr. Eisig.

The orientation of the specimens was one of the chief difficulties to be overcome. I found that the best results were given by horizontal sections perpendicular to the median sagittal plane of the larva, and sections parallel to the disc and perpendicular to the median axis of symmetry in the just metamorphosed star-fish. The planes, to which in these two cases the sections are cut parallel, viz. a median horizontal plane in the larva and the plane of the disc in the adult, make an angle of about 70° with each other; and hence it is difficult to correlate sections cut parallel to the one with those cut parallel to the other. I shall call these planes the "larval"

and "adult" planes respectively. A rudiment of the præoral lobe of the larva is retained, as we shall see, until the close of the metamorphosis, and by means of it I found it possible to determine the direction of the "larval" plane up till the adult form has been almost attained. Hence, by cutting sections parallel to the larval plane, one can follow the internal changes of the metamorphosis step by step; then when the metamorphosis is complete it is possible to correlate with less difficulty sections cut parallel to the two planes, and the further history may be followed viâ, so to speak, the adult plane. This was the course which I adopted; and I also penetrated back a considerable distance from the adult condition into the stages of the metamorphosis by sections parallel to the adult plane, and so confirmed results obtained by the other method. For the youngest stages of all, which are spherical, orientation is, of course, impossible, and one has to trust to chance to getting sections in the proper direction; but it is fairly easy to recognise from their appearance when this is so.

General Account of the Development.

The ontogenetic history of the Asterina gibbosa may be conveniently divided into three parts: first, the development of the bilaterally symmetrical larva from the egg; second, the metamorphosis of this larva into the young star-fish; and lastly, the gradual development of what we may term the young adult into the sexually mature form. I have made no observations on the segmentation of the egg, nor on the gastrulation; my work, properly speaking, commences with the completed gastrula, and my material was not suitable for observing the development of the calcareous plates. On all these points I intend, however, for the sake of completeness, to say a few words, and my authority will be Ludwig, who, in his classic research (12), has on these subjects left nothing to be desired in point of view of completeness. I may add also that the figures illustrating the changes in external form are copied from Ludwig's memoir. The three figures illustrating the relations

of the Asterid and Crinoid to their common ancestor were designed for me by my friend and colleague Mr. J. J. Lister, of St. John's: in their present form they were drawn for me by a lady friend.

The Development of the Larva.

The eggs are laid by the parent on the under surface of stones, to which they adhere by means of their vitelline membrane. I have never discovered a male, though Ludwig says that the male twists his arms round the female whilst she is depositing her ova, and then pours out his spermatozoa upon them; it is quite certain that in the English Channel, at any rate, isolated females will lay eggs which develop with perfect regularity up to the conclusion of the metamorphosis. Cuénot (4) says that young females of a certain size develop spermatozoa in their ovaries—a statement I have not been able to verify. It may, indeed, be said that Ludwig's statement that a kind of sexual congress takes place, Cuénot's observations, and the experience of the authorities of the Jersey Biological Station are irreconcilable, and that the whole subject demands renewed investigation.

The eggs are larger than those of most other Echinoderms; they are about ·5 mm. in diameter. This is a result of the yolk which they contain, and which gives them their bright orange colour. This yolk is so uniformly distributed, however, that it does not alter the type of segmentation, which is total and regular. The blastomeres, in consequence of their larger size, are more closely packed than is usual amongst Echinoderms; they are wedged into the interspaces between their neighbours, and so the strict "radial"[1] type of segmentation characteristic of the group is no longer maintained.

The result of segmentation is a hollow blastosphere or blastula, which on the second day of development becomes converted into a gastrula by embolic invagination. The embryo

[1] For a discussion of the different types of regular segmentation see "The Cell-lineage of Nereis," by Prof. E. B. Wilson, 'Journal of Morphology,' vol. vi.

is not quite spherical, its long axis exceeding very slightly its transverse axis, so that we can see that the blastopore is situated in the centre of what afterwards becomes the ventral surface. The gastrula has acquired a uniform covering of cilia, and the blastopore is a round opening with well-defined lips. This well-marked stage of development, which is easy to recognise, I have called Stage A (Pl. 18, fig. 1). The blastopore narrows in a peculiar manner, one of its lips becoming reflected over it (Pl. 18, fig. 2), and it is finally reduced to a minute pore (Pl. 18, fig. 3). This opening, which is identical with the larval anus, gradually travels back to near the posterior end of the embryo; this is effected by differences in the rate of growth of surrounding parts. During this time the embryo has been lengthening its long axis, and on the fourth day it ruptures the vitelline membrane and escapes. It then has the form shown in Pl. 18, figs. 4—6, and as this stage is also a well-marked one, I have called it Stage B.

The foregoing is Ludwig's account; my material was not suitable for such observations, which ought to be made on the living embryos, and I had not the opportunity of observing these early stages alive. As far, however, as I could make out, Ludwig is perfectly correct in his statements. I was able to recognise Stage A, for instance, with ease.

Let us turn now to the internal changes which have gone on during this time. Pl. 19, figs. 20 and 21, are two sections of an embryo of Stage A, and they form the starting-point of the changes we shall have to consider; I may here say at once that all sections which illustrate the development of the larva and its metamorphosis are to be understood to have been cut parallel to the larval plane except the contrary is distinctly affirmed. Fig. 22 is a sagittal section of a slightly older embryo; here mesenchyme cells have appeared. The large size of the archenteron is a remarkable feature, the blastocœle or segmentation cavity, usually spacious in Echinoderms, being reduced to a mere slit. Fig. 23 shows us that the archenteron becomes differentiated into an anterior thinner-walled vesicle, the cœlom, and a posterior thicker-walled gut;

and in fig. 24 we see that the cœlom has grown back in the form of two tongues, *lpc.*, *rpc.*, lying one at each side of the gut. Fig. 25 shows us a more ventral section passing through the blastopore of the same individual, and we see that in it these cœlomic lobes are absent; they are therefore still confined to the dorsal side of the embryo.

It has been mentioned above that the larva, immediately on escaping from the egg-membrane, has the form of Stage B, and it will be observed that its anterior end has the appearance of being obliquely truncated, and that the anterior surface so constituted is surrounded by a thickened rim, which is covered with specially long cilia, and to which I give the name of **larval organ**. The changes of form involved in acquiring this shape are considerable, and are undergone whilst the larva is still enclosed in the egg-membrane, though superficially the ovoid shape is maintained, the larval organ and the neighbouring ectoderm being to a large extent developed as invaginations into the interior of the larva, exactly as the Tænia head is developed on the wall of the cyst.

The histology of the embryo is illustrated in Plate 26, figs. 124 and 125. The first is a portion of section of a larva of Stage A, the same specimen as that from which figs. 20 and 21 are taken. Both ectoderm and endoderm are seen to consist of long narrow cylindrical cells, and there is no mesenchyme. Recent researches have gone to show that this is exceptional. Field (5) has proved for Asterias, and it has been long known in the case of Echinids, that mesenchyme is formed by the wall of the blastula before any invagination has taken place. Fig. 125 is taken from a slightly older gastrula. It shows the formation of the mesenchymatous cells by the division of the endoderm cells. I found no indication that mesenchyme continued to be formed when Stage B is reached. The anterior wall of the cœlom is the spot where its formation lasts longest, as in Antedon (18). The cœlomic epithelium consists of small cubical cells (see Pl. 23, fig. 95).

We must now return to Stage B, up to which we have traced the development. A stomodæum is now developed just behind

the posterior wall and ventral edge of the larval organ. This is well shown in the sagittal section, fig. 31. The larva increases in size, and the præoral portion and larval organ alter their shape, the latter changing from a circular to an elongated elliptical form, whilst the præoral lobe extends in a vertical direction (Pl. 18, figs. 7 to 9). The whole larva has now the form which Ludwig calls slipper-shaped, but which would be more correctly termed boot-shaped, the dorsal lobe of the præoral lobe representing the toe and the ventral one the heel of the boot. In the centre of the larval organ appears an elevation (*fix.*). This structure, which Ludwig did not interpret, we shall find to have a most important function during the metamorphosis; it is, in fact, the disc by means of which the animal fixes itself. Possibly this disc also functions during free life for temporary attachment, though in a different manner; thus when the larval organ is applied to the substratum, the retraction of this disc would cause a cupping action which would be relieved by its again being protruded. It has been pointed out by Ludwig, and I have myself confirmed it again and again, that the larva is able to attach itself most strongly to the substratum. The mode of life of the larva Ludwig calls "creeping." This is not strictly correct; as far as I have seen, the larva swims by means of the cilia of the larval organ. The latter is directed downwards, and for this reason Ludwig calls what I have termed the anterior surface of the animal the ventral, and the posterior end becomes for him the dorsal end. I cannot agree with this orientation; the proper longitudinal axis of any bilaterally symmetrical animal is the oro-anal one, and it is by this that I discriminate between the dorsal and ventral, the anterior and posterior surfaces. That the posterior end is held upwards is no more reason for calling it dorsal than the fact that the Cephalopod directs the apex of its visceral hump backwards is reason for calling that posterior. I should mention that Ludwig calls the whole præoral portion of the body, the præoral lobe in fact, the larval organ. I wish to avoid this, since the præoral lobe has functions which Ludwig did not suspect, and hence I confine the term "larval organ" to the

thickened ridge with long cilia, which is the locomotor organ of the larva, and is the first thing to disappear in the metamorphosis.

Stage C is the point which we have now reached, and it is characterised by the appearance of this disc for fixation. Ludwig compares the larval organ to the non-ciliated processes of the Asterid larva, the Brachiolaria. This larva appears to be merely a further stage in the development of the well-known Bipinnaria, from which it differs in the development of three stalked papillæ from the apex of the præoral lobe, which are presumably used for attachment. These papillæ arise between the anterior dorsal and the anterior ventral arms of the Bipinnaria: one of them is median and more dorsally situated than the other two, and to this arrangement Ludwig compares the occasional bifurcation of the ventral lobe of the larval organ of Asterina. Now, however, that we know the function of the adhesive disc, it is, in all probability, this which is to be compared to the papillæ of the Brachiolaria; and the larval organ with its long cilia (compare Pl. 27, figs. 133—135) in all probability represents some portion of the ciliated bands of the Bipinnaria. Garstang (6) has, in fact, recently described a Bipinnaria in which the dorsal arm of the præoral lobe executes muscular movements in the same way as Ludwig asserts for the Asterina larva. I repeat, however, that the latter can swim by ciliary action alone, without any muscular movement.

The internal changes which have occurred between Stages B and C are numerous and important. We have already referred to the appearance of the stomodæum or larval œsophagus. About the same time the primary madreporic pore is formed; it arises by a pocket of the cœlom slightly to the left[1] of the mid-dorsal line, meeting a thickening of the ectoderm (fig. 26, *mp.*) and a perforation taking place. The pocket of the cœlom is called the "pore-canal" (*pc.*, fig. 26), and is lined by cylindrical ciliated cells. By this time the two posterior

[1] This position is not shown in fig. 26; the figure represents a section which was rather oblique.

lobes of the cœlom have extended so as nearly to meet one another in the mid-ventral line; the mesentery formed by their apposition is seen in fig. 30, posterior to the gut. The opening of the gut into the cœlom has become closed ventrally (figs. 29 and 30); dorsally, however, it remains open for some considerable time yet. On the left side the cœlom becomes segmented into an anterior portion, *a.*, into which the pore-canal opens, and a left posterior portion, *lpc.*, which we may call the **left posterior cœlom** (fig. 27); this second cavity includes a large part but not all of the left cœlomic lobe mentioned above; part of this latter is, as is seen in the figure, included in the anterior cœlom. The septum between the two cavities is first formed dorsally, and then extends in a ventral direction; fig. 28 shows it in process of formation.

At the same time one can notice the first indication of that predominance of the organs of the left side which is the key to the whole ontogeny of the star-fish. We see in fig. 30 that the septum between the right and left cœlomic sacs is pushed over to the right, owing to the tendency of the left posterior cœlom to extend over to the right on the ventral side. At no time, so far as I have seen, however, does this septum break down. Some curious trabeculæ are in this stage stretched across the left cœlom. They are easily distinguished from the septum between the two sacs, as they consist of solid strings of cells, whereas the septum has two layers of epithelium with a slit of blastocœle between in this stage. These trabeculæ are very transitory; in figs. 28 and 29 (Stage B) we see them being formed, and in fig. 33 is the last trace of them (Stage C).

As development proceeds the gut becomes more completely separated from the cœlom, the larval anus closes, and the short rectum (fig. 31) disappears. Shortly before this, however, the stomodæum opens into the gut, the main portion of which constitutes the **larval stomach** (*l. stom.*), the rectum being very short; but it is only for an extremely short time that the larva possesses both mouth and anus.

Stage C is reached about the end of the fifth day, or the commencement of the sixth day. The division of the left

posterior cœlom from the cœlom of the præoral lobe, which we may now call the **anterior cœlom** (*a.*, figs. 32—35), is complete. On the right side the separation of the posterior part of the right cœlomic lobe, the **right posterior cœlom**, from the anterior cœlom has just commenced dorsally (fig. 32). On the left side the rudiment of the water-vascular system, or, as it is convenient to term it, the **left hydrocœle**, has appeared (as will be related immediately a similar rudiment appears on the right side, but "hydrocœle" alone means left hydrocœle). It originates as an outgrowth from the hinder end of the anterior cœlom; and whilst it is as yet but faintly marked off from this cavity, indications of its five primary lobes are seen. These are arranged in a curve open anteriorly, and throughout all the figures they are denoted by the Arabic numerals; the most dorsal being No. 1, the most posterior No. 3, and the the most ventral No. 5 (see figs. 32—34). Their mutual relations are well shown in the sagittal section (Pl. 20, fig. 47), though this represents a somewhat later stage.

We have seen that the division of the right posterior cœlom from the anterior cœlom has begun in exactly the same manner as happened in the case of the left posterior cœlom at an earlier stage. This division has not proceeded very far towards the ventral surface, when the anterior cœlom buds off a vesicle from its right posterior extremity. This vesicle is homologous to the water-vascular rudiment on the left side, for which reason it will be termed the **right hydrocœle**; so we see that the cœlom on the right side of the larva undergoes exactly the same changes as that on the left, only that they are retarded in their appearance. The first trace of the right hydrocœle is shown in Pl. 23, fig. 95; we see that it consists of a small vesicle of cubical cells arising as a thickening of the cœlomic wall. Its lumen is, in this stage, a minute slit; other preparations show this slit in open communication with the anterior cœlom. It is important to observe that it originates from the dorsal portion of the hinder end of the anterior cœlom, which extends further back ventrally to it, as would be seen if a more ventral section than fig. 95 were shown.

Later stages of this organ are seen in figs. 35 and 36. In fig. 35 it is a conspicuous solid bud; in fig. 36 it has acquired a lumen, and is connected with the anterior cœlom by a string of cells, which soon atrophies, and it is then left as an isolated vesicle in the midst of the mesenchyme. Bury (2), indeed, has seen it in this stage, and called it "a mesenchymatous vesicle;" and Field (5) has described what I believe to be an homologous structure in the larva of Asterias. The right hydrocœle persists in the adult as a closed sac just under the madreporite, and has been seen here by Cuénot (3), and Leipoldt (9) has described a similar sac in Echinids. It may seem rather a rash assumption to regard this organ as the fellow of the water-vascular system, but a complete proof that this is really its nature will be given when abnormal larvæ are described.

Stage D, the summit of the development of the larva, is reached on the seventh day, according to Ludwig (Pl. 18, figs. 10 and 11). The præoral lobe and the larval organ have greatly increased in size, the former having acquired a large ventral as well as a dorsal lobe. The internal changes are more striking than the external. The separation of gut from cœlom was practically complete in Stage C, the last trace of connection being shown in fig. 36. The right posterior cœlom is entirely separated from the anterior cœlom, but, strange to say, the septum between the left posterior cœlom and the anterior cœlom has become broken down in two places. This occurs by the two layers of epithelium of which it is composed fusing, and then thinning out to a film. Of these two secondary communications between the two sacs, one is situated dorsal to the left hydrocœle (Pl. 20, fig. 42), and the other ventral to it (Pl. 19, fig. 41). Figs. 42 and 43 belong to the same series; we see that the dorsal opening is formed before the separation of the right posterior cœlom is complete; the ventral opening is formed at the same time. Not having had the opportunity when I wrote my preliminary account (15) of observing younger larvæ than these, I imagined that the segmentation of the cœlom of the left side was incomplete ab initio, a mistake which was the more excusable as both the breaches in the

septum dividing the two portions of the cœlom from each other become again closed during the metamorphosis.

The left hydrocœle has become much more sharply separated from the anterior cœlom than in the last stage, though in the region of the third lobe the hydrocœle still opens widely into the anterior cœlom (Pl. 19, figs. 38—41; Pl. 20, figs. 44—46). We saw that the pore-canal in Stage B originated a little to the left of the middle line; now, however, owing to the increasing predominance of the left side, it is shifted to the right of the median plane (*pc.*, fig. 44). The stone canal (*stc.*, figs. 45 and 46) arises as a groove along the anterior face of the transverse septum forming the hinder wall of the anterior cœlom. The central portion of this groove soon becomes closed to form a canal, opening at one end into the hydrocœle between lobes 1 and 2 (fig. 46), and at the other into the anterior cœlom (fig. 45); and this opening is in this stage entirely independent of the opening of the pore-canal.

I have referred more than once to the predominance of the organs of the left side. This is strikingly shown in the stage we are considering by the narrowness of the right posterior cœlom as compared with the left. Already in Stage B we have seen that the left posterior cœlom has begun to sweep round to the right on the ventral side of the right posterior cœlom; this occurs more and more, and in the stage we are considering in the most ventral sections (fig. 41) the right posterior cœlom is entirely absent. The left not only passes under it, but to a certain extent interposes between its anterior portion and the gut (figs. 39 and 40), and here opens freely into the anterior cœlom[1] (fig. 40) by the secondary ventral communication described above. This portion of the left cœlom we may call its **right ventral horn**; it plays a most important part in the metamorphosis, and it is marked *l'p'c'.* in all the figures.

Ludwig failed entirely to recognise the left posterior cœlom

[1] I may anticipate a little by informing the reader that the anterior cœlom gives rise to the axial sinus of the adult; a space which opens to the exterior by the pore-canal and into the left hydrocœle (water-vascular ring) by the stone-canal.

as a sac separate from the anterior cœlom; he states that the mesentery between the right and left cœlomic lobes is absorbed ventrally. We have seen that only the posterior parts of the right and left cœlomic lobes are employed in the formation of the right and left posterior cœloms respectively; the anterior parts of these lobes are continuous with the anterior cœlom, and the longitudinal mesentery between them breaks down, as Ludwig observed. Hence we see that the hinder part of the anterior cœlom in Asterina is at first a double structure; in the Bipinnaria larva the anterior cœlom is at first double throughout its whole extent.

At the dorsal anterior angle of the left cœlom (fig. 37) an invagination of its wall takes place, giving rise to a thick-walled vesicle (*or. c.*), which communicates by a narrow slit with the cœlom. This structure has been strangely misunderstood. Ludwig saw it, but not its origin, and supposed it to arise as a "schizocœle," and regarded it as the rudiment of the oral blood-ring. In my preliminary account I recognised its true nature, but supposed that its upper end was the rudiment of the so-called heart,[1] with which, as a matter of fact, it has nothing to do. It is the rudiment of the oral cœlom, a space closely surrounding the adult œsophagus, the relations of which we shall study later.

Histology of the Larva.

The structure of the body-wall of the larva is shown in Pl. 27, fig. 138, and Pl. 28, fig. 144. In the first we see that the peritoneum of the left posterior cœlom consists of

[1] It will be observed that Bury, in his last paper ('Q.J.M.S.,' September, 1895), makes the same mistake. This work appeared after the present paper had been sent in for publication, and is therefore not referred to further here. The best answer to Bury's criticisms on my observations as recorded in the preliminary account (15) is the publication of full details in the present paper. Bury's observations contain much interesting matter, but also in my opinion many mistakes, which are due to the fact that the stages which he obtained in the development of most of the larvæ he studied, did not form a series without gaps; the orientation which he adopted seems to me also not that which yields the best results.

small cubical cells; the ectoderm is made up of exceedingly long and narrow cells bearing flagella, and the wall of the hydrocœle of similar cells, but I could not make out any flagella there. Fig. 144 is taken from the posterior end of the animal on the right side; the form of the ectoderm cells is well seen, and one observes occasional goblet cells (*gob.*) amongst them. The section goes through a peculiar patch of peritoneum, where the cells are actively engaged in budding off the amœbocytes which float in the cœlom. So far as I can make out, however, no cells are budded off at this stage into the blastocœle (i. e. the space between the ectoderm and the cœlomic wall), and the mesenchyme cells are as yet entirely undifferentiated. The characters of gut cells are shown in Pl. 26, fig. 126. Although this is taken from a larva in which the metamorphosis has commenced, yet the characters of these cells do not vary till the very close of the metamorphosis. They have the same general form as the ectoderm cells, but the bases of the latter are often contracted, and leave chinks between them, whereas the endoderm cells are closely apposed to one another. Fig. 126 also shows another point of interest: here and there a small round amœbocyte may be seen applied to the basal end of the gut cells, and one discovers amongst the latter also one or two rounded cells, thus suggesting that these amœbocytes may be able to pass between the gut cells like the lymph cells in the Vertebrate intestine.

Plate 27, figs. 133—135, are three sections through the larval organ which have already been alluded to. It is to be noted that in this stage the adhesive disc has short cilia, just as Seeliger (**18**) has described for the adhesive disc of Antedon. Where I have put "*nerv. larv.*" a thin strand of pale fibrous matter is observable with the highest powers. This is the only trace I can discover of a larval nervous system, and I am not perfectly satisfied about it, since it does not take the yellowish-brown tone with osmic acid so characteristic of the adult nervous system. Should my interpretation of it be correct, the larval nervous system would consist of a layer of "Punktsubstanz" underlying the larval organ.

Pl. 27, fig. 137, shows the character of the wall of the præoral lobe. The peritoneal cells have developed fine muscular tails (*musc.larv.*), and it is perfectly apparent to anyone looking at sections of a number of larvæ that it is the peritoneum which is the active agent in contraction. The ectoderm is often wrinkled (fig. 38), but the peritoneum never, though its cells vary in shape from cylindrical to flattened according to the state of contraction; thus in some cases the peritoneal cells on the left side will be cylindrical, those on the right side flattened. The cœlomic wall is in this case short and straight on the one side, and on the other bulged in to the lumen of the anterior cœlom by a great accumulation of the fluid of the blastocœle, or rather (as we must conclude from observations which have been made on other Echinoderms) the blastocœlic semi-fluid jelly. In fig. 137 we see some fine fibrils traversing the blastocœle; these, so far as I can make out, are not protoplasmic, but of skeletal nature—of the same nature, that is, as the adult fibrous tissue.

The Metamorphosis.

On the eighth day the larva fixes itself by the adhesive disc by means of a thin secretion of mucilage (see Pl. 27, fig. 136, which represents a much later stage), and remains fixed during the whole of the metamorphosis. I had the opportunity of observing this in Plymouth in 1893 and in Jersey in 1894, and it was most instructive to observe the difference between the larvæ which had thus definitely become sessile and those which, being still able to move, had attached themselves by the cupping action of the muscles of the præoral lobe, the larval organ being applied to the substratum.

In the first case, that of truly sessile larvæ, if one attempted to remove them with a pipette, one failed to move them unless very strong suction was applied or they were displaced by a needle; but once displaced they were perfectly helpless, those even which had to all appearance almost completed the metamorphosis being unable to use their tube-feet (which as yet were rudimentary); they could do nothing but feebly rotate by

the action of their general covering of cilia, and they had no power of re-attachment. In the case, however, of larvæ which were attached by what we may call voluntary muscular action, if one brought the pipette cautiously near so as not to alarm them, it was very easy to remove them from a stone, just as it is easy to kick a limpet off a stone if it is taken unawares; but if they were irritated they were excessively difficult to remove, and when one finally succeeded in getting them up into the pipette, unless one promptly re-expelled them, they attached themselves to the glass, and it was almost impossible to detach them from it.

The metamorphosis of Echinoderms is probably the most remarkable ontogenetic change known in the animal kingdom; but our knowledge of its details has been up to the present most insufficient. We possess a completely satisfactory account of only one form, viz. Antedon, for which the credit is due to the researches of Bury (1), which have been amply confirmed by Seeliger (18). As I mentioned in the introduction, I hope the account I am about to give of the metamorphosis of Asterina will compare in completeness with those I have just mentioned; and as it is of the utmost importance for the comprehension of the meaning of the anatomical structure of the Asterid that its relation to the larva should be thoroughly grasped, I shall anticipate a little what I have to say in order to make the essence of the process perfectly clear. The metamorphosis of the Asterid, then, consists in the following processes, which go on simultaneously:

(1) The constriction of the body into disc or body sensu stricto, and stalk, the latter being formed from the præoral lobe.

(2) The sharp flexure of the disc on the stalk [the former is bent obliquely downwards and to the left. This is not well shown in any of the figures copied from Ludwig; it is better seen in the diagram, Pl. 29, fig. 158 (Dec. 1895)].

(3) The preponderating growth of the organs of the left side, the left posterior cœlom and the left hydrocœle both sending out dorsal and ventral horns, which meet so as to form complete

circles, whilst the right hydrocœle and the right posterior cœlom remain small.

(4) The gradual atrophy of the stalk.

(5) The outgrowth of the adult œsophagus and the formation of the new mouth on the left side.

In the Crinoid the list would stand thus:

(1) The constriction of the animal into calyx and stalk.

(2) The displacement of the mouth and neighbouring organs, i. e. the hydrocœle, to the posterior end of the body by unequal growth.

(3) The mutual displacement of the right and left posterior cœloms, the left becoming posterior and the right anterior, both having a ring-shaped growth.

(4) The spiral growth of the intestine and formation of anus close to primary madreporic pore.

It will be seen that the Asterid metamorphosis is very different from that of the Crinoid, being much simpler: one great difference which strikes one at once being that in the former case the ends of the hydrocœle grow so as to embrace the stalk, which thus appears to spring from the oral surface; whereas in the latter case the hydrocœle is carried far away from the stalk to the posterior end of the body. Much diligent search has been made in the centre of the aboral surface of Asterids for traces of a stalk, but to anyone who has grasped the foregoing explanation it will be at once obvious how futile such search must prove. Pl. 29, figs. 158 and 159, though intended to indicate ancestral forms, illustrate the two metamorphoses outlined above very well.

The sections about to be described illustrating the metamorphosis are nearly all cut parallel to the larval plane, and as was the case with the sections of the larva, where two or three sections from the same series are figured the most dorsal is in every case placed first, and so one can clearly see their relation to corresponding sections of the larva. As one always thinks, however, of the organs of an Asterid as related to the plane of the disc or adult plane, it will be well to repeat the relation which these two planes bear to one another. The

adult plane makes an angle of about 70° or more with the larval plane; but without any very serious error, it may be regarded, for purposes of description, as at right angles to it: thus the direction right to left, according to the larval plane, becomes aboral to oral according to the adult plane, and dorsal to ventral according to the larval plane is nearly parallel to the adult plane. Here I may remark that the words "dorsal" and "ventral" will only be used with reference to the larval plane; in speaking of the adult plane the words "oral" and "aboral" will be used.

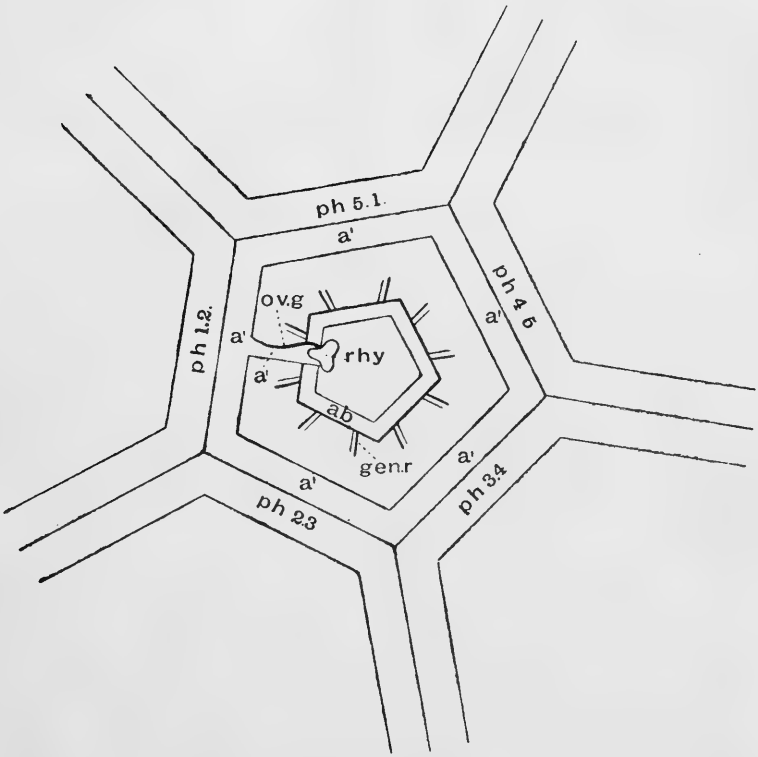
Pl. 18, figs. 12 and 13, show the appearance of a larva which has only been fixed for a short time. On the left side we see that the hydrocœle lobes have become visible externally, since they have raised the ectoderm into protrusions which, as we shall find, are the rudiments of sensory terminal tentacles of the radial water-vascular canals. Outside the curve of these rudiments is another set of protrusions, also arranged in an open curve. These are the rudiments of the arms: they are all, as we shall see, outgrowths of the left posterior cœlom, and their primary function is to form supports for the lobes of the hydrocœle, to which they later become apposed. The constriction of the præoral lobe or stalk from the body proper is hardly as yet marked, but the rounded appearance of the dorsal and ventral outgrowths of the præoral lobe is to be noticed. This is due to the disappearance of the larval organ, the opposite sides of which become approximated to each other and wrinkled, and then broken up, portions of the organ becoming invaginated into the interior and destroyed by histolysis. The appearance of the remnants of it at this stage gave Ludwig the impression that one had to do with the outgrowth of a series of protrusions homologous to the adhesive disc. This is, of course, a mistake; the adhesive disc remains single and unaltered to the end of the metamorphosis. This well-marked phase of development we may call Stage E. Pl. 20, figs. 48 to 50, are taken from a larva of this age; fig. 48 is of course the most dorsal section (see explanation of plates). In fig. 50 we notice the great growth of the left hydrocœle, lobe 3 reaching nearly to the

posterior end of the body, and we can also make out an arm rudiment, which at this stage is a mere protrusion of ectoderm filled with mesenchyme cells; it forms the extreme posterior end of the section. The rudiment of the adult œsophagus *a. œ* is also seen, and we notice the relation of the oral cœlom to it, and we may remark that the larval œsophagus is by this time disrupted from the gut. Fig. 49 shows that dorsally the hydrocœle is completely shut off from the anterior cœlom, and shows that the oral cœlom dorsally opens into the left posterior cœlom. Fig. 48 shows that the opening of the oral cœlom is in close relation to a process of the left posterior cœlom extending over to the right, dorsal to the gut. This is the **right dorsal horn** (see p. 351 for the ventral horn) of the left posterior cœlom, and it is marked *l″p″c″* in all the figures. In later stages it extends ventrally for a short way, insinuating itself between the gut and the septum dividing the anterior cœlom from the left posterior one (Pl. 21, fig. 61). The opening of the oral cœlom is later shifted so as to be connected only with the right dorsal horn, and hence it came to pass that Ludwig regarded oral cœlom and right dorsal horn of the left cœlom as one structure, and described the oral cœlom as the oral blood-ring and the dorsal horn as the "heart." In common with all other growing spaces in the larvæ, this right dorsal horn has at its growing tip an epithelial thickening, and it was this which in my preliminary account I mistook for the rudiment of the "heart."

Figs. 51—53, taken from a slightly older larva, show the appearance of the rudiments of the perihæmal spaces. It may be useful to refresh our memory of the arrangement of these spaces in the adult; this the annexed woodcut is intended to do. They are usually described as consisting of a canal situated just aboral to each radial nerve, and divided by a longitudinal septum (Pl. 29, fig. 155). These radial canals open into a circular canal surrounding the mouth, inside which is another inner ring-canal. The longitudinal septa of the radial canals are inserted in the septum separating these two ring-canals. Into the inner of the circular canals a vertical canal opens

which is the axial sinus, embedded in the wall of which is the stone-canal (Pl. 25, figs. 110—118). This axial sinus

Fig. 1.



ph. 1.2, &c. Rudiments of the outer perihæmal ring. *a'*. Axial sinus and its outgrowth the inner perihæmal ring. *ab.* Aboral sinus. *gen.r.* Genital rachis.

was supposed to open at its upper end into an aboral perihæmal ring or pentagon, from which in each interradius two canals branched off to go to the genital organs. As is well known, these spaces were called "perihæmal" by Ludwig (10), because he imagined that he had discovered the true blood-system in the form of curious tracts of tissue embedded in the longitudinal septa of the radial canals, and in the septum separating the two circular canals. He further supposed that that curious

so-called heart, which projects along with the stone-canal into the axial sinus, was connected with this system, and that a string of tissue lying in the aboral ring and connected with the "heart" was also part of the vascular system. We shall, however, see later that these two latter structures ("heart" and aboral string) are of totally different nature from the oral ring, being composed of primitive germ-cells, and have, as a matter of fact, no connection with it. The radial tracts are absent in Asterina, but the oral circular tract is well represented, and we shall study its development later.

The woodcut shows us that the foregoing description is not quite correct. In the first place, we see that one can hardly speak of an outer perihæmal ring, because this space is broken up into five compartments by the prolongations of the longitudinal septa of the radial canals; secondly, apart from the mistake we just pointed out in reference to the nature of the "heart" and aboral ring, we see that the axial sinus (a') does not open into the perihæmal aboral ring; and, further, that to the upper end of the axial sinus is closely apposed a small closed sac, the right hydrocœle.

Returning to figs. 51—53, we see that each of the five compartments of the outer oral perihæmal ring arises separately as a wedge-shaped outgrowth of the cœlom. I have numbered these rudiments according to the numbers of the lobes of the hydrocœle between which they occur—*ph.* 1.2, *ph.* 2.3, *ph.* 3.4, *ph.* 4.5, and *ph.* 5.1; the last, however, arises later, and is not seen in these figures, and the first is an outgrowth of the anterior cœlom (Pl. 20, fig. 51, Pl. 21, fig. 54): all the rest arise from the left posterior cœlom. The shape and relations of these rudiments are well shown in the enlarged drawing given of one of them (Pl. 27, fig. 139); we see that the base of the wedge is directed outwards, and that its basal angles tend to insinuate themselves between the ectoderm and the hydrocœle. As a matter of fact, each angle grows out till it meets the adjacent one of the next rudiment. The two then become apposed to each other, and their walls, which meet, form the longitudinal septum of the radial canal, and

both spaces grow out together underneath the growing lobe of the hydrocœle, and thus the radial perihæmal canal itself is formed; we shall find later that the inner perihæmal ring arises as an outgrowth from the oral end of the axial sinus or anterior cœlom, and hence it is marked *a'* in the woodcut.

Fig. 53 shows us that the fourth and fifth lobes of the hydrocœle have extended over to the right; this being the result of the tendency of the two ends of the hydrocœle, which have become entirely shut off from the anterior cœlom, to approach one another. We also see from the obliquity of the right posterior cœlom (compare figs. 44—46 with figs. 52 and 53) that the lateral flexure of the body on the stalk has commenced. The flexure in a downward direction cannot be well shown by sections.

Pl. 29, figs. 54—57, are sections of a larva rather older than Stage E. We see that the differentiation of the stalk from the body has been initiated by the dorsal constriction of the neck of the præoral lobe. In consequence of this the anterior cœlom becomes divided into a stalk portion *a*, and a body portion *a'*, the latter forming the axial sinus. We see, further, that the ventral horn of the left posterior cœlom *l'p'c'* has pursued its growth, extending obliquely to the right under the gut, and then upwards in a dorsal and anterior direction, and on its course the last of the five arm rudiments appears, viz. V. Fig. 57 shows the outgrowth of septa destined shortly to close the ventral communication between this right horn of the left posterior cœlom and the anterior cœlom. The primary lobes of the hydrocœle have each by this time given rise to two lateral lobes, the rudiments of the first tube-feet, the primary ones themselves being destined to form the terminal tentacles of the water-vascular system.

Figs. 58 and 59 represent a larva about midway between Stages E and F. We see the final division of the hydrocœle from the anterior cœlom, the last connection being in the neighbourhood of lobe 3, and also the separation of the axial sinus from the stalk cœlom. We see also the remains of the larval œsophagus (*lœ.*), which already in Stage E has broken off

from connection with the gut; the relative position of the adult œsophagus (*a.œ.*) is also well shown. Fig. 60 is from a larva of about the same age; it shows the formation of the fifth perihæmal rudiment (*ph.* 5.1) as an outgrowth of the ventral horn of the posterior cœlom: this lies beyond the fifth hydrocœle lobe, and will therefore come to lie between this and No. 1 lobe when the two ends of the hydrocœle meet. We also see the process of destruction of the stalk going on, the ectoderm of its anterior surface being invaginated in patches, and, as we shall see, each patch as it is invaginated becomes destroyed by histolysis. Fig. 61 is from a larva which has nearly attained Stage F; it shows how the dorsal horn (*l''p''c''*) of the left posterior cœlom wedges itself in between the gut and the hinder wall of the anterior cœlom (*a'*). In this wall we see running from left to right (i. e. from oral to aboral sides of the disc) from the second lobe of the hydrocœle, the stone-canal. The ciliated cylindrical epithelium of this has now become continuous with that of the pore-canal, but only on one side; the conjoined tubes still open to the anterior cœlom, and this opening persists in the adult, a fact which Ludwig did not observe (to see this, a more dorsal section than fig. 61 would have to be shown). The reader will remember that the pore-canal is formed by a dorsally directed outgrowth of the anterior cœlom fusing with the ectoderm, and a perforation occurring at the point of contact, and that the stone-canal is at first a ciliated groove running along the posterior wall of the anterior cœlom. This groove we found became converted into a canal opening into the hydrocœle on one side, and the anterior cœlom on the other just below the inner opening of the pore-canal (woodcut 2).

We have now arrived at Stage F, the external appearance of which is shown in Pl. 18, figs. 14—16. We notice that the præoral lobe or stalk has become very much reduced, and that the two ends of both curves, that of the hydrocœle lobes (numbered in Arabic figures) and that of the arm rudiments (numbered in Roman numerals), have become very much approximated to each other.

At the same time we see that oral and aboral parts of the

future star-fish are decidedly oblique to one another, being closely apposed posteriorly, but anteriorly separated by the thick base of the stalk. We see also that a lateral shift of the arm rudiments has commenced, No. V having passed beyond the hydrocœle lobe No. 5, and so also in the case of the others. A second pair of rudiments of tube-feet has grown out from each lobe of the hydrocœle, so that they are now 5-partite.

Figs. 62—69, Pl. 21, are taken from a most instructive series of sections of a larva of this age, and are intended to give a clear conception of its internal anatomy. We are struck at once by the great reduction of the stalk, although ventrally (fig. 66) the stalk cœlom still communicates with the axial sinus. In fig. 65 we see the last trace of the secondary ventral communication between the left posterior cœlom (*l'p'c'*) and the axial sinus *a'* (anterior cœlom) just closing. The secondary dorsal opening persists much longer, but fig. 63 shows us that it also is beginning to be closed. Comparing figs. 64 and 65, we see that the adult œsophagus has acquired two lateral outgrowths, one directed anteriorly, the other posteriorly ; there is also a third horn directed dorsally, which of course cannot be seen in the sections. Fig. 67 shows how the oral cœlom (*or.c.*) now half encircles the adult œsophagus. As to the arm rudiments, the most interesting thing is to notice the wide separation of No. V from the hydrocœle lobe No. 1. When the intervening tissue shrinks, a change which involves a reduction in size of the axial sinus (compare *a.'*, Pl. 22, figs. 75 and 76), the metamorphosis will be complete. The incipient shift of the other rudiments is seen, especially in the case of Nos. II and III, the latter falling between lobes 3 and 4.

By a continuation of the processes referred to above, viz. the constriction of the base of the stalk, the increasing flexure of the body on it, and the continued growth of the hydrocœle and left posterior cœlom, we soon reach Stage G, which is represented in Pl. 18, figs. 17 and 18. We notice the great reduction of the stalk (which is now usually directed downwards almost at right angles to the disc, though the extent of the angle between the two varies) and the completion

of the circle of arm rudiments, though No. I is not quite adjusted to hydrocœle lobe No. 2, and the hydrocœle ring is as yet incomplete. Here is a fitting place to give in a word or two the gist of Ludwig's observations on the calcareous plates. On the oral side (fig. 17) we notice ten small calcareous stars, two at the base of each primary hydrocœle lobe, situated on the inner side of the first pair of tube-feet rudiments. These are the beginnings of the first ambulacral ossicles (*amb.*). On the aboral side we notice eleven plates, one central (*C.*), five situated in the arm rudiments and destined to form the terminals (*T.*) (the plates which protect the terminal tentacles of the water vascular system), and five interradially situated, the basals (*B.*), one of which becomes the madreporite. The name "basal" is given on account of an imagined homology with the basals of Crinoids; the groundlessness of this assumption I shall point out later. All these plates make their first appearance simultaneously, rather earlier than Stage F. Fig. 19 shows the aboral surface of a young star-fish about sixteen days old. We see that the anus has been formed close to the central; that a plate has been interposed between each terminal and the central, the former maintaining its position in the tip of the growing arm, and that finally a pair of plates has appeared in each interradius, peripherally situated with regard to the basals, the latter retaining their position in the centre of the disc. These paired interradial plates are homologised by Ludwig with the interambulacrals of Echinids.

Plate 22, figs. 70 and 71, are two sections of a larva of Stage G. As in all the figures the stalk is placed as nearly as possible in the same position, one can see at a glance the very great lateral flexure which the disc has undergone with reference to the stalk. We see the relation of the rudimentary larval œsophagus to the permanent one; we further see that the oral cœlom is commencing ventrally to open into the left posterior one (this is of course a secondary communication, and I may say at once that the oral cœlom does not give rise to a separate space in the adult, but merely forms the part of the cœlom abutting on the inner side of the buccal membrane), and finally

we observe the incipient bifurcation of the posterior end of the pyloric sac (which is formed from the larval stomach) to form the pyloric cæca.

Fig. 79 is a section parallel to the adult plane of a slightly younger larva; it shows beautifully the mutual relations of the water-vascular ring (*wvr*), the axial sinus, and the oral cœlom. If one compares this figure with Pl. IV, fig. 53, in Ludwig's paper, one sees at once that his supposed rudiment of the oral blood-ring is only the oral cœlom. Figs. 75 and 76 show the completion of the metamorphosis by the apposition of arm rudiment No. V covering the tip of the ventral horn of the left cœlom (*l'p'c'*) to hydrocœle lobe No. 1. As compared with the larva represented in Pl. 21, figs. 62—69, we notice the much smaller size of the axial sinus (*a'*). Fig. 75 shows also the bifurcation of the anterior end of the pyloric sac into two cæca. Comparing it with fig. 76, which is a more ventral section from a larva of the same age, we see also that the spaces between the pyloric cæca (*py*) and the aboral body-wall are continuations of the right posterior cœlom.

Fig. 76 shows also the first trace of ovoid gland ("heart") (*ov.g.*) arising as a ridge of epithelium including blastocœlic jelly and fibres and amœbocytes, projecting into the axial sinus. By comparing this figure with Pl. 21., fig. 61, the shift of arm rudiment No. V can be clearly made out. Figs. 80 and 81 are sections parallel to the disc of a larva rather older than Stage G. Fig. 80 shows how the oral cœlom almost surrounds the œsophagus, and also that the axial sinus is commencing to form the inner perihæmal ring by growth from its lower end (compare woodcut). In fig. 81 we see at the point marked * the closing of the water-vascular ring by outgrowths from the hydrocœle lobes Nos. 1 and 5 respectively. We also notice what we have already seen in fig. 76, that the septum between the oral cœlom and the left posterior cœlom is breaking down; and in fig. 82, which is from a young star-fish in which the metamorphosis is just complete, we see that from the remnants of this septum the retractor muscles of the œsophagus or "stomach" are formed. The remaining figures on the plate show the finishing touches

of the metamorphosis. In fig. 72 the adult mouth is formed, and the sessile mode of life has been given up, the stalk being reduced to a small solid rudiment. We see also the first trace of the eye as a small knob at the base of hydrocœle lobe No. 3. Fig. 78 shows the permanent anus; if we compare its position with that which the larval anus occupied, we find that they are by no means the same: the larval anus, if it had persisted, would be situated at the point ×, though both occupy a position on the mesentery dividing the left from the right posterior cœloms. Fig. 77 from the same larva shows that the left posterior cœlom now forms a complete ring by the breaking down of the partition between its right ventral and right dorsal horns (*l'p'c'*. and *l''p''c''*.).

In fig. 73 a dorsal section, and in fig. 74 a ventral section, we see the incipient bifurcation of the right posterior cœlom in order to form the outgrowths connected with the two dorsal and the ventral pyloric cæca respectively. We see, therefore, that of the five pyloric cæca, two are formed from the dorsal end of the pyloric sac or larval stomach, and two from its ventral end, and that their suspensory mesenteries are outgrowths from the mesentery separating right and left posterior cœloms. The fifth cæcum is directed dorsally and posteriorly. In Pl. 22, fig. 82, and Pl. 23, figs. 83, 84, we have three sections parallel to the adult plane of a specimen which had just completed the metamorphosis. Once the mouth is open, the trifid form of the adult œsophagus changes, we get the five slightly bifid lobes of the adult "stomach." In fig. 83 we see the first trace also of the bifurcation of the pyloric cæca; I remind the reader that in each arm of the adult there are two cæca; the characteristic appearance of the axial sinus, stone-canal, and right hydrocœle in a section parallel to the disc are also shown, the right hydrocœle having a crescentic form. Fig. 84 shows us the relation of the rectum and the rudiment of the rectal cæcum to the pyloric cæca; we see that the mesentery which binds the bases of the pyloric cæca together is only the original mesentery between the right and left posterior (oral and aboral cœloms); and, further, that the mesenteric band connecting the inter-

radius of the stone-canal with the stomach is a part of this same original mesentery, with which, however, is continuous a piece of the wall between dorsal and ventral horns of the left cœlom, these two horns being still separated by this wall near their right sides (aboral surfaces).

Histological Changes during the Metamorphosis.

Up to Stage G the histology has little changed from that of the larva before metamorphosis. The most striking alterations are those connected with the destruction of the præoral lobe. Pl. 27, fig. 136, gives a specimen of them. This figure, which is taken from the larva represented in figs. 62—69, shows that the ectoderm becomes invaginated into pockets, and then these pockets completely closed, so that no breach in the continuity of the skin is made. The invaginated portion is then destroyed by amœbocytes as shown in the figure. The peritoneum lining the stalk cœlom contracts violently, the cells becoming cylindrical instead of flattened, and the larval muscles very apparent. So far as I can make out, these cells are destroyed by amœbocytes of the cœlom.

In the larva the whole hydrocœle rudiment is lined by cylindrical cells (Pl. 27, fig. 138); but as metamorphosis proceeds, and the hydrocœle increases in size, the cells are stretched so as to become flattened (Pl. 27, fig. 139); they retain their original character only in the rudiments of the tube-feet (Pl. 28, fig. 149) and terminal tentacles. The first trace of the adult nervous system appears in Stage F in the ectoderm covering the water-vascular ring,—that is, the portion of the hydrocœle between the primary lobes. The ectodermal cells become long and filamentous, with their nuclei set at different levels, and amongst their bases (Pl. 28, fig. 140) appears a tangle of fine fibrils of excessive tenuity, so that the highest magnification is required to make them out; this is the first trace of the adult nervous system.

Ludwig talks of cells stretched parallel to the surface under the ectoderm, which he supposed to become the bipolar ganglion cells of the nerve-cord; but the cells in question, if I

rightly identify what he means, are only the epithelial lining of the perihæmal spaces which at a later period become closely apposed to the ectoderm. The first trace of muscles in the body-wall appears much earlier. Pl. 28, fig. 145, shows the formation of a well-marked muscular band from the wall of the right posterior cœlom of a larva of Stage E. We see that it consists of indubitable myo-epithelial cells. I have traced this band into the oldest specimen I have examined for histology; and so far as I can see it appears to become a dilator of the anus. It is very strange that it should appear long before any other muscles of the body-wall; it forms quite a conspicuous feature in sections of all well-preserved metamorphosing larvæ. The same figure shows the first trace of histological differentiation in the mesenchyme; we see the first formation of that fibrous intra-cellular substance which gives firmness and tenacity to the adult body-wall.

The cells of the gut remain unchanged till the very end of the metamorphosis, but in Stage G we can trace some differentiation. Pl. 26, figs. 127, 128, show part of the lining of the adult œsophagus and of the pyloric sac of such a larva. The cells of the former are very long and narrow, and their outer portions take a clear yellow tone with osmic acid; those of the latter are ordinary cylindrical epithelium cells.

Abnormal Larvæ.

I mentioned above that the demonstrative proof that the sac I have termed the right hydrocœle is of that nature is obtained from the study of abnormal larvæ. I suppose that about one in thirty of the larvæ I examined were abnormal, though in very different degrees. The commonest abnormality results from the unusually great development of the organs of the right side, and the consequent checking of the metamorphosis.[1] The larva of which the two sections are given in figs. 85 and 86 had about attained Stage D. The left hydrocœle is perfectly normal, but the right, though not much larger than usual, is

[1] The reader will remember that in the analysis of the metamorphosis which I have given on p. 355, one of the main factors recognised is "the preponderating growth of the organs of the left side."

divided into distinct rounded lobes lined by cylindrical epithelium (*rhy.*), in all respects similar to those of the left, and the rudiment opens by a narrow but distinct slit into the anterior cœlom. This larva also exhibits another very common abnormality, which I do not in the least understand; this consists of the breaking up of the gut epithelium into a mass of cells having the appearance of mesenchyme, which choke up the lumen, but leave the walls almost denuded of epithelium, consisting chiefly of the basement membrane. This curious change can take place at any stage from the commencement of the differentiation of the cœlom, up to young adults a month old: in one such specimen it affected the pyloric cæca. As to what its meaning is, I confess I am entirely in the dark.

Figs. 87 and 88 represent a most remarkable larva. The development of the left posterior cœlom would indicate that it had reached Stage E, but the left hydrocœle consists only of four lobes, and is poorly developed. There are two rudiments of a hydrocœle on the right side; the more ventral has three distinct lobes lined by cylindrical epithelium (*r'hy'.*, fig. 88), and opens by a distinct opening into the anterior cœlom; the more dorsal is perfectly normal (*rhy.*, fig. 87); but, as if to emphasise the fact that, in spite of the presence of the other rudiment, it does in fact represent a hydrocœle, we find in connection with it a second small stone-canal and pore-canal (*p'c'. st'. c.*). The relation of these to the right hydrocœle may seem unusual; instead of the canal (conjoined stone and pore-canal) leading from the hydrocœle to the anterior cœlom and thence to the exterior, it appears to lead from the anterior cœlom to the hydrocœle and thence to the exterior. This apparent difference may be reconciled with the arrangement on the left side by observing the angle which stone-canal and pore-canal make with one another. Woodcut 3, p. 370, shows that this is an acute instead of an obtuse angle, and hence that stone-canal and pore-canal have coalesced laterally; Woodcut 2 shows for the sake of comparison the normal stone-canal and pore-canal and their relationship to the left hydrocœle and the axial sinus or anterior cœlom.

Fig. 89 is a section of a larva of Stage D; both hydrocœles are well developed—the right, in fact, better than the left; the

Fig. 2.

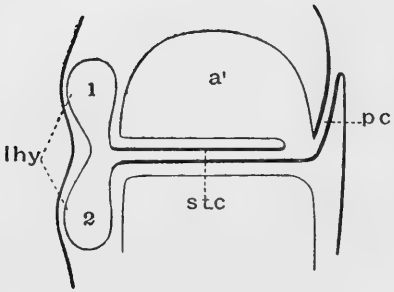
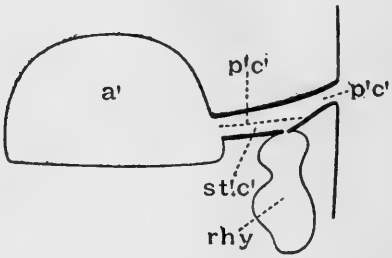


Fig. 3.



right hydrocœle appears on the left side of the figure, since by an oversight the section was drawn from the wrong aspect. It took me some time in this larva to determine which side was which, but the right hydrocœle is rather more dorsally situated, and opens by only a narrow slit into the anterior cœlom. It is also curved somewhat differently, the most posterior lobe being No. 4, not No. 3, as on the left side. Fig. 90 shows a most remarkable variation. We see a pore opening directly from the hydrocœle to the exterior. If, as I shall attempt to show later, the anterior cœlom may be compared to the proboscis cavity of Balanoglossus, and the two hydrocœles to the collar cavities of that animal, we see that what we may term a collar-pore may arise as a variation. Figs. 91—94 are sections taken from a larva of Stage G. Its only abnormality is that in connection with the right hydrocœle, which is of normal character, a second pore-canal and stone-canal are developed. Fig. 92 should show the opening of the second stone-canal into the hydrocœle, but the lithographer has unfortunately not brought out the slit-like opening; fig. 93 the opening of conjoined pore-canal and stone-canal (compare woodcut 3) into the axial sinus. Fig. 91 shows that the two pore-canals unite, to open by a common median pore. The above are not by any means all the variations observed, but they are sufficiently typical to indicate their nature.

The History of the Young Star-fish.

The just metamorphosed Asterina gibbosa has a disc of about ·6 millimetre in diameter; if we take R to denote the length from the tip of the arm to the centre of the disc, then R equals ·36 millimetre. A larva such as that figured in figs. 51—53 may be ·8 millimetre from the tip of the adhesive disc to the posterior end, and measured obliquely from the dorsal end of the præoral lobe may exceed a millimetre in length. There is, therefore, a considerable diminution in size during the metamorphosis, the reason of which is evident when we consider that no nutriment is taken during this time. A full-grown specimen may have a diameter one hundred times greater than that of the just metamorphosed star-fish,—that is, it may exceed the latter one million times in bulk. The young star-fish, however, rapidly increases in size, and by the time R equals 3·7 millimetres the ovaries are visible. This is the oldest stage I have examined; my account of the histology is, however, taken from smaller specimens, in which R equals ·8 mm.

The changes we shall have to consider are (1) the formation of the primitive germ cells, the ovoid gland, genital rachis, and ovaries; (2) the dermal branchiæ; and (3) general histological differentiation.

We have already in Fig. 76 seen the first trace of the ovoid gland. It there appears as a ridge projecting into the axial sinus; inside this ridge there is as yet to be found only amœbocytes, jelly and fibres, as is the case with the other blastocœlic spaces in the larva. Later, a thickening of peritoneum takes place on the wall of the left posterior cœlom opposite the aboral end of this ridge—and from this thickened patch a cord of cells grows into the ridge, gradually forcing its way in an oral direction; this is the characteristic core of the ovoid gland.

From this same thickening of peritoneum a cord of cells grows out in a direction parallel to the disc; this is the origin of the genital rachis. By the outgrowth of a flap of peritoneum it is enclosed in a space which is called the aboral sinus. The genital rachis and the space enclosing it both give off branches

one at each side of each arm. Local thickenings of these branches of the rachis constitute the genital organs. The surrounding spaces, the genital sinus (*ab gon*, figs. 122 and 123), is shut off from the aboral sinus by the outgrowth of a septum.

Fig. 99 is the marginal portion of a section vertical to the disc of a larva of Stage G. We see the rudiment of the ovoid gland (*ovg.*) as a fold projecting into the axial sinus. Further up we notice a thickened patch of peritoneum, which is invaginated into the septum separating the axial sinus from the left posterior cœlom (*pr. germ. inv.*). This is the rudiment from which, on the one hand, the genital rachis and, on the other, the core of the ovoid gland are derived. Figs. 100—103, similar sections to fig. 99, from a just metamorphosed star-fish, illustrate this. We see that from this rudiment a cord of primitive germ cells has grown out and filled the fold which is the rudiment of the ovoid gland. The last two sections cut a more oral portion of the fold, since they are slightly oblique; we see (figs. 102 and 103) that this core has not as yet penetrated to the oral end of the fold, and, further, that the fold is attached to the oral side of the inner perihæmal ring, or, in other words, that it traverses the lower end of the axial sinus, and is attached to its lower side. The original invagination to form the germ cells is situated at the very tip of the right dorsal horn of the left cœlom, where it meets the right ventral horn, but at this level the two horns do not open into each other (see p. 367). Figs. 104—106, again representing sections vertical to the margin of the disc, are taken from a young star-fish, in which R equals ·4 millimetre. Fig. 104 shows the cord of cells which arises from the peritoneal invagination and penetrates the dorsal organ, and the relation of this cord to the right hydrocœle and the axial sinus. We see that now this core of cells reaches to the oral end of the ovoid gland, and penetrates also a prolongation of the same, which is prolonged as a fold, hanging from the aboral wall of the inner perihæmal canal (figs. 105 and 106).

Pl. 25, fig. 110, which represents a similar section to figs. 99—106, shows practically the adult condition of the ovoid

gland and neighbouring organs. We see that the madreporic pore has commenced to be divided into two by the ingrowth of a fold. It is not the case in Asterina, as far as I can make out, that the numerous pore-canals found in the fully grown adult are derived from fresh perforations, as Cuénot has stated (3). Rather the statement which he quotes from Perrier seems to give the actual method of their formation.[1] We see that the openings of the stone-canal proper and the pore-canal into the axial sinus are still maintained. The ovoid gland with its core is seen to reach right down to the oral end of the axial sinus, and to be attached to its oral wall. Embedded in the septum dividing the inner perihæmal ring-canal (lower end of the axial sinus—see woodcut 1) from the perihæmal spaces proper is the so-called oral blood-ring (*sang. circ.*). This is a ring-shaped tract of peculiarly modified connective tissue; the section shows that it is of a different nature from the ovoid gland, and has no connection with it. In Asterias this ring gives off radial prolongations traversing the longitudinal septa of the radial perihæmal canals, but these do not exist in Asterina. The development of this structure as far as its histology is concerned is shown in Pl. 24, figs. 107—109, which represent small portions of sections parallel to the disc. The first two sections are taken from the same specimen as figs. 82—84; in this specimen as we have already learned (see above, p. 366) the metamorphosis has just concluded. We see that the mesenchymatous tissue between the outer and the inner perihæmal rings has undergone differentiation. Most of it has become converted into fibrous tissue, but at one level no fibres have been formed, the whole of the mesenchyme cells becoming amœbocytes (*sang. circ.*); this part is the rudiment of the blood-ring. In fig. 109, taken from a specimen in which R equals ·45 millimetre, we see that the ring is completely formed;

[1] Durham, in a paper on "Wandering Cells in Echinoderms" ('Quart. Journ. Micr. Sci.,' vol. xxxiii), has described the communication of the axial sinus and stone-canal in a young Cribrella. He also insists that we have no blood-vessels, but rather "hæmal strands" in Echinoderms, but makes the common error of supposing the ovoid gland to belong to this category.

the intercellular jelly or plasma has acquired staining properties. To Leipoldt (9) is due the credit, in a careful paper on the anatomy of "the so-called excretory organ of the sea-urchin," of emphasising the fact that the ovoid gland and the oral blood-ring are of totally different nature; he describes branches from the blood-ring ramifying on the external surface of the ovoid gland.

The question arises, what is the true nature of this blood-ring? Cuénot (3) answers that it is a lymphatic gland, or centre for the formation of amœbocytes; and there is a great deal to be said for this view. We must, however, remember that structures of similar nature are found accompanying the alimentary canal in Echinids and Holothurids. Ludwig (13) has given a splendid description of their arrangement in the last group. He brings out with great clearness that they are tracts of connective tissue in which the fibres are sparse. The close relation of these "vessels" to the alimentary canal suggests forcibly that we may have here the first attempt at forming blood-vessels. There is certainly no propulsive organ or proper circulation, but the staining properties of the plasma show that it has been chemically altered, and the idea is suggested of some secretion from the gut-cells propelling itself along these tracts by the vis a tergo force of secretion. In the Asterid no close connection with the gut is observable,—the oral cœlom, in fact, intervenes between the œsophagus and the ring, as we have seen (p. 365); but the altered character of the plasma suggests that perhaps here some substance is formed necessary for the well-being of the organism, which then diffuses out into the neighbouring cœlomic spaces. The blood-spaces of the higher animals are known in many cases to be remnants of the blastocœle or segmentation cavity of the embryo; this has been shown in the case of Balanoglossus with great clearness by Spengel (21). Strictly speaking, therefore, the blood and lymph spaces of other forms are represented in Echinodermata by all the spaces in the body-wall unoccupied by fibrous tissue and dermal ossicles, and traversed by amœbocytes; but the blood-ring, gut vessels, &c., may be a first attempt at specialisation.

Figs. 113—117 are intended to illustrate the formation of the genital rachis; and they all represent portions of sections cut parallel to the disc; those portions, in fact, which are transverse sections of one of the five interradial folds of the body-wall which in the star-fish project into the body-cavity. As we see in Pl. 23, fig. 83, the axial sinus, right hydrocœle, and the stone-canal, are embedded in one of these folds. It follows that the cœlomic wall of this particular fold represents the larval septum between the anterior cœlom and the posterior cœloms; and its interradial position in the star-fish becomes explained when we remember that the stalk with its contained anterior cœlom lies opposite an interradius of the water-vascular ring; which interradius is constituted by the outgrowth of processes of the two lobes situated at the ends of the hydrocœle, which is as yet an imperfect ring. These outgrowths meet, so to speak, above the neck of the stalk. Figs. 113 and 114 are from the same specimen as fig. 109. We see the appearance of the rudiment of the germ cells in a section parallel to the adult plane, and notice the remains of the cavity of invagination (fig. 114, *pr. germ. inv.*). Fig. 113 shows us that one horn of the right hydrocœle has become embedded in the ovoid gland, and this is one reason why it is extremely difficult to trace the continuity of the primitive germ cells by sections taken parallel to the adult plane, since the cord of cells is in some spots so narrow, and is therefore difficult to distinguish from the epithelium lining the right hydrocœle. Longitudinal sections, such as fig. 104, show it much better. In figs. 115 and 116 (taken from a specimen in which R equals ·7 millimetre) we see the formation of the genital rachis; this takes place by a lateral outgrowth from the primitive patch of invaginated peritoneum, from which we have seen the core of the ovoid gland originating as an orally directed outgrowth; the aboral sinus which surrounds it (*ab.*) is formed at the same time, it is a portion of the cœlom shut off by the outgrowth of a fold of peritoneum. Fig. 117, taken from a much older specimen, shows the genital rachis in its complete form

in continuity with the original rudiment of the primitive germ cells.

It is, then, not quite correct to speak of the genital rachis as being an outgrowth from the ovoid gland, as Cuénot has done (3). This statement, nevertheless, marked a step in advance in our knowledge, for it gave a hint as to the meaning of the ovoid gland. Cuénot found specimens of Astropecten with the ovoid gland, but without the genital rachis, and noting the identity of the character of the cells in the two structures, stated that the rachis was an outgrowth from the gland, though he found no intermediate stages. These were first found by me (14) in the Ophiurid Amphiura squamata, and at the same time I demonstrated the epithelial origin of both gland and rachis. It is the genital rachis which of course was formerly known as the aboral blood-vessel; in most Asterids and Ophiurids it later undergoes partial degeneration, giving rise to cells containing violet pigment. Ludwig, however (11), and Haman (7) have pointed out that the central core remains unaltered; the latter was the first to point out that in all Echinoderms, except Holothurids, a genital rachis exists, of which the genital organs are local outgrowths. In Amphiura squamata, however, and in Asterina gibbosa, according to Cuénot (3), the whole genital rachis remains unaltered through life; this is only one of the many points in which Asterina shows itself to be one of the most primitive of Asterids. In the plans given in text-books of the blood system, two vessels are shown proceeding from the aboral ring in the interradius of the madreporite to the pyloric sac. These are two mesenteric bridles, remnants of the piece of septum left at this level between the two horns (right dorsal and right ventral) of the left cœlom. At this spot the right (aboral) cœlom breaks through into the left (oral) cœlom, perforating the piece of tissue referred to, and leaving only the mesenteries. The peritoneum covering them seems to be peculiarly modified, and is possibly a place where the amœbocytes of the cœlomic fluid are formed.

The genital rachis gives off, as it passes each interradius, two branches enclosed in corresponding branches of the aboral

sinus (*gen. r.*, woodcut 1); one of these branches runs in an oral direction down each side of the interradial septum. This septum is an ingrowth of the body-wall, which has by this time become marked, though its first beginnings date back to the end of the metamorphosis (Pl. 23, fig. 84).

A section of one of these branches in an older specimen is given in Pl. 26, fig. 119). These genital branches are formed as the rachis reaches each interradial septum before it has formed a circle; in one specimen I have observed a rachis reaching only to the next interradius, and there giving off one genital branch. Figs. 120 and 121 (taken from the same specimen as fig. 119) show the first rudiments of the genital organs. The branch of the rachis ends in a swelling accompanied by a dilatation of the aboral sinus, and we see the beginning of a septum tending to shut off the main aboral sinus from this dilatation. This septum was first described by Cuénot (3), and in it the genital duct is formed. This is shown in fig. 123, taken from the oldest specimen I examined, in which R equals 3·7 millimetres. We see that the genital duct is formed by a core of primitive germ cells burrowing its way through the body-wall. Fig. 122, from the same specimen, shows the continuity of the rachis and the ovary. We notice also the formation of follicles from the indifferent germ cells.

We are now in a position to compare the arrangement of the ovoid gland and genital rachis and their accompanying spaces in Amphiura squamata with that found in Asterina gibbosa. In the former I described the genital rachis issuing from the oral end of the gland and accompanied by three spaces, which I named sinus *a*, sinus *b*, and sinus *c* (Pl. 25, fig. 112). This figure is a diagram of a section parallel to the long axis of the stone-canal. Fig. 111 is a diagram of a similar section of Asterina, but it is not quite accurate, since it shows both the ovoid gland and the stone-canal, and these two structures do not lie in the same radial plane in Asterina. In order to avoid obscuring the opening of the stone-canal into the axial sinus, it is necessary to indicate part of the ovoid gland by dotted lines.

Comparing figs. 111 and 112 we see that the axial sinus of Asterina is represented in Amphiura by sinus *c*, the so-called "ampulla." The aboral sinus (*ab*, fig. 111, sinus *a*, fig. 112) is also obviously homologous in both.

[Since my paper (14) was published, and since the present work was sent in for publication, I have made a careful re-examination of my sections of Amphiura squamata, and have arrived at a more complete comprehension of the structure and development of the ovoid gland and the neighbouring spaces in that animal. The space marked sinus *b'* (fig. 112) is not, as I formerly supposed, a part of sinus *b*, but is quite distinct. Sinus *b'* probably represents the right hydrocœle; it is already present in the youngest specimens I examined. Sinus *b** is a portion of the cœlom shut off by the outgrowth of a flap of peritoneum; from the inner wall of this sinus the cells which at the same time give rise to the ovoid gland and to the genital rachis take their origin; it is obviously homologous to the cavity of the invagination of the primitive germ cells (*pr. germ inv.*, figs. 110 and 111), only in Asterina this space disappears.—December, 1895.]

We observe that the arrangement in Amphiura might be obtained from that in Asterina by rotating the stone-canal and accompanying structures outwards and downwards through an angle of 180°. That this is what has occurred in phylogeny is indicated, not only by the fact that in the young Amphiura the madreporite is near the edge of the disc and the stone-canal almost horizontal, whereas in the adult the madreporite is situated far in towards the mouth on the oral surface, but also by the curious undulating course of the genital rachis, which is aboral in the interradii and oral in the radii. This points to the conclusion that the aboral parts of the interradii

* In my paper on this subject (14) sinus *b* is referred to as the axial sinus—it was formerly supposed to be continuous with sinus *c*, though Ludwig knew this was not so. At that time the meaning of the axial sinus in Asterids which Bury first suggested (2) was not generally known, and his interpretations were not accepted, and hence two different spaces were called axial sinus, one in Asterids and the other in Ophiurids.

have greatly developed, and have grown in between the radii on to the oral surface, forcing the original oral plates to the extreme centre of the disc; and so the stone-canal has been swung round and the genital rachis pulled out of shape. Now in Asterina gibbosa there is a trace of this process; the rachis does not, as Hamann (7) has described in Asterias, lie in one plane, but pursues an undulating course, being much more aboral in the radii than the interradii. I am inclined to look upon this as the primitive condition from which the Asterid and Ophiurid arrangements have been derived. I may as well mention here some other facts which indicate the primitive nature of Asterina. Chief among them is, that in the family of which it is a member we meet with the most rudimentary form of those characteristic Asterid organs the pedicellariæ. We have in Asterina the aboral surface covered with small spines, arranged in twos and threes, and acting on irritation like pedicellariæ. It is true that some Asterids have no pedicellariæ, but here the evidence from allied genera (cf. Luidia and Astropecten) suggests that they have been lost; Asterina, however, shows us pedicellariæ in statu nascendi. The simple biserial tube-feet also constitute a primitive character.

Fig. 118 represents ovoid gland and stone-canal in the latest stage examined by me. The gland is attached by an exceedingly narrow pedicle to the wall of the axial sinus. Its surface is thrown into deep folds, and the peritoneal lining of the axial sinus, which forms its outer covering, is modified, consisting of cylindrical cells with projecting rounded ends. The interior of the gland is filled with a mass of primitive germ cells supported by fibres, doubtless of mesenchymatous origin. I was unable to find any trace of a tube lined by primitive germ cells, such as was discovered by Hamann in the young Asterias.

What, we may finally ask, is the function of this strange organ? Cuénot, as usual, maintains that it is a lymphatic organ. This I am disposed to doubt very strongly; the cells which it contains are of quite a different nature from the amœbocytes of the oral blood-ring, and the evidence that

Cuénot brings to show that they escape by diapedesis into the axial sinus is quite insufficient. The cells of outer epithelial lining are not flattened but cylindrical, and I strongly suspect that he has mistaken their freely projecting ends for escaping amœbocytes; and I may remark that this curious outer epithelium shows its distinctive character from the time the first rudiment of the ovoid gland appears. Whatever its function may be now, there is no doubt that the ovoid gland was primitively a part of the genital organ, and probably is a remnant of the arrangement of the reproductive cells before the radial symmetry was acquired. It is interesting to notice that it originates from the left posterior cœlomic wall, whereas an analogous organ in Crinoids arises in the right or aboral cœlom, so that they are not strictly homologous.

If Hamann is, as there is strong reason to suppose, right in stating that the primitive germ cells wander along the rachis into the genital organ, it seems very probable that, at any rate in the young adult, the ovoid gland is a centre of formation of the primitive germ cells; and its relation to the axial sinus may have to do with its aëration, for it must be remembered that the pore-canal opens into the axial sinus, and the current in this is, as we shall see, inwards. In the fully grown adult it no doubt undergoes, to some extent, the degenerative change noted above in the genital rachis of other genera. What the meaning of this change is, is very obscure. Observations on the histology of the gland at different seasons might elucidate its meaning.

Turning now to the stone-canal, we see, in fig. 118 (a section transverse to the axial sinus and stone-canal), the beginning of that curious T-shaped ingrowth which is so marked a feature of the stone-canals of Asterids, but which is much less developed in Asterina than in other genera. It is covered by short cilia, the rest of the epithelium bearing long flagella.

Cuénot asserted that the stone-canal was a functionless rudiment, the current being neither outwards nor inwards. Ludwig[1]

[1] Ludwig, "Uber die Function der Madreporenplatte und des Steinkanals der Echinodermen," 'Zool. Anz.,' 1890, p. 377.

subsequently showed that in the stone-canal of Holothurids and Echinids the direction of the current is inwards. He examined the stone-canal cut out of the living animal; I have confirmed his result by a somewhat more satisfactory method. I kept Amphiura squamata living for several days in sea water, carrying in one case carmine, and in another lamp-black in suspension; and on cutting sections I found these particles in the pore-canal, and in some cases apparently ingested by the cells lining it. In view of Ludwig's researches Cuénot comes in a later paper (4) to what I believe to be the correct solution of the question of function. He there suggests that the flagella lining the stone-canal are always tending to produce an inward current, and that thus the turgidity of the whole water-vascular system is kept up. [This is practically the old view; except that he does not assert a continuous inward current.—December, 1895.]

It is obvious from the structure of the valves of the tube-feet that, in consequence of the ambulatory movements, there must be a slow loss of fluid. The ampulla and the tube-foot are shut off from the canal leading into the radial water-vascular canal by a pair of valves opening only inwards. Consequently during the contraction of either ampulla or tube-foot the two act together as a closed system, since no fluid can escape into the radial canal. The existence of the valves however shows clearly that fluid occasionally enters the tube-foot, and this can only be rendered possible by a slow loss of turgidity owing to the osmosis of the contained fluid when under pressure. This is confirmed by considering the case of Ophiurids, where, the tube-feet having lost their ambulatory function, the madreporite has only one or at most two pores, and the calibre of the stone-canal is exceedingly narrow.

The dermal branchiæ arise when the star-fish has reached a diameter of about 1·5 millimetres (R equal ·85 millimetre). We see that the branchia is only a very thin piece of the body-wall produced into a finger-like process (Pl. 23, fig. 98). Around the base of the branchia is a peribranchial space lined by flattened epithelium: this space, as Cuénot has rightly

observed, is the only one of the great "schizocœlic" spaces which Hamann (8) has described in the body-wall which has any real existence, the others being merely artefacts produced by the process of decalcification. I have found one specimen showing the first trace of a dermal branchia (figs. 96 and 97). We see a slight thickening of the peritoneum, and above it the peribranchial space. Fig. 96 shows that the latter is a diverticulum of the cœlom. As I have only one section illustrating this I do not speak with absolute certainty on the point; but, with this possible though very improbable exception, there is no schizocœle whatsoever in Asterina gibbosa: all spaces lined by epithelium are of cœlomic origin.

Histological Differentiation.

The cells of the gut-wall have undergone some change since the close of the metamorphosis. Specimens of the epithelium from different regions are given in Pl. 26, figs. 129—132. These are all taken from a young adult in which R equals ·85 millimetre. The cells of the lateral walls of the stomach (i. e. the adult œsophagus) have become exceedingly long and narrow; their outer ends are refracting and take a light yellow tone with osmic acid (fig. 129). The cells of the aboral wall, on the contrary, have developed numerous gland cells filled with globules; interspersed amongst them are some very narrow filamentous cells. Fig. 130 shows the spot marked × where the stomach opens into the pyloric sac and the abrupt change in the character of the epithelium. The pyloric sac is lined by uniform columnar cells; the nucleus is generally near the base of the cell, and is never placed further up than the middle, and the protoplasm is uniformly granular (fig. 131). The cells lining the rectal cæcum (fig. 132) are similar in form but smaller, and the protoplasm is clearer, with the outer part more refringent. It is at least a plausible suggestion that the gland cells of the stomach secrete the poison which paralyses the prey, and that the cells of the pyloric sac give rise to a digestive ferment.

The differentiation of tissues which has gone on in the

body-wall is illustrated in Pl. 28, figs. 146 and 147. These sections are taken from young adults in which R equals ·4 mm. and ·86 mm. respectively, and they pass through the same region as fig. 145, which is from a larva in Stage E, and which we have already described. In fig. 146 we see that the muscular fibres of the muscle we may call the dilator ani are still connected with the peritoneal cells; but in fig. 147 they have become quite distinct, and the cells of the peritoneum have become quite flattened. The ectoderm has entirely changed its character, the numerous larval goblet cells have almost disappeared, and the cells in general have become shorter; many of them are inversely wedge-shaped, and are apparently about to become converted into gland cells, probably of the same histological character as those of the aboral wall of the stomach. Here and there is a narrow cell ending in a fine hair, one of the scattered sense-cells of the aboral surface; these are shown in fig. 148, a piece of ectoderm from another individual of the same age. All observers agree in maintaining that the ectoderm of the adult retains its ciliated covering; but though I have been able to make out easily the cilia, or rather flagella of the metamorphosing larva, I have not been able to do so with any certainty in the aboral wall of these young adults. Probably the cilia are very delicate and fragile. The tissues of the mesenchyme have undergone marked differentiation. So far as my researches have extended it seems that three fates are open to mesenchyme cells, all of which are illustrated in fig. 147. They may remain practically unchanged as amœbocytes or wandering cells (*amœb.*), or they may become embedded in bundles of fibres so as to form connective-tissue cells (the fibres being intercellular, not outgrowths of cells); or, finally, they may fuse to form a syncytium having the form of a meshwork (*calc.*). This is the skeletogenous tissue; lime is deposited in the interstices of the meshwork. There is a fourth fate, which is not reached by any as far as I have gone, but which obviously must be the lot of some, and that is to form the muscles moving the spines or rudimentary pedicellariæ. The superficial position of these muscles renders it exceedingly

unlikely that their muscles are of peritoneal origin, and their position in other Asterids where, as in Asterias, for example, they occur on the skin covering the spines, growing even from their tips, makes such a supposition almost impossible. Therefore we must postulate some muscles of mesenchymatous origin for Asterina, although all those which I have examined are of epithelial origin.

The development of the **nervous system** has advanced greatly, and has reached, as soon as the metamorphosis is complete, its final form; this is shown in fig. 141, taken from the same specimen as fig. 146. The ectoderm cells have increased immensely in number, and become excessively filamentous, so that the nuclei are many layers deep; the fibrillar layer has increased very much in thickness. It is traversed by vertical fibres which sometimes branch and sometimes have small nuclei on them; these are in continuity with the ectoderm cells, but are probably of non-nervous character. Sections parallel to the disc show that numerous little bipolar cells are embedded in the mass of fibrils (Pl. 24, fig. 109, *bip. gang.*). Since these cells are not present in the just metamorphosed form, they must be ectoderm cells which have passed in, and occasionally one sees a cell just at the boundary of the fibres apparently in the act of passing in. The perihæmal spaces become closely apposed to the nerve-cord, no mesenchyme being left between (*ph.* fig. 141); the vertical fibres do not, however, arise in connection with the epithelium of these cavities, since they are present before this close apposition takes place. Cuénot states that all the ectoderm cells of the nerve-cord end in the vertical supporting fibres described above. This is a bold statement which it is quite impossible to prove by sections, and which is most improbable. As a matter of fact these vertical fibres are not present in nearly large enough number to account for all the ectoderm cells; and Hamann's statement (8) is probably correct, that many of these end in fine processes which lose themselves in the mass of fibrils.

The sense-organs of Asterina are all developed in connection with the appendages of the water-vascular system. The eye

arises at the base of the terminal tentacle of the radial canal; two stages in its development are given in Pl. 28, figs. 142 and 143. In the first we see a simple ectodermic involution; in the second we see a pit surrounded by columnar cells, probably retinal, and filled up by closely fitting polygonal cells, which correspond to the layer of vitelligenous cells in an Arthropod eye. The existence of these cells has been vigorously denied by Cuénot (3), who maintains that we have only polygonal cuticular plates. My sections, however, remove all doubt on the subject; they show with perfect clearness that we have to do with cells, and their nuclei can be made out. This pit is the first only of the numerous pits which cover the "eye" of the adult, which is really essentially a small rounded swelling at the very tip of the radial nerve. The method of preservation employed seems to have dissolved the pigment.

The remaining sense-organs are the tips of the tube-feet and the terminal tentacle. A longitudinal section of a tube-foot is given in Pl. 28, fig. 150. This is taken from a specimen in which R equals ·4 millimetre, but it holds true for specimens of a radius of a millimetre or more,—that is, for probably the first two months after the metamorphosis. Comparing it with fig. 149, a similar section taken from a larva in Stage F, we see that the ectoderm at the tip has become thickened, and underneath it we can make out on each side a mass of nerve-fibrils. A powerful nerve leaves the radial nerve-cord to supply each sense disc; it would be more correct to speak of these branches as actual prolongations of the nerve-cord with its cells and fibrils; they are, indeed, the only conspicuous branches which it gives off. Some of the ectoderm cells of the sense disc have a peculiar regular cylindrical form, which recalls that of the retinal cells.

The facts above related justify the view that the whole radial canal with its tube-feet is to be looked on as one large branched tentacle, the main function of which was probably originally prehensile and therefore also sensory; and since a plexus of nerve-fibrils is in the adult found under the ectoderm all over the body, the central nervous system may be said to be a local

concentration of this in the neighbourhood of a greatly developed sensory tentacle. The support of this tentacle by the arm is a secondary matter, as we have already learned—a fact which comes out still more clearly in Crinoid development. There the primary hydrocœle lobes develop into long free tentacles covered with sensory hairs. At a very late period (later than any which Seeliger observed) these primary tentacles, according to Perrier (17) become applied to five outgrowths of the body-wall; these latter immediately bifurcate to form the ten arms, and so the free tips of the tentacles are situated each in the angle between a pair of arms. Seeliger (18) adduces this last fact to show that the primary tentacles are not the same as the primary hydrocœle lobes of Asterids, forgetting that the point where a pair of arms diverge corresponds to the tip of the Asterid arm, since in Antedon there are ten arms which have arisen by dichotomy from five.

The epithelium of the water-vascular system in fig. 150 shows an interesting feature; the cells have developed muscular tails which are arranged longitudinally, and the important point is that these myo-epithelial cells persist as such for a considerable period of free life.

Pl. 29, figs. 151—154, show us that the aboral wall of the perihæmal space also gives rise to muscles. These connect one ambulacral ossicle with its fellow of the opposite side, and serve, by approximating these to one another, to close the ambulacral groove. Figs. 151 and 152 show us that here again we have, in the first instance, to do with myo-epithelial cells. Muscles connecting one ossicle with its successor and predecessor are also present, but very much more feebly developed. In Ophiurids, however, as is well known, they are most powerful, and this point gives the key to nearly all the peculiarities of this group as compared with Asterids. Presuming, as we fairly may, that these muscles are developed from the perihæmal wall as in Asterids, we are brought face to face with a most interesting effect which this produces on the nervous system. Fig. 156 gives a section of the radial nerve-cord of an Ophiurid. We notice two great masses of cells and fibres on

the aboral side of the nerve-cord, and Hamann (8) has shown that the nerves for the ambulacral muscles arise entirely from these.

Now it has been for a long time suspected, and Cuénot has finally proved it (4), that there is a similar but feebler development of what we may call "cœlomic nervous tissue" takes place in the Asterid. None of my specimens were old enough to show this, though one can see (fig. 141) that the perihæmal epithelium has come into intimate connection with the nervous matter. Pl. 29, fig. 155, represents a transverse section of the nerve-cord of a young Asterias; we see in it that this epithelium has become thickened on each side of the median septum; one requires, however, a section of the nerve of a fully grown adult to see the cœlomic nervous fibrils. So we may say that from their aboral wall the perihæmal spaces give rise to muscles, and from their oral wall to the corresponding nervous tissue. I ought to mention in this place that Cuénot describes a canal leading from the perihæmal space into the cœlom at the level of each ambulacral ossicle; also five pores leading from the outer perihæmal ring to the cœlom. If these communications exist, they are certainly secondary, as there is no trace of them in my specimens; but as Cuénot's results were founded on injection I am exceedingly sceptical as to the existence of such openings.

I have said above that the increasing importance of the ambulacral muscles is the explanation of the evolution of Ophiurids from Asterids. The Ophiurids have substituted the quick powerful movements of these muscles for the slow motions of the tube-feet. In correlation the nervous system has become better developed, the radial cords becoming gangliated, and the whole is removed from the exterior by invagination, and thus the subneural space is really a neural canal. The ambulacral ossicles have become firmly united, each to its fellow, to form a series of vertebræ, and thus the cavity of the arm is reduced, and this, with the simpler food, accounts for the disappearance of the pyloric cæca.

We have already pointed out that the lessened activity of

the tube-feet, consequent upon the loss of the locomotor function, explains the reduced stone-canal and madreporite, though probably their increased sensitiveness has helped in developing the nervous system.

Literature consulted.

An account of the earliest publications on Echinoderm development is not given here, since a résumé of them will be found in the papers I quote; and I hold it to be a waste of time to reiterate with each new paper the whole history of the growth of our knowledge ab initio. I mention here only those authors on whose results I have, so to speak, built, or from whom I have found it necessary to differ. Ludwig's work on the anatomy of Asterids (**10**) laid the foundation of our knowledge of the hæmal and perihæmal systems; though, as we have seen, many of his ideas about these structures were incorrect. Subsequently in treating of Ophiurids (**11**) he discovered the genital rachis. Hamann (**7**) extended this result, and pointed out the amœboid nature of the primitive germ cells. Then we had Ludwig's great work on the development of Asterina gibbosa (**12**), the first account of the metamorphosis of any Echinoderm which had any pretence of completeness, and to which I have constant occasion to refer. His account of the changes in external form and of the development of the calcareous plates can hardly be improved upon. Owing, however, to the imperfect methods in vogue at that time he failed to penetrate with equal success into the course of the internal changes. He saw nothing of the segmentation of the cœlom or of the ring-like growth of the left cœlomic vesicle; he saw nothing also of the origin of genital organs, ovoid gland, or oral cœlom. He did not observe the right hydrocœle or find the origin of the perihæmal spaces. He missed the fixed stage, and he does not seem to have had any clear conception of the relation to each other of the larval and adult planes of symmetry. We owe to him, however, the clear distinction of pore-canal and stone-canal, and the recognition of the fact that the pore-canal is completely independent of the

hydrocœle. Bury (1) may be said to have introduced modern conceptions of Echinoderm development by his work on the development of Antedon; there he distinguished between anterior cœlom and hydrocœle, and showed that the stalk was the præoral lobe. Then he made a series of observations on Echinoderm larvæ (2), and showed that generally speaking the cœlom on each side becomes segmented into two vesicles, an anterior and a posterior. He, however, regarded the hydrocœle as an essentially unpaired structure, an outgrowth from the anterior cœlom, and was greatly distressed to find that it originated from the posterior vesicle in Ophiurids, and that in Asterina the stone-canal, which in other forms represented the original neck of communication between anterior cœlom and hydrocœle, was apparently an independent perforation. The last difficulty has been answered by Ludwig;[1] as to the former, the proof I have brought that the hydrocœle is paired shows that there are really three primary divisions of the cœlom on each side, viz. the anterior cœlom, single in Asterina, but primitively paired in Asterias; the right or left hydrocœle, and the posterior cœlom (right or left as the case may be); the apparent formation therefore of the hydrocœle from the anterior or posterior vesicle is a mere question as to whether the septum between the posterior cœlom and the hydrocœle or the septum between the hydrocœle and the anterior cœlom is formed first.

In speaking of the Bipinnaria, Bury says that in a future paper he intends to prove that the anterior cœlom becomes the axial sinus, but up till now he has published nothing further on the subject.[2] He made a few observations on Asterina

[1] Bury had not seen the stage of development when the stone-canal is an open groove.

[2] Since the preliminary account (15) of the present paper was published, a paper on the "Organogeny of Stellerids," by M. Achille Russo, has appeared in the 'Atti della Accadema reale di Napoli' for 1894. In this work (to which I only obtained access some considerable time after the present paper was finished) M. Russo gives a description of the ontogeny and anatomy of the ovoid gland and axial sinus in Asterina gibbosa and an Ophiurid. He combats my statements about the origin of these structures in Amphiura squamata. The origin of the axial sinus in Asterina has been correctly described; it is about the only thing that is correctly described in the paper.

larvæ of Stage D, and saw the completely closed cœlomic vesicle on the right, and the imperfect transverse septum on the left side, and was at a loss how to interpret these appearances; the right hydrocœle he calls a mesenchymatous vesicle.

It is curious to see how unable many zoologists have been to grasp Bury's idea of the anterior cœlom; thus Seeliger, who has confirmed his work on Antedon and amplified it till it may be said that we have an exhaustive knowledge of the subject, objects to consider the structure Bury named anterior cœlom as such, on the supposition that Bury meant by that a fellow of the hydrocœle, which it obviously is not. Seeliger calls it the "parietal canal," but the structural facts he so accurately relates are convincingly in favour of Bury's interpretation. The weak point in Bury's observations on Plutei and other larvæ was that in no case were any more than a few stages taken at random examined; but I hope the account I have given in this paper will provide a more solid basis for the idea of segmentation of the cœlom in Echinoderms. Field (5) has published a short paper on the development of the Bipinnaria; he carries it up only to a stage corresponding to midway between Stages B and C of Asterina. The chief points of interest in the paper are that many of the larvæ had two madreporic pores, and he suggests that this is a normal stage in the ontogeny; also that the two ciliated rings characteristic of the Bipinnaria are derived from one, and that there is a præoral sense-organ comparable to that in Antedon.

This paper does not contain the discovery that the water-vascular rudiment is paired; for, as a matter of fact, in the oldest larva examined no trace of the left hydrocœle was present. The "schizocœlic space," near the madreporic pore, may represent the rudiment of the right hydrocœle; needless to say, it was not recognised as such.

Theel (22) has recently succeeded in following the metamorphosis in Echinocyamus pusillus so far as the external features are concerned. He finds that already in the blastula

M. Russo's technique was obviously not equal to dealing successfully with such difficult subjects as Echinoderm larvæ.

a præoral sense-organ is present; this subsequently becomes incorporated with the ciliated ring, and if this organ is homologous with that of the Bipinnaria, we may conclude that the ciliated band of the Pluteus corresponds only to the posterior of the two bands of the Bipinnaria, since in the Bipinnaria the sense-organ is situated between præoral and post-oral ciliated bands, and this spot corresponds to a constriction in the original longitudinal ciliated ring, not to a position on its anterior edge.

Our knowledge of Echinoderm histology is largely due to Hamann (8) and Cuénot (3 and 4). The latter, as we have seen above, was the first to suggest that the ovoid gland gave rise to the genital rachis. The first account of the development of ovoid gland and rachis is given in my paper on Amphiura squamata (14), and I have there collected the fragmentary notices on this subject, which had till then appeared.

[I regret that when I sent in this paper for publication I did not mention the well-known paper of Metschnikoff ("Studien über die Entwickelung der Echinodermen und Nemertinen," 'Mémoires de l'Académie Impériale de St. Pétersbourg,' tome xiv, No. 8), in which he describes a right hydrocœle in Amphiuria squamata. He there says that the right cœlomic vesicle becomes divided into anterior and posterior portions just like the left; the anterior portion sometimes atrophies but sometimes develops into a regular five-lobed hydrocœle. It has been the fashion to ignore this work, since it was not accomplished by modern methods; but after my experience with Asterina I feel morally certain that Metschnikoff was right, though of course he did not distinguish between hydrocœles and anterior cœlom. Bury (2) seems to have missed the importance of this observation.—Dec., 1895.]

General Considerations.

On reviewing the developmental history recorded in this paper, two main questions present themselves: first, what light does it throw on the affinities of the Asterids with other Echinoderms? and second, does it suggest any direction in

which we may look to find the origin of the group Echinodermata as a whole?

In answer to the first question, we must observe that the stalks of Asterina and Antedon are morphologically equivalent,[1] both being formed from the præoral lobe, and, so far as one might judge from the different shape of the latter in the two cases, the adhesive discs by which they fix themselves are situated in precisely the same position. Now no one doubts that Antedon had a fixed ancestor; it is, in fact, one of the very few Crinoids which do not remain fixed throughout their whole life. If Asterids ever had an ancestor in common with Crinoids which could be called an Echinoderm at all, it must have been one represented by the fixed larva of Antedon before it has fully acquired radial symmetry, since, as we have already pointed out, the metamorphoses of Antedon and Asterina pursue different courses. In the first case the mouth is shifted backwards and upwards, and a precisely similar thing happens to the larvæ of Entoproct Polyzoa, Ascidians, and Cirripedes when they fix themselves. In the second case, however, the disc is flexed obliquely downwards on the stalk, so that the left cœlomic sac and the hydrocœle both come to encircle the base of the stalk; and as consequence the aboral poles in the two cases are not homologous, for in the first case this pole is the cicatrice left by the rupture of the stalk, whereas in the second case the point where the stalk passes into the disc is quite remote from the aboral pole. The apparent correspondence of the calcareous plates of the calyx in Antedon and the so-called calyx in Asterina is simply due, in my opinion, to the

[1] Since the present paper was sent in for publication, my attention has been called to some observations of Perrier's which I regret having overlooked. In his account of the Echinoderms collected by the "Mission Scientifique du Cap Horn," he describes the larvæ of Asterias spirabilis, which adhere to the buccal membrane of the mother. They are attached by a pedicle which Perrier compares to the stalk of the Antedon larva and to the præoral lobe of the Asterina larva. He points out that both in the case of Asterias spirabilis and of Asterina gibbosa the pedicle arises from the oral surface, whereas in Antedon it is aboral in its origin, but he offers no explanation of this difference in position.

fact that their arrangement is in both cases dominated by the prevailing pentamerous symmetry of the adult.

The reason why the change in the position of the mouth takes place in Antedon is that this animal, like the others in which a similar change occurs, feeds on swimming or floating prey, and, so to speak, turns the mouth upwards to receive it. Asterids and their allies, on the other hand, find their food on the substratum, and therefore we must suppose that in the fixed ancestor of Asterids the body was flexed downwards so as to bring the substratum within reach of the tentacles. The difficulty suggests itself that a fixed form finding its food on the substratum might very soon devour all within its reach; and the suggestion may be made that perhaps the ancestor of Asterids never was fixed, but that the divergence from Crinoids took place when the common ancestor was a creeping form, since we may reasonably conclude that creeping habits formed the transition stage between a free-swimming and a fixed mode of life. In this case, however, the difficulty meets us of accounting for that radial symmetry which is so deeply impressed on the organisation of Asterids and other forms. It would be rash to say that fixed life is the direct cause of radial symmetry when we consider the case of Brachiopods, Cirripedes, &c., but this symmetry is only, so far as our knowledge goes, developed in connection with a fixed life.[1]

The proximate cause of the radial symmetry of Asterids is the immense preponderance of the organs of the left side, and it is difficult to see how this could have gone on to the extent it has done in an animal which moved about with a definite part directed forwards. The motion of the Asterid when metamorphosed is vague,—that is, any part is directed forwards; and it seems to me that a fixed stage must intervene between this and the mode of motion in which the head went first.

[1] Some might object that Ctenophores and Medusæ are radially symmetrical, but the first are not truly so; and as to the second, I hold very strongly the view that the Medusa is only a specialised bud, which has secondarily acquired locomotive powers in order to disperse the ova. Its radial symmetry has been inherited from fixed ancestors.

Therefore I feel that we are shut up to the supposition that Asterids had a fixed ancestor, and we must suppose that this form lived under conditions where enough food drifted along the bottom to meet its demands. Pl. 29, fig. 157, represents the characters which I consider the common ancestor of all Echinoderms possessed when it became fixed. Figs. 158 and 159 show how these characters became modified in the cases of the Asterid and Crinoid respectively.

It is probable that a fixed stage occurs in the life history of all Asterids. The larvæ of Echinaster and Asterias Mülleri, which are carried in brood-pouches, certainly possess one, and the three papillæ on the Brachiolaria larvæ are generally interpreted as an apparatus for fixation.

The fixed stage has, however, been lost so far as we know in all other Echinoderms; and it is instructive to note in this connection that Asterids alone retain the great præoral lobe. This has completely atrophied in the Plutei both of Ophiurids and Echinids; and in the latter case, as I have indicated above, (page 391) there is some evidence to show that a præoral ciliated band has likewise disappeared. The Auricularia still retains a trace of the præoral lobe, and it has been regarded as an exceedingly primitive form because it retains the undivided longitudinal ciliated band, and because the larval mouth becomes the adult one. The internal anatomy of this larva shows that, except in these two points, it is the most modified of all; the anterior cœlom so conspicuous in the Bipinnaria is represented, as Bury has shown (2), by a bud of cells which forms the secondary madreporite on the stone-canal, and the whole mode of segmentation of the cœlom is most erratic.

I have dwelt on this subject at some length because some have regarded the Holothurids as the primitive group of the Echinoderms, and Sémon (19) has even attempted to show that the primary hydrocœle lobes in them became the oral tentacles, whilst the so-called radial canals were really interradial outgrowths. Ludwig (13) has, however, shown the incorrectness of this; in the Synaptidæ alone do the oral tentacles spring direct from the ring-canal, and it was the development of

Synapta on which Sémon based his theory. In all Holothurids the buccal tentacles spring like the buccal tube-feet of Echinids from the proximal portion of the radial canals. It is, however, difficult for me to see how anyone can doubt that the Asterids are the least modified group of the Echinoderms. I have already dealt with their relations to Ophiurids, and have also pointed out that the Asterid central nervous system is really a concentration of the diffuse nervous plexus in connection with what must be regarded as a great sensory tentacle,—that, in fact, the whole radial water-vascular canal is to be regarded as a pinnately branched tentacle for which the arm is a secondary support. Sémon himself has suggested this (**20**), and it comes out even more clearly in Crinoid development than in the case of Asterids. Now the long radial canals in Echinids, ending in degenerate sense tentacles, clearly at one time had arms to support them; but these supports have been drawn back into the body. The Holothurids have been probably derived from the primitive Echinids; their calcareous nodules are most likely plates and spines atrophied in order to allow of free muscular movement. The terminal sense tentacles of the radial canals have entirely disappeared, and the forward shift of the madreporite and genital opening is no more difficult to understand than the varying position of the anus in Echinids. In the Asterids alone is locomotion entirely dependent on the tube-feet, and in them only we have the nervous system exposed.

On the second question, viz. that of the affinities of the Echinodermata as a whole, much light is thrown by the development of Asterina gibbosa. It is of course well known that the Tornaria larva of Balanoglossus shows a strong resemblance to the Bipinnaria in the course of its ciliated bands, and in possessing a præoral cœlom opening by a pore on the left. The adult Balanoglossus has five cœlomic cavities, and Bateson has shown that these arise as separate pouches of the gut. The question arises whether it is legitimate to homologise with these the five cœlomic cavities of the Asterina larva which arise by division of pouches already formed, but

which still persist in the adult as sharply separated cavities, only the most posterior pair, viz. the right and left posterior cœloms (oral and aboral) of the adult having partially fused with each other. The development of Antedon seems to answer this question in the affirmative. In its case the hydrocœle is budded off quite independently of the posterior cœlomic sacs.

Adopting, then, the view that the cœlomic sacs of the Enteropneusta and Asterids correspond, we find that the hydrocœle represents the collar cavity. Now in Cephalodiscus the collar cavities are produced into long pinnately branched tentacles, comparable to the radial water-vascular canals, and further a branch from the central nervous system accompanies each tentacle, just as the radial nerves accompany the radial canals in Echinoderms. Now, if we suppose that the two hydrocœles of Asterina were equally developed and approximated in the mid-dorsal line, the fusion of the anterior portion of the two nerve "rings," which of course would in this case be only open curves (since a ring-form is attained through the preponderating growth of one side) would give rise to a mid-dorsal nervous system like that of Cephalodiscus. Nor is that all; Professor Spengel (**21**) has shown in his monograph of the Enteropneusta that the currents in the proboscis-pore and collar-pore are inwards, and that by this means the animal inflates the proboscis and collar so as to render them efficient locomotor organs. We have seen that the function of the stone-canal is a similar one.

We conclude, then, that the free-swimming ancestor of Echinoderms, for which we may adopt the name Dipleurula, and the Tornaria ancestor of Balanoglossus, were closely allied. This involves the assumption that they were allied to the Protochordata, for, as I have elsewhere stated (**16**), Professor Spengel's attempt to refute the Chordate affinities of Balanoglossus has been, in my opinion, futile. Although it may seem somewhat fanciful, I cannot help seeing hints of Vertebrate peculiarities in the anatomy of Echinoderms. Where else among all animals of higher grade than the Cœlenterates do we find calcareous ossicles in the dermis? Where else

is the removal of the nervous system from the surface effected by invagination leading to the formation of a neural canal?

When we come to try and picture the characters which the Dipleurula possessed, we see at once that it must have been far more primitive than any existing form. In point of fact an Asterid is about the most undifferentiated animal above the level of Cœlenterates which exists. No proper blood-vessels, no specialised excretory organ, a central nervous system which is really a local concentration of a diffuse skin plexus, perfectly simple generative ducts, a most feebly developed muscular system, the fibres being for a considerable time simply myo-epithelial cells,—where is such a state of things to be found outside the Cœlenterata? When we further add that in the Crinoid the ambulacral nervous system nearly atrophies in the adult, and is replaced by a new system developed in a totally different position, we see that we are at about as low a level as one could well imagine, since the central nervous system in all higher forms is a most persistent structure.

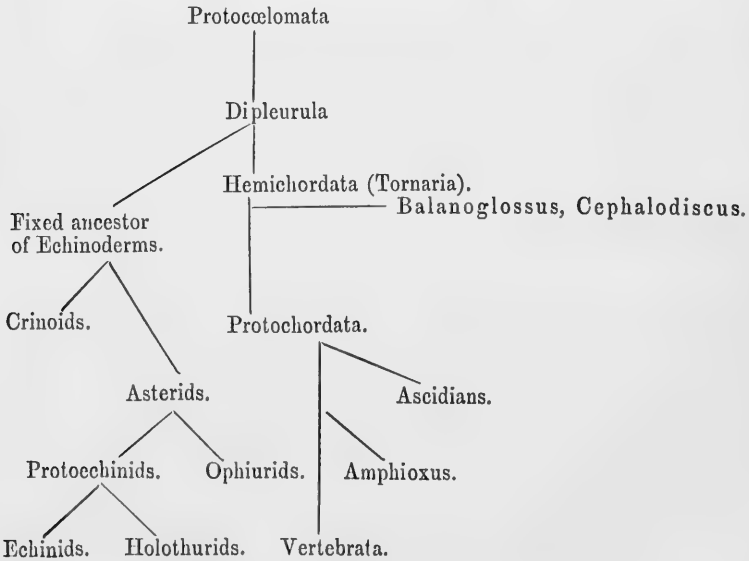
Assuredly Platyhelminths, which have been usually regarded as the basal group in the Cœlomata, or better, Triptoblastica, are far more highly specialised. To say nothing of their cephalic ganglia, we have their highly developed muscular wall and their complicated excretory and genital organs to prove this.

We shall not, then, go far astray in assigning the Dipleurula and the Tornaria to a group, the Protocœlomata, which were not far removed from the Cœlenterates; the cœlom was divided into three parts on each side, but of these the most anterior were usually fused to form an unpaired vesicle. The Dipleurula differed from the Tornaria chiefly in possession of an aperture, the stone-canal, in the wall separating the proboscis cœlom from the collar cœlom. This may have been the primitive arrangement, or it may have been a secondary arrangement acquired in consequence of the Dipleurula having lost the collar-pores, one of which may, however, as we have seen, be developed as a variation in the Asterid larva. At the apex of the præoral lobe was a more or less developed sense-organ with associated

nervous tissue. The collar cavities were probably prolonged into tentacles with which nervous tissue was associated.

If this supposition is correct, the group Protocœlomata was a pelagic cosmopolitan one, and it is in accordance with what we know of wide ranging groups that some of its members should adopt changed habits and modified structure. The Echinodermata, then, represent the earliest offshoot which took to a sessile life and acquired radial symmetry. A little later the Hemichordata branched off, a burrowing life being adopted and consequent degeneracy resulting. The main stem, however, remained pelagic and gave rise to the Chordata. The Ascidians were the next offshoot, and then came Amphioxus. We see, therefore, that the track of the great Chordata phylum through past ages is traced by examining those of its members who at very different periods of its history, and at different stages in its evolution, have forsaken their high vocation, and taken to a sessile or burrowing life, with the inevitable consequence—degeneracy.

The following diagram may represent these relationships a little more clearly :



I hope in a future paper to be able to show that the Trochophore larva is also related, though much more distantly, to the Dipleurula.

Zoological Laboratory,
Cambridge.

March 8th, 1895.

List of Works referred to in this Memoir.

1. Bury, H.—"The Early Stages in the Development of Antedon rosacea," 'Phil. Trans. Roy. Soc.,' 1888.
2. Bury, H.—"Studies in the Embryology of Echinoderms," 'Quart. Journ. Micr. Sci.,' 1889.
3. Cuénot, L.—"Contributions à l'Étude anatomique des Asterides," 'Arch. pour Zool. Exp.,' 2me series, tome v bis.
4. Cuénot, L.—"Études morphologiques sur les Echinoderms," 'Arch. de Biol.,' tome xi, fascicles 1 and 2.
5. Field.—"The Larva of Asterias," 'Quart. Journ. Micr. Sci.,' 1892.
6. Garstang, W.—"On some Bipinnariæ from the English Channel," 'Quart. Journ. Micr. Sci.,' 1894.
7. Hamann, Otto v.—"Die Wandernden Urkeimzellen und ihre Reifungsstätte bei den Echinodermen," 'Zeitsch. für wiss. Zool.,' 1880.
8. Hamann, Otto v.—'Beiträge zur Histologie der Echinodermen,' 4 Hefte, Jena, 1889.
9. Leipoldt, H.—"Das angebliche Excretiensorgan der Seeigel," 'Zeitsch. für wiss. Zool.,' 1893.
10. Ludwig, H.—"Beiträge zur Anatomie der Asteriden," 'Zeitsch. für wiss. Zool.'
11. Ludwig, H.—"Neue Beiträge zur Anatomie der Ophiuriden," 'Zeitsch. für wiss. Zool.,' Bd. xxxiv, 1880.
12. Ludwig, H.—"Entwickelungsgeschichte der Asterina gibbosa, Forbes," 'Zeitsch. für wiss. Zool.,' Bd. cxxxvii.
13. Ludwig, H.—'Bronn's Thierreich,' new edition, article "Echinodermata."
14. MacBride, E. W.—"The Development of the Genital Organs, &c., in Amphiura squamata," 'Quart. Journ. Micr. Sci.,' 1892.
15. MacBride, E. W.—"The Organogeny of Asterina gibbosa," 'Proc. Roy. Soc. London,' vol. liv, 1893.

16. MacBride, E. W.—"Review of Professor Spengel's Monograph on Balanoglossus," 'Quart. Journ. Micr. Sci.,' 1894.

17. Perrier.—'Mémoire sur l'organisation et le développement de la Comatula de la Mediterranée,' Paris, 1886.

18. Seeliger.—"Studien zur Entwickelungsgeschichte der Crinoiden," 'Zool. Jahrbücher, Abt. für Anat. und Ontog. der Thiere,' Bd. vi.

19. Sémon.—"Die Entwickelung der Synapta digitata und ihre Bedeutung für die Phylogenie der Echinodermen," 'Jenaische Zeitschrift,' 1889.

20. Sémon.—"Die Homologien Innerhalb des Echinodermenstames," 'Morph. Jahrbuch,' 1889.

21. Spengel, J. W.—'Die Enteropneusten des Golfes von Neapel und der angrenzenden Meeresgebieten' (Fauna and Flora of the Gulf of Naples), 1894.

22. Théel.—"The Development of Echinocyamus pusillus," 'Transactions of Roy. Soc. of Upsala,' 1892.

EXPLANATION OF PLATES 18—29,

Illustrating Mr. E. W. MacBride's paper on "The Development of Asterina gibbosa."

(The outlines of all the sections figured were drawn with the camera lucida.)

List of Abbreviations used.

a. Anterior body-cavity and the part of it persisting in the stalk. *a'.* Axial sinus. *ab.* Aboral sinus. *ab. gon.* Dilatation of branch of aboral sinus round the gonad. *amb.* Ambulacral ossicle. *amœb.* Amœbocytes. *a. œ.* Adult œsophagus or "stomach." *B.* Basal plate. *bip. gang.* Bipolar ganglion-cells. *branch.* Dermal branchia. *C.* Centro-dorsal plate. *calc.* Calcigenous tissue. *ect.* Ectoderm. *end.* Endoderm. *fibr.* Fibrous tissue. *fix.* Disc on prœoral lobe for attachment of larva. *gen. r.* Genital rachis. *gob.* Goblet cells. *hist.* Involution of ectoderm to facilitate histolysis. *lhy.* Left water-vascular rudiment or hydrocœle; its lobes are numbered with Arabic numerals. Roman numerals I, II, III, IV, V, denote the arm rudiments. *lo.* larval organ. *lœ.* larval œsophagus. *lpc.* Left posterior cœlom. *l'p'c'.* The right ventral horn of the same. *l''p''c''.* The right dorsal horn of the same. *l. stom.* Larval stomach. *mes.* Mesenchyme. *mp.* Madreporic pore. *musc.* Muscular

tissue. *musc. amb.* Ambulacral muscles. *musc. larv.* Larval muscles. *musc. retr.* Retractor musclesof adult œsophagus or stomach. *nerv.* Nervous tissue. *nerv. circ.* The nerve-ring. *nerv. larv.* Larval nervous tissue. *or. c.* Oral cœlom. *pbr.* Peribranchial space. *p. c.* Pore-canal. *p'c'.* Additional pore-canal in abnormal larva. *per.* Peritoneum. *ph.* Perihæmal space. The perihæmal rudiments are numbered 1.2, 2.3, 3.4, 4.5, and 5.1, according as they originate between the hydrocœle lobes 1 and 2, 2 and 3, 3 and 4, 4 and 5, and 5 and 1 respectively. *pr. germ.* Primitive germ cells. *pr. germ. inv.* Involution of the peritoneum whence these cells arise. *py.* Pyloric sac and its cæca. *ret.* Retinal cells. *rhy.* Right hydrocœle. Its lobes when they exist are numbered like those of the left hydrocœle, &c. *r'hy'.* Extra right hydrocœle present in abnormal larva. *rpc.* Right posterior cœlom. *sang. circ.* Oral blood-ring. *stc.* Stone-canal. *st'c'.* Extra stone-canal present in abnormal larva. *T.* Terminal plate. *tr.* Trabecula. *vit.* Cells forming crystalline body (Glaskörper). *wv.* Radial water-vascular canal. *wvr.* Water-vascular ring-canal.

PLATE 18.

All the figures are reproduced, though in a somewhat simplified form, from Ludwig's memoir on the development of Asterina gibbosa. The various figures have, however, been enlarged or reduced as the case demanded so as to bring them to one uniform scale of magnification, viz. 85 diameters.

Fig. 1.—A gastrula with wide blastopore. Stage A. This stage is reached on the second day.

Fig. 2.—A slightly older gastrula. The blastopore is commencing to be narrowed, and one of its lips is reflected over it.

Fig. 3.—A still older gastrula.

Fig. 4.—Lateral view of larva three days old which has just escaped from the egg membrane. The "larval organ" (*l. o.*) or præoral ridge of ectoderm with long cilia has appeared. Stage B.

Fig. 5.—Ventral view of the same larva.

Fig. 6.—Dorsal view of the same larva.

Fig. 7.—Lateral view of larva of six days. The disc for adhesion (*fix.*) has appeared in the centre of the larval organ. Stage C.

Fig. 8.—Antero-lateral view of the same larva of six days.

Fig. 9.—Anterior view of the same larva of six days.

Fig. 10.—Left view of fully developed larva of seven days. Stage D.

Fig. 11.—The same drawn in the position it assumes in life.

Fig. 12.—Left view of larva in which metamorphosis has commenced, and which has fixed itself. The Arabic figures denote the primary lobes of the water-vascular system or hydrocœle, the Roman figures the rudiments of the arms. The larval organ has disappeared. Stage E.

Fig. 13.—Right view of the same larva.

Fig. 14.—Ventral view of larva of about nine days. The arm rudiments form a nearly complete circle. The lobes of the water-vascular system have developed two pairs of accessory lobes each. Stage F.

Fig. 15.—Right view of the same larva of about nine days.

Fig. 16.—Left view of the same larva of about nine days.

Fig. 17.—Oral view of just metamorphosed star-fish about ten days old. *amb.* Ambulacral ossicles. Stage G.

Fig. 18.—Aboral view of another specimen of the same age. *C.* Central plate. *B.* Basal. *T.* Terminal. The curve of the arm rudiments has become a circle, No. V coming to be apposed to the lobe No. 1 of the water-vascular system. *mp.* Madreporic pore. Stage G.

Fig. 19.—Aboral view of a young star-fish sixteen days old. Notice the anus, the additional calcareous plates, and the spines.

PLATE 19.

All the sections represented in this plate are magnified 80 diameters, and, except where otherwise stated, they have been cut parallel to the "larval plane," i.e. they are horizontal longitudinal sections. Where several sections from the same series are figured, the most dorsal is in every case put first. The darkest shade represents the epithelium of the gut; the intermediate shade represents ectodermic and cœlomic epithelium, including the lining of the derivatives of the cœlom; the lightest shade represents the cavity of the blastocœle with all its contained structures, jelly, fibres, cells, &c., and also the muscular tails of the epithelial cells lining the water-vascular system. In Fig. 27, however, a portion of the gut opening into the cœlom, and in Figs. 30, 31, 34, 39, 40, and 41 the larval œsophagus, have been printed (through oversight) in the intermediate tint.

Figs. 20 and 21.—Two sections of a gastrula a little older than Stage A. No mesenchyme has as yet appeared.

Fig. 22.—Sagittal section of a gastrula about the same stage as Fig. 3. *mes.* Mesenchyme cells.

Fig. 23.—Section of an embryo older than that shown in Fig. 3. It shows the differentiation of the archenteron into gut and cœlom.

Figs. 24 and 25.—Two sections of an embryo somewhat younger than Stage B, and still enclosed in the vitelline membrane. The cœlom has grown back at each side of the gut, forming two posterior lobes, *lpc.*, *rpc.* Fig. 25 shows, however, that these lobes do not as yet extend ventral to the gut.

Fig. 26.—Section of larva rather older than Stage B, to show the formation of the madreporic pore. *pc.* Pore-canal ending blindly in contact with the ectoderm. *l. stom.* Larval stomach. *mp.* Thickening of ectoderm where the primary madreporic pore will be formed.

Figs. 27—29.—Three sections of a larva slightly older than the preceding. Fig. 27 shows that on the left side the cœlom is divided into an anterior cœlom *a*, and a left posterior cœlom *lpc*. Fig. 28 shows that this division only extends about halfway to the ventral side. Fig. 29 shows that the separation of the cœlom from the archenteron commences ventrally, since here the cœlom is shut off from the gut. *tr*. First trabecula.

Fig. 30.—Section of a larva rather older than that shown in Figs. 27—29. *læ*. Larval œsophagus. *tr*. Trabeculæ cords of cells spanning the left posterior cœlom.

Fig. 31.—Sagittal section of larva about Stage B, to show the formation of the larval œsophagus. It is clearly seen that this is a stomodæum which has not as yet joined the gut.

Figs. 32—34.—Three sections of a larva younger than Stage C. The segmentation of the cœlom on the left side is complete; on the right side it has begun dorsally (Fig. 32). The left water-vascular rudiment or hydrocœle (*lhy*.) has appeared as an outgrowth of the anterior cœlom, its lobes numbered as in Pl. 18. Fig. 32 shows Nos. 1 and 2; Fig. 33, No. 3; and Fig. 34, Nos. 4 and 5.

Fig. 35.—Section of a larva of Stage C. The first trace of the right hydrocœle (*rhy*.) has appeared.

Fig. 36.—Section of a slightly older larva than preceding. The development of the right hydrocœle is more advanced.

Figs. 37—41.—Five sections of a larva of Stage D, or slightly younger. In Fig. 37 we see a section of the pore-canal (*pc*.) and the origin of the rudiment of the oral cœlom (*or. c*.). In Fig. 38 the fully developed form of the right hydrocœle (*rhy*.) is shown. In Figs. 39 and 40 we see the left posterior cœlom extending obliquely beneath the right posterior cœlom (*rpc*.); this is the right ventral horn (*l'p'c'*.) of the left posterior cœlom. In Fig. 41 we see it opening into the anterior cœlom.

PLATE 20.

The same remarks apply to this as to Plate 19, but in addition it is to be remarked that the epithelium of the pore-canal and of the stone-canal is distinguished by a cross-striation.

Figs. 42 and 43.—Two sections of a larva rather younger than Stage D. *stc*. Rudiment of the stone-canal. Fig. 42 shows the septum between the anterior cœlom and the left posterior cœlom broken down dorsally; and Fig. 43 shows that the septum between the anterior cœlom and the right posterior cœlom is still incomplete ventrally.

Figs. 44—46.—Three sections of a larva of Stage D. Fig. 44 shows the opening of the pore-canal into anterior cœlom; Fig. 45, opening of the stone-canal into the same; and Fig. 46, the opening of the stone-canal into the

hydrocœle. It shows also that the hydrocœle has a wide opening into the anterior cœlom independent of the stone-canal.

Fig. 47.—Sagittal section of a larva of Stage D, to show the relations of the lobes of the left hydrocœle to each other.

Figs. 48—50.—Three sections of a larva of Stage E. The larva has suffered an injury, a piece of ectoderm in the præoral lobe indicated by the dotted line being missing. Fig. 48 shows relation of rudiment of oral cœlom (*or. c.*) to the right dorsal horn of left posterior cœlom (*l''p''c''.*). Fig. 50 shows the great growth of the left hydrocœle (compare Fig. 40). *a. œ.* Adult œsophagus; rudiment of the "stomach" of the adult. In Fig. 49 the *o* of *or.c.* has failed to print.

Figs. 51—53.—Three sections of a larva slightly older than the preceding, to show rudiments of the perihæmal spaces (*ph.*). These are numbered according to the lobes of the hydrocœle between which they occur: *ph.* 1.2, *ph.* 2.3, *ph.* 3.4, and *ph.* 4.5. *ph.* 1.2 arises from the anterior cœlom, the rest from the left posterior cœlom. The lobes of the hydrocœle are commencing to be trifid.

PLATE 21.

The same remarks apply to this plate as to the two foregoing.

Figs. 54—57.—Four sections of a larva about midway between Stages E and F. Fig. 54 shows the incipient dorsal constriction of the anterior cœlom into a stalk portion (*a.*) and a body portion or axial sinus (*a'.*); also the origin of the perihæmal rudiment (*ph.* 1.2) from the anterior cœlom. Fig. 55 shows the growing tip of the right ventral horn of the left posterior cœlom, and over it the arm rudiment No. V; it shows also the stone-canal opening into the hydrocœle and the perihæmal rudiment insinuating itself between the hydrocœle and the ectoderm. Fig. 56 shows the axial sinus and the stalk cœlom continuous with each other, and also the anterior cœlom opening into the right ventral horn of the left posterior cœlom. Fig. 57 shows that this right ventral horn is commencing to be again divided from the anterior cœlom ventrally by the outgrowth of a septum.

Figs. 58 and 59.—Two sections of a larva slightly older than the preceding, to show the separation of the axial sinus ventrally on the one hand from the stalk cœlom, and on the other hand from the hydrocœle. *Py.* Rudiment of the pyloric sac of adult. *lœ.* Last trace of the larval œsophagus.

Fig. 60.—Section of larva about the same age as preceding, to show the fifth perihæmal rudiment (*ph.* 51) which intervenes between lobes 5 and 1, as yet widely separated.

Fig. 61.—Section of larva about Stage F, to show the mutual relations of the stone-canal, the axial sinus (*a'.*), the right dorsal horn of the left posterior cœlom (*l''p''c''.*), and the right hydrocœle (*rhy.*).

FIGS. 62—69.—Eight sections of a larva slightly older than Stage F, to show the relation of the arm rudiments to the lobes of the hydrocœle. Fig. 63 shows the incipient healing of the breach in the septum between the anterior cœlom (axial sinus) and the left posterior cœlom. Figs. 64 and 65 show that arm rudiment No. V is still widely separated from hydrocœle lobe 1 by the base of the stalk, and also that the right ventral horn (*l'p'c'.*) of the left posterior cœlom is not completely separated from the axial sinus (*a'.*). Fig. 65 also shows the complete separation of the hydrocœle from the axial sinus. Figs. 66 and 67 show relation of the oral cœlom (*or. c.*) to the adult œsophagus (*a. œ.*). Fig. 69 shows the adhesive disc of the stalk (*fix.*) attached to a piece of Alga (*x*), and the rest of the ectoderm of the præoral lobe being invaginated (*hist.*) to undergo destruction. It also shows that each primary lobe of the hydrocœle has developed two pairs of secondary lobes.

PLATE 22.

The same remarks apply to Figs. 70—78 as to the contents of the three foregoing plates. Figs. 79—82 are sections cut parallel to the disc of the star-fish or "adult plane," the magnification being the same, viz. 80 diameters.

FIGS. 70 and 71.—Two sections of a larva of Stage G. Fig. 70 shows the relationship which the adult and the larval œsophagus occupy with regard to one another, the latter being a mere rudiment unconnected with the gut; it also shows the outgrowths from the adult œsophagus. Fig. 71 shows the oral cœlom opening into left posterior cœlom ventrally by breaking down of partition between them; also the first trace of the pyloric cæca as outgrowths from the pyloric sac.

FIG. 72.—Section of larva rather older than Stage G. The adult mouth is formed, and the oral cœlom opens widely into the left posterior cœlom. The stalk has become a small solid rudiment. The dotted line shows the boundary between the pyloric sac and the adult "œsophagus" or "stomach."

FIG. 73.—Section of a larva of the same age as the preceding; it shows the two dorsal pyloric cæca already formed, also the so-called heart or "ovoid gland" (*ovg.*), as a fold projecting into the axial sinus (*a'.*).

FIG. 74.—Another section from the same series as Fig. 72. Shows the two ventral pyloric cæca; it is seen also that their suspensory mesenteries are derived from the mesentery separating the right posterior cœlom from the left (compare Fig. 75). Note also that the tube-feet have acquired their suckers. The animal has broken loose from its attachment, which accounts for the rudimentary condition of the stalk.

FIG. 75.—A section of a larva of Stage G. Shows the dorsal pyloric cæca and their suspensory mesenteries.

FIG. 76.—A section of another larva of Stage G. Compare with Pl. IV, fig. 61, and note that the arm rudiment No. V (not marked in the figure) has

now become applied to the lobe No. 1 of the hydrocœle. The stone-canal is seen opening into lobe No. 2, and the perihæmal rudiment 1.2 has grown out into a canal insinuating itself between the ectoderm and the hydrocœle.

Figs. 77 and 78.—Two sections of a rather older larva. Fig. 77 shows that the right ventral and right dorsal horns (*l'p'c'.* and *l''p''c''.*) of the left posterior cœlom have coalesced, and that the left posterior cœlom has thus acquired a ring-like form. Fig. 78 shows the formation of the anus of the adult.

Fig. 79.—Section parallel to the adult plane of a larva of Stage F. Shows the relationships of the axial sinus, oral cœlom, and water-vascular ring (*wvr.*), the last being still incomplete; also four perihæmal rudiments alternating with the five hydrocœle lobes.

Figs. 80 and 81.—Two sections in same plane as Fig. 79 of a larva of Stage G. Fig. 80 shows the axial sinus (*a'.*) in process of growth to form the inner perihæmal canal. Fig. 81 shows the completion of the water-vascular ring at the spot marked by the asterisk between the hydrocœle lobes Nos. 1 and 5; it also shows the trifid form of the adult œsophagus before the mouth is formed, and the oral cœlom opening into the left posterior cœlom.

Fig. 82.—Similar section of older larva in which mouth is formed. The five interradial lobes of the "stomach" are present, the trifid shape having disappeared; and the retractor muscles of these lobes are formed from remnants of septum between oral and left posterior cœlom. The distance (R) from tip of arm to centre of disc ·36 millimetre.

PLATE 23.

Figs. 83 and 84.—Two more sections from the same series as Fig. 82. Fig. 83 shows the pyloric sac with its five cæca just beginning to be bifid, and the mutual relations of the right hydrocœle and axial sinus; also the stone-canal opening into the latter. Fig. 84 shows the point of origin of the rectum and the rudiment of rectal cæcum and the relation of right posterior cœlom to the pyloric cæca. In Fig. 83 (*pr. germ. inv.*) is the involution of peritoneum from which the primitive germ cells are formed.

Figs. 85—94 represent sections of abnormal larvæ. These sections are cut parallel to the larval plane, except Fig. 90, which is rather oblique to that plane. Magnification the same as before.

Figs. 85 and 86.—Two sections of a larva of Stage D, or slightly younger. *rhy.* Right hydrocœle developed into two distinct lobes lined with cubical epithelium.

Figs. 87 and 88. Two sections of a larva between Stages D and E. *p'c'.*, *st'c'.* Pore-canal and stone-canal of right side in connection with normal right hydrocœle, *r. hy.* Their openings into this are in another section. *r'hy'.* A second, more ventrally situated hydro-

cœle rudiment on the right side, with a distinct opening into cœlom. The left hydrocœle is feebly developed for the stage which the larva has reached, and has only four lobes.

Fig. 89. Section of a larva of Stage D, in which the right hydrocœle has five lobes, and is larger than the left. This section is drawn from the ventral aspect, and hence appears reversed.

Fig. 90. Section of a larva of Stage G, showing a "collar pore" opening from the left hydrocœle between lobes 2 and 3, directly to the exterior.

Figs. 91—94. Four sections of an almost normal larva of Stage F, or somewhat older. *p. c.* Normal pore-canal, opening into axial sinus, the septum between the latter and the left posterior cœlom being still incomplete dorsally. *p'. c'.* Pore-canal, *st'. c'.*, and stone-canal in connection with the right hydrocœle. Fig. 93 shows the opening of the second pore-canal into the axial sinus. Fig. 92 ought to show the opening of the second stone-canal into the right hydrocœle, but the slit-like opening has not come out in the figure. Fig. 91 shows the two pore-canals uniting to open by a common pore. (Compare Woodcut 3.)

Fig. 95.—Section parallel to the larval plane from larva of Stage C, showing the first trace of right hydrocœle. (Compare Plate 19, fig. 35.) Note its relationship to the anterior cœlom, which extends obliquely beyond it posteriorly, passing under it and to the right of it. Magnification 1000 diameters; Leitz's immersion $\frac{1}{12}$.

Figs. 96 and 97.—Two sections of body-wall of young star-fish, cut perpendicular to disc, in which R equals ·8 millimetre. Fig. 97 shows first trace of "papula" or dermal branchia (*branch.*). Fig. 96, origin of its peribranchial space, *p.br.* Magnification 400 diameters.

Fig. 98.—Section of body-wall of young star-fish, in which R equals ·88 millimetre, showing dermal branchia and its peribranchial space. Magnification about 400 diameters.

PLATE 24.

Figs. 99—106 illustrate the development of the so-called heart or "ovoid gland." The sections represented are perpendicular, or nearly so, to the disc of the star-fish, and the magnification is 350 diameters.

Fig. 99. Section of larva of Stage G. *ov.g.* Fold projecting into the axial sinus, the rudiment of the ovoid gland. *pr. germ. inv.* Invagination of peritoneum, whence the primitive germ cells are formed. *Calc.* Calcigenous tissue in the body wall.

Figs. 100—103. Four sections of a specimen older than preceding. Fig. 101 shows the growth of the primitive germ cells into the rudiment of the ovoid gland. Figs. 102 and 103 show that they do not yet extend through its whole extent. Fig. 103 shows that the ovoid gland

rudiment is at one point attached to the oral wall of the axial sinus. (Compare Plate 25, fig. 110.)

Figs. 104—106. Three sections of a young star-fish, in which R equals ·4 millimetre. Fig. 104 shows the primitive germ cells arising from the involution of the peritoneum. Figs. 105 and 106 show that they now extend throughout the whole extent of the ovoid gland; these figures also show the relation of the oral end of the axial sinus to the perihæmal spaces.

Figs. 107 and 108.—Two sections from same series as Figs. 82—84, magnified 350 diameters. They show the development of the oral "blood" ring, *sang. circ.*, as a modification of the mesenchymatous tissue of the blastocœle. *fibr.* Fibrous tissue.

Fig. 109.—Similar section of a young star-fish, in which R equals ·45 millimetre. Same magnification. The blood-ring is fully formed. Notice also the minute cells amongst the nerve-fibres (*bip. gang.*).

PLATE 25.

Fig. 110.—Longitudinal section of the stone-canal of young star-fish, in which R equals ·8 millimetre. *sang. circ.* Oral "blood" ring. *wvr.* Water-vascular ring-canal. *musc. amb.* Muscles of ambulacral ossicles. Notice the incipient division of madreporic pore into two, and entire independence of ovoid gland and blood-ring. Magnified 350 diameters.

Fig. 111.—Diagram showing the relative positions of the ovoid gland, stone-canal, and various sinuses in proximity. *gen. r.* Genital rachis. *ab.* Aboral sinus (or *sinus a.*). *pr. germ. inv.* Primary peritoneal involution to form germ cells. The cavity of this is probably the same as *sinus b* in next figure. The axial *sinus a'* is *sinus c.* The dotted lines show the continuity of two parts of the ovoid gland in a different plane to that of the stone-canal.

Fig. 112.—Similar diagram of Amphiura squamata. Accompanying spaces, *sinus a*, *sinus b*, and *sinus c*, as in the author's paper (**14**).

Figs. 113—118 illustrate the development of the ovoid gland and genital rachis. They are all taken from sections cut parallel to the disc; they are, in fact, transverse sections of the interradial septum in which the axial sinus is embedded.

Figs. 113 and 114. Two sections from a star-fish, in which R equals ·45 millimetre. Fig. 113 shows the manner in which the right hydrocœle is enclosed in the upper part of ovoid gland; Fig. 114, the primitive peritoneal involution, the pore-canal, and the crescentic form of right hydrocœle. Magnification 350 diameters.

Figs. 115 and 116. Two sections from star-fish, in which R equals ·7 millimetre. *ab.* Aboral sinus containing the rudiment of genital rachis. Fig. 115 shows that the sinus is a portion of the cœlom shut off by the outgrowth of a flap from the body-wall.

Fig. 117. Section from star-fish, in which R equals 2·2 millimetres, showing the continuity of rachis and ovoid gland, and that the rachis now extends in both directions. Magnified 150 diameters.

Fig. 118. Section from star-fish, in which R equals 3 millimetres, showing fully developed ovoid gland and changed form of stone-canal. Magnified 350 diameters.

PLATE 26.

Figs. 119—121.—Portions of three sections from the same series as Fig. 118. Fig. 119 shows the genital rachis enclosed in the branch from the aboral sinus, *ab.* Fig. 120 shows the passage of the genital rachis into the rudimentary genital organ, and the outgrowth of septum which cuts off the perihæmal space surrounding this rudiment from the "genital vessel." *ab.* A branch of the aboral ring. Fig. 121 shows the development of the cavity of the genital organ. Magnification 350 diameters.

Figs. 122 and 123.—Two sections of young ovaries, from a specimen in which R equals 3·7 millimetres. Fig. 122 shows the continuity of the ovary and rachis, Fig. 123 the outgrowth of germ cells to form the genital duct.

Fig. 124.—Portion of body-wall of gastrula figured in Plate 19, fig. 21. Notice the absence of mesenchyme. *end.* Endoderm. Magnification 600 diameters.

Fig. 125.—Similar view of the body-wall of slightly older embryo, to show the formation of mesenchyme. Same magnification.

Fig. 126.—Portion of the gut epithelium of larva figured in Plate 20, figs. 51—53. Same magnification.

Fig. 127.—Epithelium of the adult œsophagus of the larva shown in Plate 22, fig. 76, Stage G. Magnified 480 diameters.

Fig. 128.—Epithelium of the pyloric sac (larval stomach), from the same section as foregoing.

Fig. 129.—Epithelium of the lateral wall of the stomach of a star-fish, in which R equals ·8 millimetre. Magnified 480 diameters.

Fig. 130.—Epithelium of aboral wall of the stomach from the same section as foregoing. At × it passes into the epithelium of the pyloric sac.

Figs. 131 and 132.—Epithelium of the pyloric sac and of the rectal cæcum respectively. From the same section as fig. 130.

PLATE 27.

Figs. 133—135.—Three sections of the ectoderm of the anterior surface of præoral lobe of larva of Stage D. *Fix.* Disc for fixation. *l. o.* Larva organ. *nerv. larv.* Larval nervous tissue. Fig. 133 is through the dorsal part of præoral lobe; Fig. 135 through its ventral tip. Magnified 480 diameters.

Fig. 136.—Section of the adhesive disc of larva shown in Plate 21, figs. 62—69. *x*. A small piece of alga, to which it adheres by a secretion of mucus. *hist*. Involutions of neighbouring portions of ectoderm to undergo destruction by amœbocytes, *amœb.*; by this means the præoral lobe is reduced in size. Magnified 480 diameters.

Fig. 137.—Section of the lateral wall of præoral lobe of larva of Stage D. *musc. larv*. Larval muscles derived from the peritoneal cells. Magnified 1000 diameters.

Fig. 138.—Section through the ectoderm and hydrocœle wall of a larva of Stage D, to show the characters of the various larval epithelia. Magnified 1000 diameters.

Fig. 139.—Similar section from a larva between Stages E and F. A perihæmal rudiment is shown. Magnified 1000 diameters.

PLATE 28.

Figs. 143, 149, and 150 are magnified 600 diameters, the rest 1000 diameters (Leitz's immersion $\frac{1}{12}$).

Fig. 140.—Similar section from a larva of Stage F (that shown in Figs. 62—69). *nerv*. The incipient nervous tissue developing as a fine plexus amidst the bases of the ectoderm cells.

Fig. 141.—Similar section from a young star-fish, in which R equals ·4 millimetre. *Nerv. circ*. Nervous ring. *calc*. Calcigenous tissue. *fibr*. Fibrous tissue. *retr. musc*. Retractor muscles of stomach.

Fig. 142.—Developing eye of same star-fish. A simple ectodermic pit is seen.

Fig. 143.—Eye of star-fish from which Figs. 129—132 are taken. *ret*. Visual cells. *vit*. Cells functioning as "Glaskörper."

Figs. 144—148 illustrate the differentiation of tissues in the body-wall.

Fig. 144. From the right side of a larva of Stage D. At * a cell is seen in the act of dividing, to form one of the amœbocytes of the cœlom.

Fig. 145. From larva of Stage E (that shown in Figs 51—53). *gob*. Goblet cells. *musc*. Developing muscles; as yet they are simply tails of the cœlomic epithelium. *fibr*. First rudiment of fibrous tissue.

Fig. 146. From the young star-fish from which Fig. 141 is taken *calc*. Small portion of calcigenous tissue.

Fig. 147. From the young star-fish from which Fig. 143 is taken, and also Figs. 129—132.

Fig. 148. Ectoderm of another specimen of same age, to show the sense-cells.

Fig. 149.—Tube-foot of the larva shown in Figs. 62—69.

Fig. 150.—Tube-foot of the star-fish from which Figs. 141 and 146 are

taken. *nerv.* Nervous tissue under the sensory epithelium at the tip. *musc.* Muscular tails of hydrocœle epithelial cells.

PLATE 29.

Figs. 151—154 show the development of the transverse muscles, which extend from one ambulacral ossicle to its fellow of the opposite side.

Figs. 151 and 152.—Two sections perpendicular to the disc from a star-fish, in which R equals ·4 millimetre. *sang. circ.* Oral "blood" ring. *musc. amb.* Ambulacral muscles; the reference line (in Fig. 151) is too long. *ph.* Perihæmal space; the reference line (in Fig. 151) is too short. Magnified 350 diameters.

Fig. 153. Similar section from star-fish in which R equals ·63 millimetre.

Fig. 154. Similar section from star-fish of the same size as the preceding, but more advanced in the development.

Fig. 155.—Transverse section of the radial nerve-cord of a young Asterias, to show the feeble development of cœlomic nervous system.

Fig. 156.—Similar section of nerve-cord of an Ophiurid, to show the great ganglia of the cœlomic nervous system.

Fig. 157.—Diagram of the hypothetical ancestor of Asterids and Crinoids. The hydrocœle is a paired structure.

Fig. 158.—Diagram of a stage in the evolution of Asterids from this ancestor. Notice the growth of both left hydrocœle and left posterior cœlom to form rings. The hydrocœle encircles the base of the stalk. This drawing does not properly represent the oblique position which the disc acquires in reference to the stalk. The mouth ought to be half turned towards the observer.

Fig. 159.—Diagram of stage in evolution of Crinoids. Notice that the hydrocœle is carried entirely away from the stalk.

These last two diagrams are only hypothetical, in so far as they represent as co-existing structures which succeed one another in ontogeny; otherwise they represent the actual fixed stage in both Asterid and Crinoid ontogeny.

taken. *nerv.* Nervous tissue under the sensory epithelium at the tip. *musc.* Muscular tails of hydrocœle epithelial cells.

PLATE 29.

FIGS. 151—154 show the development of the transverse muscles, which extend from one ambulacral ossicle to its fellow of the opposite side.

Figs. 151 and 152.—Two sections perpendicular to the disc from a star-fish, in which R equals ·4 millimetre. *sang. circ.* Oral "blood" ring. *musc. amb.* Ambulacral muscles; the reference line (in Fig. 151) is too long. *ph.* Perihæmal space; the reference line (in Fig. 151) is too short. Magnified 350 diameters.

Fig. 153. Similar section from star-fish in which R equals ·63 millimetre.

Fig. 154. Similar section from star-fish of the same size as the preceding, but more advanced in the development.

FIG. 155.—Transverse section of the radial nerve-cord of a young Asterias, to show the feeble development of cœlomic nervous system.

FIG. 156.—Similar section of nerve-cord of an Ophiurid, to show the great ganglia of the cœlomic nervous system.

FIG. 157.—Diagram of the hypothetical ancestor of Asterids and Crinoids. The hydrocœle is a paired structure.

FIG. 158.—Diagram of a stage in the evolution of Asterids from this ancestor. Notice the growth of both left hydrocœle and left posterior cœlom to form rings. The hydrocœle encircles the base of the stalk. This drawing does not properly represent the oblique position which the disc acquires in reference to the stalk. The mouth ought to be half turned towards the observer.

FIG. 159.—Diagram of stage in evolution of Crinoids. Notice that the hydrocœle is carried entirely away from the stalk.

These last two diagrams are only hypothetical, in so far as they represent as co-existing structures which succeed one another in ontogeny; otherwise they represent the actual fixed stage in both Asterid and Crinoid ontogeny.

MAR 1896

The Early Development of Amia.

By

Bashford Dean, Ph.D.,
Columbia College, New York.

With Plates 30—32.

Ganoids, or more accurately Crossopterygians and Chondrostean Actinopterygians, must be looked upon as, in many ways, a transitional and intermediate group. For, on the one hand, the evidence is becoming conclusive that the Teleosts are to be regarded as its highly differentiated descendants; and, on the other hand, its most primitive members have certainly the closest ties of kinship with both the early sharks and lungfishes.[1]

Amia calva is doubtless, at the present day, the sole survivor of the race of Mesozoic Ganoids of which Caturus or Megalurus may be taken as a type. It claims, therefore, an especial interest as most nearly the ancestral form of some, if not all, of the recent Teleosts; for its structures are peculiarly Teleostean, and its closely kindred forms occurring from the Oolite to the Cretaceous provide the actual stepping-stones to the Clupeoids.

But in embryology the Ganoid and the Teleost still stand widely separate, and there has even been a tendency to look

[1] The writer refers to the structural nearnesses of the early Crossopterygians (e. g. Gyroptychiids), Phaneropleurid Dipnoans, and Pleuracanth sharks. He also notes that decidedly shark-like features are now found to be present in the early development of the sturgeon, and especially of the gar-pike.

upon these kindred forms as representing distinct phyla, early divergent from a primitive chordate ancestor. And it is therefore evident that, before Teleosts can be conclusively shown to be of Ganoidean descent, it becomes necessary to demonstrate that well-marked transitional characters exist not only in their structures, but in their ontogeny.

It has accordingly been my object in the study of Amia to determine its developmental relationships. For in the ontogeny of this most Teleostean Ganoid there seemed evidently the key to the solution of the entire problem—on the embryological side—of Teleostean descent; and, conversely, the degree of its developmental unlikenesses to the types of sturgeon and gar-pike could not fail to prove suggestive.

Embryonic material of Amia was to be obtained at Pewaukee, Wisconsin, a locality which has long been known as an exceptionally favorable spawning ground. It was here that Allis, Ayers, Strong, Ecclesheimer, and Fülleborn had succeeded in securing developmental material, and this locality appeared, therefore, far more reliable than Black Lake, St. Lawrence County, New York, where, from my preceding studies on Lepidosteus, I was but moderately sure of success. In order to undertake the collecting trip, I was enabled through the kindness of President Low, of Columbia College, to leave my duties as early as May 14th of the present year. Proceeding directly to Wisconsin, I was fortunate enough to secure eggs and larvæ by May 17th, and on May 19th had the opportunity of observing the spawning fish and to secure the earliest cleavage stages. Cold and rainy weather then proved favorable to my studies, for it retarded the development of the eggs, and gave me an opportunity to observe the living material, and to prepare the figures of those stages especially which in surface view (as my studies of Acipenser and Lepidosteus[1] had taught) could not well be examined in the fixed material.

By the time of my visit, however, the spawning season had

[1] 1895, Dean, "On the Early Development of Gar-pike and Sturgeon," 'Journal of Morphology,' vol. xi, No. 1.

practically ended. Indeed, so late was the time of my arrival at the spawning ground that it was altogether due to the kindness and skilful efforts of Mr. Henry Meyer, of Oconomowoc, that my trip proved successful. I found it impossible, accordingly, to secure at the same time both male and female fish in spawn for purposes of artificial fertilisation, and I was unable to employ the method of caring for the eggs which had proved so helpful in the studies of Acipenser and gar-pike. Fortunately the eggs of Amia were found to be especially hardy; they might be removed from the nests and retained in floating hatching cases, often even kept in the laboratory in pans and trays without serious losses. From their adhesive membrane, however, I have no doubt that the same method of procedure would have succeeded as in the case of the other Ganoids. In fixing the eggs, alcoholic (50 per cent.) picro-sulphuric mixture was generally used.

As to the general habits of Amia, but little need be said in the present paper; the notes of Dr. Fülleborn[1] regarding them have been fully confirmed. The fish is especially abundant in the Wisconsin lakes, and as it is worthless as food, and persists in taking any and all baits, it is not looked upon kindly by the local fisherman—especially as it not infrequently breaks both his rod and line. Luckily for him, perhaps, it feeds mainly during the evening and night,—but even then he meets it continually when using the jack-light. The strength and apparent clumsiness of the fish are to be emphasised; when disturbed in the shallows it will break through the water noisily in its strong efforts to escape.

The themes of the present paper have been arranged as follows:

[1] 1895, Fülleborn, "Berichte über eine zur Untersuchung der Entwickelung von Amia, Lepidosteus, und Necturus unternommende Reise nach Nord-America," 'Sitzungsberichte der Akademie der Wissenschaften zu Berlin,' Bd. xl, ss. 1057—1070.

PAGE

I. The breeding habits of Amia 416
II. The early development 421
A. Egg and egg membranes 421
B. Rate of development 423
C. Segmentation (first to eighth cleavage) . . . 424
D. Blastula 429
E. Gastrula: its relations to Lepidosteus, sturgeon, and Teleost 429
F. Mode of formation of the embryo . . . 436
G. Origin of the germ layers and early embryonic structures 437
III. Conclusions 440

I. The Breeding Habits of Amia.

The account of the spawning habits of Amia, as recently given by Fülleborn (op. cit.), has been entirely confirmed by the present writer. A number of additional notes[1] are presented in the following pages.

In the beginning of the spring Amia makes its way from the deeper water, where it has remained sluggish during the winter season, to the shallows in the vicinity of its spawning ground. This is usually in the swampy end of a lake, where the water is well filled with reeds, stumps, Chara, Potamogeton rootlets, here and there broken by little clear channels or inlets several feet in depth. In this neighbourhood the fishes are early seen, often in numbers, sunning themselves near the surface. They are at this period active, and may not be closely approached. Like the gar-pike, they are then usually in companies, the fish well separated.

The spawning season is an early and somewhat extended one, and is apparently induced by the first warm days of spring. The time of spawning in 1894, as recorded by Fülleborn, extended from the beginning of May to the first days of June. In the spring of 1895, however, although the previous winter had been unusually severe, a few warm days in April appear to have been the cause of earlier spawning. As with the gar-pike

[1] These include observations made by the present writer at Pewaukee, Black Lake, and in the South Carolina rivers.

the spawning then appeared, and has been almost simultaneous,—a general "run," after which the season was concluded by an intermittent spawning, with a nest here and there. At the height of a "run" as many as a half-dozen nests, as fishermen stated, were found to occur within the space of a few square yards. The fish were observed depositing their eggs as early as April 25th, and before the 1st of May the spawning appeared to have been generally completed. By the middle of this month larvæ were abundant, and from their uniform size in the different localities, and in different lakes, the spawning time could have been varied but little throughout the entire region.[1]

Immediately before spawning it is said that the fish divide themselves into parties, each comprising a female and several males, and that they then circle about nearer and nearer the shallows. A spawning place is selected—a well-sheltered spot with a water depth of about a foot—and a nest is there prepared. And it seems evident that nests are prepared sometimes well in advance of spawning, for several were noted by the writer which were occupied by the fish for a number of days before the eggs were deposited. The mode of building a nest is in some ways doubtful: fishermen state that the spawning party prepares it by constant circlings before the time of spawning, and this view seems entirely corroborated by a careful examination of the newly made nest; the soft weeds and rootlets appear bent and brushed aside in a way that gives it somewhat the appearance of a crudely finished bird's-nest.

The mode of depositing the eggs appears to be entirely similar to that described by the present writer in the case of the gar-pike. The spawning fish leave the nest from time to time, returning in company. The eggs and milt are emitted simultaneously. The fishes apparently rub closely together, since scales are found scattered in the nest bottoms, as noted by Fülleborn, and now confirmed by the present writer. The eggs become instantly adhesive, sticking to any portion of the

[1] The present writer finds the spawning region very general: nests were found in La Belle Lake (cf. Fülleborn, op. cit. p. 1059).

weedy nest which at the moment they happen to touch (e.g. Potamogeton or bulrush rootlets, Fig. 1). The writer has seen a nest in which—judging from the wide difference in the cleavage stages—the oviposition must have taken place during

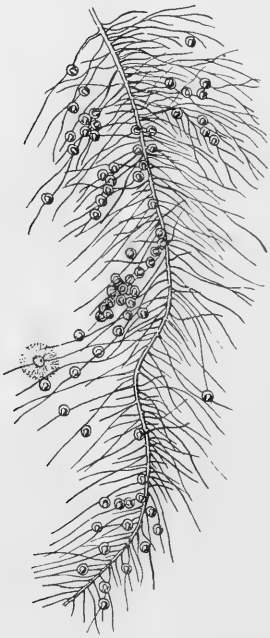


Fig. 1.—Eggs of Amia. Shown as collected, attached to root of bulrush. × about $\frac{7}{8}$.

a period of about twelve hours. Another nest, on a somewhat warmer day, appeared to have been filled with eggs within about half an hour, since all cell stages were notably uniform. The number of eggs the writer roughly estimates as in the neighbourhood of a million.

Shortly after oviposition a single male takes his position on the nest—whether by driving the others away or not the writer has been unable to determine. Here he remains until the eggs are hatched, sometimes in the nest, circling slowly about, sometimes in the adjoining "runway," as in Fig. 2, his

head and pectorals projecting over the nest. It is evident that his constant breathing is an important source of the eggs' aëration: his movements, moreover, aid, no doubt, in sweeping the nest free of sediment, from which, on account of their position, the eggs might otherwise suffer.[1]

Shortly after the eggs are hatched the entire swarm of larvæ

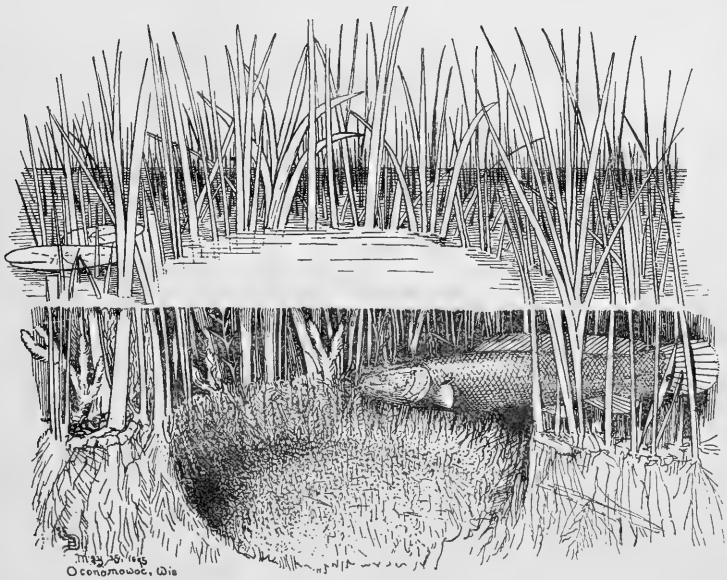


FIG. 2.—Nest of Amia.

leaves the nest. A fine nest of eggs, from which the writer had expected to get the young just before the time of their hatching, was found to be entirely deserted at a time when the young could not have been older than twenty-four hours. The closest search in and about the nest revealed no trace of their whereabouts, although from their larval habits it was thought that they should surely be found attached to the neighbouring weeds or deep in the mass of root-fibres and detritus of the nest bottom. They had evidently left the nest in a body, and were

[1] The few remaining eggs of nests which had been "robbed," and to which the male did not return, were found to become destroyed by fungus.

nowhere in the immediate neighbourhood. It was plausibly suggested by Mr. G. W. Kosmak, who then accompanied the writer, that they had been taken away by the male fish, attached to him by their sucking dishes.

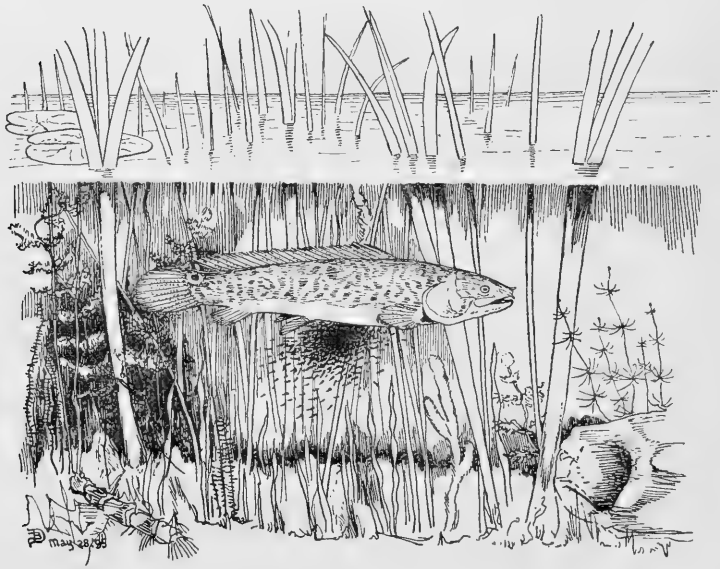


Fig. 3.—Young of Amia attended by male.

It is certain that when the male reappears it is with a swarm of nestlings; but they are now well grown ($\frac{5}{8}$—$1\frac{1}{4}$ inches). With these he remains for a time in the neighbourhood of the spawning ground; then he appears to gather them together with constant circlings and slowly takes his way to the neighbouring shallows (Fig. 3). The fish is courageous in the protection of his charges, remaining with them, facing the danger, until the boat approaches within a couple of yards; in one instance the writer has seen the fish actually pushed aside with the handle of the spear.

II. The Early Development of Amia.

A. The Egg and Egg Membranes.—The egg, shortly after its deposition (15″—30″), presents the general appearance of Pl. 30, fig. 1. It has assumed an oblong form, averaging 2·2 × 2·8 mm. The germinal area, even in freshly deposited eggs, was well defined as a whitish cap reaching down to about one third of the egg's longer or vertical axis. Its yolk pole region is pale greyish in colour (resembling that of the freshly laid sturgeon egg), and retains this colour throughout earlier development, appearing dingier and browner, however, as the outer membranes become soiled.

A single micropyle probably occurs, as in Lepidosteus and the Teleosts. Of this, however, the writer is by no means sure, as his only observation was made hastily during a collecting trip, and he neglected to immediately harden his material.

The egg membranes are essentially similar to those of Lepidosteus and Acipenser; they appear, however, relatively thinner and more intimately associated with the egg. They are not to be removed by needles until the embryo has become well established; even then the process is a difficult one. There is present a well-marked zona radiata and villous layer; these, in the younger stages, are approximately of equal thickness. The radiata is more compact in structure than in the other Ganoids; the villous layer, on the other hand, is of a far looser texture, its elements crumpled and intertwined, the heads of the villi oblong and swollen. At the point of the egg's attachment, e. g. the stem of waterweed, furthermore, as may be seen in the adjoining figure (Fig. 4), the villi become enormously elongated, their heads firmly fixed to the attaching object. A. granulosa occurs irregularly; it sometimes appears as a cell tract of considerable size, at other times it is almost wanting; it has certainly no such important relation as in Acipenser. In Pl. 30, fig. 1, the egg is shown attached to the waterweed; its membranes show a broad jelly-like base consisting of the elongated villi, and the mottling roughnesses of the egg surface represent patches of the granulosa.

The germinal area, as above noted, is readily to be determined by the unaided eye, even at a very short time after the egg is

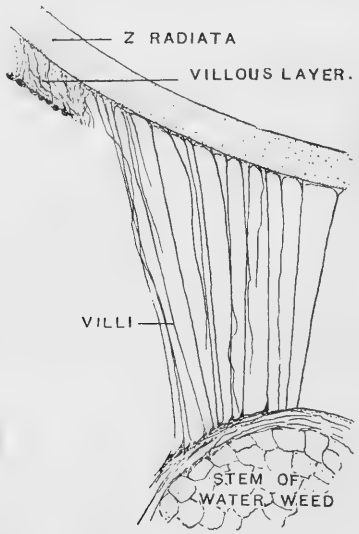


FIG. 4.—Egg membranes of Amia. × about 180.

deposited; its margins fade gradually into the yolk somewhat above the equatorial region of the egg. Its marked appearance is doubtless the reason of the oblong form which the egg early assumes. This shape, moreover, would seem to bear with it a specialised developmental character in that it does not, in the event of the egg's displacement, permit the germinal region to rotate backward into its vertical position, as it so readily does in the case of the sturgeon and gar-pike. The writer has found, however, in the early stages of segmentation that the cleavage planes occur in the normal way when the position of the egg was reversed. In these instances there was a slight attempt at the rotation of the germ disc, but in no case was this complete as far as the present observations went. The power of rotation in the eggs of Amia, it might here be noted, becomes more perfect at the subsequent stages of development; toward the close of gastrulation the egg's outline becomes more

nearly spherical, and the writer has observed that the blastopore of inverted eggs could slowly rotate downward.

Deutoplasm and germ region are more early to be distinguished in Amia than in other Ganoids. Studied in vertical sections (Pl. 31, fig. 21) the line of union of yolk and germ may be traced in the plane passing through the lower rim of the germ area; this plane of demarcation, however, on closer examination, proves to be broader than it at first appears; the yolk spherules may be traced, becoming smaller and smaller, well into the germ area. The nucleus, small in size, takes its position in the lower portion of the germ.

"Pigment" is practically absent in the late as well as in the earlier stages; in this regard the eggs of Amia and the garpike closely correspond.

B. The Rate of Development.—The following summary of the rate of development relates to a particular set of embryos. It is evident, however, that the time-proportion between the different stages is relatively uniform for a longer or shorter period of development. The position with regard to the upper and lower egg poles assumed by the growing embryos was observed by the writer to correspond with those he has already recorded in the Lepidosteus. Disturbance in the actual position of the egg, notably during the stages earlier than gastrulation, is attended with variations of position; the lack of the power to rotate backward into its normal position has already been recorded, p. 422.

Hours after oviposition.	Hours.	Hours after oviposition.	Hours.
First cleavage	1	Early gastrula	47
Second cleavage	2	Blastopore closes	80
Third cleavage	3	Embryo's length 90°	68
Fourth cleavage	$4\frac{1}{4}$	,, ,, 180°	80
Fifth cleavage	$5\frac{1}{2}$	,, ,, 270°	124
Sixth cleavage	$6\frac{7}{8}$	,, ,, 320°	158
Seventh cleavage	$7\frac{1}{8}$	Egg hatches	192

The rate of hatching, as recorded by Fülleborn, varied between eight to fourteen days. The present writer finds, however, that

the eggs hatched far more rapidly than this during the latter part of the present season,—in one case (May 24) within four days, in all cases not longer than eight. A sudden increase in the water temperature, as in the Lepidosteus, and, for that matter, doubtless as in all other fishes, hastens the rate of development.

C. Segmentation.—The segmentation stages of Amia are readily studied in the living egg, the transparency of its membranes permitting the cleavages to be followed. They are, however, far more obscure than those of the other Ganoids, and impress the observer with their marked Teleostean characters. They are readily reduced to the plan of those of Lepidosteus; and the accompanying figures (Pl. 30, figs. 1—9) may be instructively compared with those in the 'Journal of Morphology,' vol. xi, pl. i, figs. 1—9. And, on the other hand, they closely suggest the plan of segmentation of the Teleost, e.g. that of Serranus.[1] In the following description, accordingly, it will be the writer's purpose to emphasise these more important comparisons.

In general it may be said that the egg of Amia is meroblastic, that its (earlier) cleavages are confined entirely to the germinal area, that the compact blastomeres are Teleost-like, and that the segmentation cavity is practically wanting.

First Cleavage (Pl. 30, fig. 2).—In the living egg the first cleavage passes at once vertically through the germ area, causing the resulting blastomeres to be closely opposed. In the preserved material, however, the rim of contact (as in Pl. 30, fig. 3) appears somewhat rounded. At this stage the lower rim of the germ disc presents a more definite line of contact with the yolk, and the cleavage plane rounds off the corners of the blastomeres. In the transverse vertical section shown in (Pl. 31, fig. 21) are illustrated the depth and the character of the cleavage plane; it has passed slightly deeper than the niveau of the nuclei, but leaves below it a well-marked layer of the germinal protoplasm; the germ disc in this region has

[1] H. V. Wilson, "The Embryology of Serranus atrarius," 'Bull. U. S. Fish Comm.,' 1891, pp. 209—277.

become slightly deeper. The first cleavage was observed in several instances to divide the germ disc into blastomeres of unequal size; this abnormality, however, as in Lepidosteus,[1] Teleosts,[2] as well as in other Chordates, was found to in no way influence the subsequent developmental stages.

The writer, it might be here noted, has taken especial care to verify his observations on the meroblastic character of the cleavages of Amia. During the first few cleavages several hundred living eggs were examined with a view of determining holoblastic variations. These, however, did not occur, nor were there found even by the most favorable means of illumination, traces of what might be construed as surface furrows traversing the yolk region of the egg. In no case did a marginal cleavage pass below the rim of the germinal disc.[3]

Second Cleavage (Pl. 30, fig. 3) passes in a vertical plane at right angles to the first cleavage. This it closely resembles in depth and marginal limits; and in this stage the nuclei retain the same niveau with similar relations to the yolk. Immediately below animal pole the blastomeres slightly separate, giving rise to the beginnings of the segmentation cavity. In an examination of a number of eggs at this stage but very few (2 per cent.) variations were observed, the second plane in these cases intersecting the first at an angle of about 70°. Polflucht was in no instance noteworthy.

Third Cleavage (Pl. 30, fig. 4).—This cleavage plane is

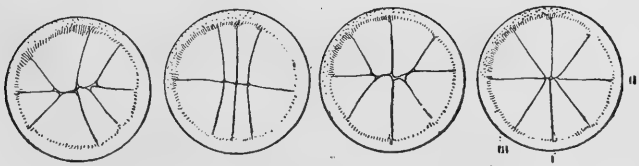


Fig. 5.—Variations in the stage of third cleavage.

again vertical, and, as in Lepidosteus and Teleosts, at right angles to the preceding plane (i. e. parallel to the first cleavage). In this stage variations were found to be common (20 per cent.),

[1] Dean, op. cit., p. 16.

[2] Ryder (cod), H. V. Wilson (Serranus), Whitman, and others.

[3] Cf. the somewhat different view of Fülleborn, op. cit., p. 1061.

and noteworthy Polflucht occurred. Several variations are shown in the adjoining figure (Fig. 5), of which the most frequent, as in Lepidosteus, is the symmetrical meridional form shown at the left. The segmentation cavity takes its definite origin at this stage; in the region of the animal pole the blastomeres are separated from the underlying yolk—the germ disc by a narrow fissure, which has been found to arise in the cleavage planes of the animal pole. Thus in a section of the germ disc passing through the points *—* of Pl. 30, fig. 4, it will be seen (Pl. 31, fig. 23) that the blastomere, which is cut nearly transversely (*a*), is separated below from the yolk region of the germ disc (*yg.*) ,and at the sides partly, from the adjacent blastomeres by the fissure-like segmentation cavity (*sc.*). And it will be further noted that the yolk region of the germ disc (*yg.*) is still in common, marginally, with the blastomeres. With these conditions should be contrasted the more shark-like features of the corresponding stage of Lepidosteus (Dean, op. cit., p. 17, and pl. ii, fig. 26).

Fourth Cleavage (Pl. 30, fig. 5).—The plane of the fourth cleavage is again vertical, resembling the third cleavage in essential regards. Its general direction is parallel to the second cleavage. This stage usually results in the division of the germ area into blastomeres of approximately equal size; often, however, as in the figure (Pl. 30, fig. 5), the cleavage passes nearer or further from the animal pole than in the preceding stage: in this event the central blastomeres appear at the surface rectangular instead of square. Many cleavage variations at this stage were recorded, in which noteworthy Polflucht occurred, and in which meridional planes often replaced the transverse cleavages; in no instance, however, were horizontal cleavages observed. The segmentation cavity in this stage does not markedly differ from that noted in the earlier stage. In Pl. 31, fig. 24, a section is shown passing through the central blastomeres in the direction of the (first or) second cleavage plane; its direction is slightly oblique, and it is for this reason that five blastomeres appear. In this section the extent of the segmentation cavity may be followed, and by

a study of serial sections it may be determined that the central blastomeres are now separate from the underlying germ-yolk, but that the marginal blastomeres are unseparated from it; the nuclei still remain in the low region of the blastomeres. The dilated spaces separating the sides of the blastomeres might perhaps be regarded as artefacts.

Fifth Cleavage (Pl. 30, fig. 6).—The stage of thirty-two cells is the first in which horizontal cleavages have been noted. These occur, however, only by variations in the divisions of the central blastomeres, and are by no means common. The typical conditions of this stage are shown in the above figure. From the study of living eggs the fifth cleavage was observed to take place in the following manner:—The five central blastomeres of the sixteen cells divide vertically in somewhat meridional planes, forming together a compact mass in the region of the animal pole, separated from the marginal blastomeres by a sharply cut trench; a few minutes later the marginal blastomeres undergo vertical division in meridional planes. That this is the normal plan of cleavage has been verified by serial sections of the late sixteen-cell stages where the nuclear figures have been clearly followed. Thus it appears that there occurs a noteworthy difference from the normal mode of the fifth cleavage in Lepidosteus (Dean, op. cit., p. 17). Variations, however, are numerous; the horizontal cleavage of the central blastomeres has been already noted, and the tendency of the cleavage planes of the marginal blastomeres to pass obliquely seems to suggest an approach to the conditions of the gar-pike. This cleavage of Amia, therefore, is not as nearly of the Teleostean plan as in the latter form. An interesting condition in this stage is the mode of origin of the newly formed marginal blastomeres; these appear to be budded out directly from the germ-yolk (Pl. 31, fig. 25, *gyb.*), their nuclei at first lying below the plane of the segmentation cavity; the central blastomeres as before are separated from the germ-yolk by the segmentation cavity.

Sixth Cleavage (Pl. 30, fig. 7).—The lineage of the cells of this stage could not be definitely followed; numberless

variations were found, and every attempt to reduce them to a common type was unavailing. Numerous horizontal cleavages occur in all blastomeres; increments to the cell disc are not lacking from the floor of the segmentation cavity. In the section in Pl. 31, fig. 26, a cell is seen to be budding out of the germ-yolk region at *m*, and at *m'* a dividing nucleus in the same region is clearly comparable to a merocyte. A study of living material demonstrates one of the steps in the transition from the fifth cleavage, i.e. the usual mode of division of the marginal blastomeres. These may be seen to bud off their polar ends, which, in turn, join the central cell mass, and leave as their outer boundary a furrow similar to that outlining the central blastomeres in the preceding stage. It is evident from the section of Pl. 31, fig. 26, that the area of the segmentation cavity, *sc.*, has greatly enlarged, although as a cavity it is no longer marked as in Pl. 31, fig. 25; the blastomeres are now directly apposed to the germ-yolk area.

Seventh Cleavage (Pl. 30, fig. 8).—Horizontal and vertical cleavage planes pass irregularly through the cells of the germinal disc. Nuclei occur (Pl. 31, fig. 27) in the germ-yolk area (*m, m, m.*) and bud off blastomeres to the overlying cell disc, and are seen to be undergoing direct division. The segmentation cavity (*sc.*) has now a flooring of a single layer of irregular blastomeres derived mainly from the germinal yolk.

Eighth Cleavage (Pl. 30, fig. 9).—By this stage the blastomeres have so subdivided that in surface view they can be but obscurely defined; marginally, however, they extend no further than in the earlier cleavages. Sections of this stage show that the cell-cap has increased in thickness (Pl. 31, fig. 28); a number of irregular blastomeres, yolk-laden, are seen in process of being budded off from the floor of the segmentation cavity, and numerous yolk nuclei are apparent. The segmentation cavity (*sc.*) extends irregularly among the blastomeres.

Subsequent stages of segmentation correspond closely with that last described, the blastomeres continuing to subdivide, and at the same time to encroach slowly on the yolk region of the egg.

D. Blastula.—A typical stage of the blastula is figured in vertical section in Pl. 31, fig. 29, and a somewhat later stage in surface view in Pl. 30, fig. 10, and in section in Pl. 31, fig. 30. The latter, contrasted with the sections of figs. 28 and 29, indicates clearly the downgrowth and the greater depth of the blastoderm; its cells have greatly increased in number, and build a dome-shaped cell cap of nearly twice the thickness of that of fig. 28; its cells are small, spherical, and of uniform size; those of the surface layer, however, have compacted into a firm cell stratum, *e'*, and those in the lowermost part of the blastoderm are slightly larger, yolk-laden. In this region the space between the loosely associated cells (*sc.*) is evidently to be compared with that (*sc.*) of the former figures. The segmentation cavity will be noted to extend irregularly between the blastomeres as far as the outermost cell stratum. Its floor is flattened, and bears a tier or more of irregular, upwardly projecting yolk-cells, and below them a merocyte-bearing zone of yolk.

The conditions of the blastula of Amia present interesting resemblances to those of Lepidosteus, and especially to those of the Teleosts.

The resemblances to the Teleostean blastula (e. g. cf. H. V. Wilson, op. cit, fig. 27) include—the lenticular shape of the blastoderm, the general uniformity of its elements, the differentiation of the outermost cell stratum, the apparent relations of the yolk nuclei to mode of growth of the blastoderm. In the latter regard it cannot be doubted that the closest functional kinships to the periblast are present; cell increments are being constantly made in the plane of the base of the blastoderm through the agency of a layer of nucleated elements derived from the yolk region.

E. Gastrula.—Typical conditions of the gastrula have been figured in surface views in Pl. 30, figs. 12, 13; an early stage in fig. 11, and two of the closing stages in figs. 14, 15. Like the blastula, it proves of considerable interest in comparison with the conditions of the older Ganoids on the one hand, and of the Teleosts on the other.

The transition from the stage last described to that of the early gastrulation may first, however, be followed. In Pl. 30, fig. 10, is figured in surface view a late blastula; the light coloured dome-shaped blastoderm appears sharply marked off from the yolk; its marginal rim, as a somewhat irregular line, seems as if in bold relief against a somewhat dark-coloured zone of the yolk; in surface view, in fact, it appears to overhang the yolk, and thus to represent the beginnings of gastrulation. That this observation, however, is incorrect is demonstrated both by sections and by a closer examination of the surface view of the object; the darker colour of the yolk zone is probably due to its merocyte-bearing character. It is in the stage figured in Pl. 30, fig. 11, that gastrulation actually begins. The downgrowth of the blastoderm is accompanied by the slight overlapping of its rim at one side—at the left in the figure; the remainder of the clearly defined margin has not as yet separated from the yolk. By the stage of Pl. 30, fig. 12, the downgrowth of the blastoderm has separated its entire rim from the yolk; and that portion which initiated the process of separation is now separated most widely as the dorsal lip of the blastopore. This region at a very similar stage has been shown in the following figure, Pl. 30, fig. 13, as exhibiting a variation [1] of considerable interest; at the rim's dorsalmost point a slight indentation is present, which may be supposed to correspond to the true blastopore of the ancestral Ganoid and Elasmobranch, the remaining portion of the rim representing the circumcrescence margin (O. Hertwig).

Later surface views of the gastrula are seen in Pl. 30, figs. 14, 15. In the former the continued growth of the blastoderm has greatly reduced the size of the blastopore; this now appears as a circular opening, its margin slightly nicked on both ventral and dorsal sides, and shows dorsally the whitened tract which marks the appearance of the embryo. In the

[1] Variations in the outline of the closing blastopore are not uncommon (cf. Lepidosteus, Dean, op. cit., p. 22). Oval blastopores were noted: in some one or both of the marginal indentations had disappeared; the dorsal one, however, is usually persistent.

latter is figured the stage of the closing blastopore; the embryo is here faintly outlined as a white flattened cell mass; at its hindmost region the blastopore, as a funnel-shaped pit, is enclosed by its thickened and constricting margin.

It is, however, from the study of gastrulæ in sagittal sections that the most interesting comparisons may be made with the conditions in gar-pike, sturgeon, and Teleosts. Some of these have been figured in Pl. 32, figs. 31 (=Pl. 30, fig. 11); 32 (=Pl. 30, fig. 12); 33 (=Pl. 30, fig. 14); 34 (slightly earlier than Pl. 30, fig. 15). But before comparisons may be established, the advancing changes should briefly be reviewed. The early gastrula, Pl. 32, fig. 31, it is to be noted, occupies approximately the area of the egg's surface as the blastula of Pl. 31, fig. 30; it has, however, the following advancing characters:—the loose cells of the blastoderm, now of minute size, have flattened into a compact cell mass, ectoblast (*o*) still presenting a well-marked surface layer (*o'*); the segmentation cavity (*sc.*) has accordingly become greatly depressed, and is now fissure-like; below it are several tiers of loosely compacted cells, which, by regular transition, appear to take their origin in large, vaguely defined yolk cells (*yc.*) arising from merocytes (*m'*); the rim of the blastoderm at *dl.* is the region where the blastoderm is early separated from the yolk; there is here, however, no cavity apparent separating the dorsal lip of the blastopore (*dl.*) from the yolk (*y.*); both are closely apposed, and at the point * their elements can no longer be distinguished; the entoblast (*i.*), arising from the undifferentiated tissue of the dorsal lip, is here composed of compact elements, but in the central region of the blastoderm becomes equivalent to the loose cellular layer already noted as continuous with yolk-cells and merocytes; at the opposite point of the blastoderm's margin, the blastopore's ventral lip, no separation of the germ layers from the yolk has as yet occurred.

In a following stage (Pl. 32, fig. 32) the blastoderm is shown enclosing about 285° of the egg's vertical circumference. The ectoblast has greatly thinned out, is thickest at its perimeter and at its dorsal lip; the segmentation cavity is fissure-like;

the ventral lip of the blastopore is now separate from the yolk, but is closely apposed to it, as in the case of the dorsal lip, making the cœlenteron (*c.*) fissure-like; the inner germ layer is in contact with the yolk-cells near the point *; in the central region of the blastoderm it no longer exhibits the entoblast cells; their well-marked layer has apparently become merged with the yolk-cells.[1]

In a still later gastrula (Pl. 32, fig. 33) the growth changes include:—the greater thickness of the lips of the blastopore, the retraction of the yolk-mass, the appearance of the cœlenteron as a distinct cavity (*c.*) and its extension forward under the dorsal lip (as far as the point *), the origin of the ectoblastic head mass, and of the middle germ layer. In the last regard this stage merits special attention: the mesoblast is found to arise peristomal (*m.*); on the dorsal side it arises from the undifferentiated tissue (of the tail mass), thence extends forward as a separate cell layer, and finally appears to be blended with the loosely associated cells of the entoblast; ventrally the mesoderm (*m.*), although distinctly to be recognised, is not to be separated from the cellular elements of the entoderm.

And finally, in the stage of the closing blastopore (Pl. 32, fig. 34), the embryo having surrounded about 180° of the egg's circumference, the following characters appear:—The greatly increased size of the ectoblastic head mass (*h.*), and of the cœlenteron (*c.*) together with the complete differentiation beneath the embryo of the middle germ layer (*m.*). The latter appears to have been differentiated in situ from the loosely associated cell mass shown in the preceding figure; it is separate anteriorly as far as the region immediately below the head terminal. The cœlenteron, now a deep cavity beneath the dorsal lip, extends forwards below the entire head; its hinder dilation (*k.*), immediately below the dorsal lip, is to be interpreted as representing Kupffer's vesicle;[1] beneath the ven-

[1] No critical attempt has ever been made to follow the actual mode of the growth of the embryo, as, for example, has been done by Morgan in the case of Ctenolabrus (1895, 'Journal of Morphology,' vol. x, No. 2).

[2] Dean, op. cit., p. 42.

FIGS. 6—13.

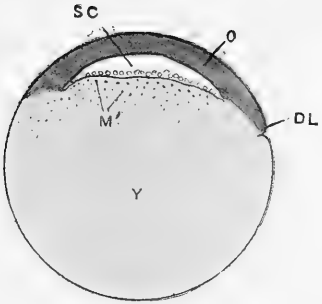


FIG. 6.

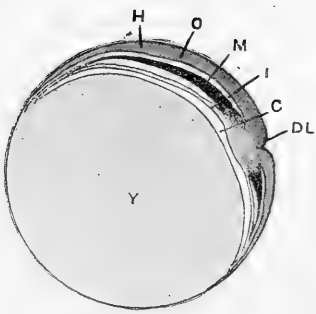


FIG. 7.

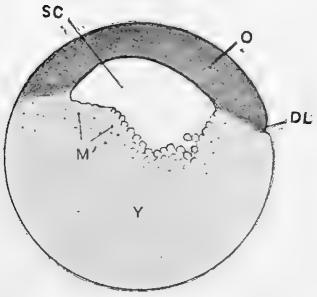


FIG. 8.

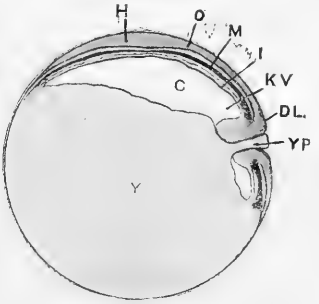


FIG. 9.

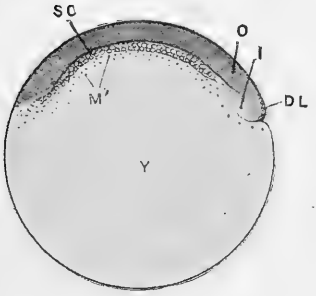


FIG. 10.

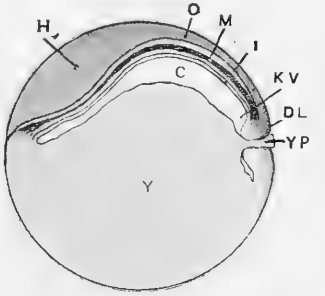


FIG. 11.

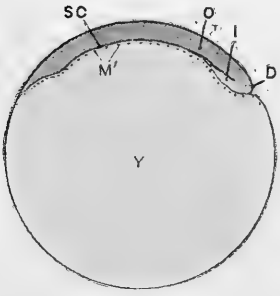


FIG. 12.

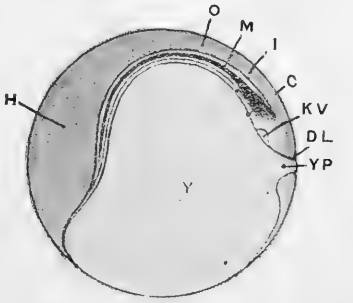


FIG. 13.

Sagittal sections of early and late gastrulæ of Ganoids and Teleosts. 6, 7. Lepidosteus. (In Fig. 7 the blastopore has just closed.) 8, 9. Acipenser. 10, 11. Amia. 12, 13. Teleost. C. Cœlenteron. DL. Dorsal lip of blastopore. H. Head (= cephalic thickening of neuron). I. Inner germ layer. KV. Kupffer's vesicle. M. Middle germ layer. M'. Merocytes (= periblast in Fig. 12). O. outer germ layer. SC. Segmentation cavity. Y. Yolk region. YP. Yolk plug.

A Comparison of the Gastrulation of Lepidosteus, Acipenser, Amia, and Teleost (cf. figs. 6—13).

	Lepidosteus.	Acipenser.	Amia.	Teleost.
Early Gastrula. Dorsal lip of blastopore appearing.	Surrounds about 160° of the egg's circumference. Well-marked meniscus-shaped segmentation cavity, with a smooth flooring of merocyte-yolk, overlaid by an irregular tier of loosely associated entoblast cells. Entoblast of dorsal lip undifferentiated.	Surrounds about 170°. Segmentation cavity large, deep, irregular; its roof thick, its flooring of loosely associated cells merging gradually into the yolk. Entoblast of dorsal lip undifferentiated.	Surrounds about 150°. Segmentation cavity flattened, fissure-like, its roof slightly thicker (relatively) and its elements larger than in L.; its floor a double tier of loosely associated cells, with below a yolk-cell zone. Entoblast of dorsal lip cannot well be distinguished from the loosely associated cells of the floor of the segmentation cavity, i. e. cœlenteron tends to merge with segmentation cavity.	Surrounds about 90°. Segmentation cavity flattened, fissure-like; its roof slightly thicker (relatively) and its cellular elements larger than in Am., its floor a smooth merocyte-bearing yolk layer (= periblast = tier of loose associated cells of Am.). Entoblast of dorsal lip closely apposed to, but now separate from periblast; cœlenteron merges, therefore, with segmentation cavity.
Late Gastrula. Blastopore closing.	Germ layers of nearly uniform size and thickness. Cœlenteron flattened, extending far forwards and also notably under ventral lip. Randwulst not marked. Embryo hardly noticeable (surrounds 90°). Mesoblast appears late, peristomal and gastral.	Outer germ layer thickest. Cœlenteron deep, extends anteriorly in front of the head region, but does not extend far under the ventral lip. Randwulst greatly marked (Kupffer's vesicle appearing). Embryo noticeable (surrounds 110°). Mesoblast appears early, largely peristomal.	Outer germ layer thickest, deepening in the sagittal plane. Cœlenteron flatter than in Ac., extends not as far forward or hindward. Randwulst not as marked as in Ac. (Kupffer's vesicle appearing). Embryo more noticeable (surrounds 190°). Mesoblast appears earlier, and is (very largely, if not entirely) peristomal. But two germ layers clearly distinguishable in ventral lip.	Outer germ layer very thick in the sagittal plane. Cœlenteron flattened between embryo and yolk, appearing as a cavity only under the dorsal Randwulst, which is now less prominent than in Am. as Kupffer's vesicle. Embryo well marked (surrounds 220°). Mesoblast (probably) peristomal. Layers undifferentiated in ventral lip.

tral lip the cœlenteron is no longer prominent. The closure of the blastopore is effected very much as in Lepidosteus or Acipenser, the constricting of its margin is the apparent cause of a greatly enlarged Randwulst; this ingrowth is attended by the protrusion of a slender yolk-plug (*yp.*), which on the blastopore's closure appears to be largely withdrawn.

A comparison of the gastrulation of Amia with similar stages of kindred forms may now be made. And this will be seen to become of especial interest since the intermediate characters of its gastrulation enable a far clearer understanding of this complicated growth stage of fishes than has yet been given. On the one hand the gastrula of Amia present decidedly Ganoidean features, while on the other its structures are clearly to be compared with those of the Teleost. An accompanying series of figures (figs. 6—13) enables comparisons to be more readily drawn; they present sections (nearly sagittal) of the earliest and of the latest stages of the gastrula of Lepidosteus, Acipenser, Amia, and Teleost; in the earliest stages the dorsal lip is coming to be formed; in the latest, the blastopore is closing.

From the foregoing comparison it seems to the writer evident that well-marked transition exists in the structures of corresponding stages. These he believes may, as in his figures, most conveniently be followed, starting with the archaic plan of gastrulation of Lepidosteus, passing to that (somewhat divergent) of the ancient sturgeon, thence to the more modern type of Amia, and finally to that of the highly specialised and recent Teleost. And it seems clear to him, furthermore, that the puzzling features of the gastrula of the specialised bony fishes (Teleocephali) may be interpreted as altogether due to a process of advancing or accelerated (precocious) development. Thus the advancing growth changes appear to be indicated in the following processes.

1. The invagination tends to begin at an earlier stage when the blastodisc covers a smaller area of the egg's surface, and the elements of the early gastrula tend to become relatively larger, and the roof of the segmentation cavity relatively thicker.

2. The segmentation cavity tends to become flattened and to recede centrad.

3. The embryo tends to become larger, and its structures more precociously differentiated, concentrating its substance more and more early in the sagittal plane of the dorsal lip (and leaving, therefore, the remaining region—e. g. ventral lip undifferentiated), and growing notably in the head region.

4. The embryo, pari passu with its more precocious development, has come to acquire more perfect relations with the yolk. The growth of the embryo in the generalised condition is derived from deep merocyte layers of the yolk; in the more specialised conditions, however, the more peripheral layers of deutoplasm become of service. Cellular increment is derived in Amia from a double tier of cellular zone of the floor of the segmentation cavity, which in the Teleost becomes clearly homologous with the periblast. In such an event the cœlenteron becomes clearly confluent with the segmentation cavity.

From the foregoing discussion the writer's views as to the homologies of the structures of the Teleostean gastrula are clearly apparent; and they will be found to correspond with those which he had formerly expressed in his paper on the gar-pike and sturgeon (p. 52). His interpretation requires, accordingly, the Ziegler-Wilson conception of the gastrula to become modified as follows:[1]—The cœlenteron extends under the rim of the blastoderm, from the free end of the "primitive hypoblast" to that of the "ventral mesoderm" of H. V. Wilson, the periblast yolk losing its ancestral cellular connection with the embryo, on account of acquiring its indirect but more highly important (and specialised) method of furnishing its cellular increments.

F. The Mode of Formation of the Embryo.—The embryo has already made its appearance by the time of the closure of the blastopore. It is there recognised in the flattened and opaque cell mass extending in front of the blastopore, and enclosing about 180° of the egg's circumference (Pl. 30,

[1] H. V. Wilson, op. cit., p. 264.

fig. 15, and Pl. 32, fig. 34). Its substance is insunken in the egg.

The mode of establishment of the early embryo's outward form may be followed in Pl. 30, figs. 16—20. In the first of these figures (fig. 16) the embryo surrounds about 185° of the egg's circumference, and the blastopore has disappeared; a whitened line on the egg's surface represents the neural axis, its enlarged terminal the brain tract; a slight darkening in the axial line is due to a shallow trench-like insinking of the neural plate. The embryo is not as yet noticeable above the surface curvature of the egg. In Pl. 30, figs. 17 and 18, the head and tail regions of a slightly older stage, the following changes have taken place:—The embryo, a rod-like surface thickening, surrounds about 195° of the egg's circumference; its head, prominent and enlarged, rises slightly above the surface curvature of the egg; its trunk tapers hindward, ridge-like in form, closely apposed to and slightly insunken in the egg mass; two mesoblastic somites have appeared. In the stage of Pl. 30, figs. 19 and 20, six somites are present, and the embryo has enclosed about 220° of the egg's circumference; the neural axis, now more sharply marked, rises slightly above the egg; the tail in its growth is now separating from the egg surface; the brain regions are defined, optic vesicles are appearing; and the head region, growing slightly forward, has in front the beginnings of the stomodæum.

In the features of early outward growth Amia again presents marked transitional characters between Ganoids and Teleosts. Like the latter, its form growth is early concentrated in the sagittal plane, and the embryo deeply sunken in the egg mass. It later resembles the Ganoids in its uplifting from the yolk and in the details of organogeny. A few further notes regarding the origin and growth of its structures are given in the following section.

G. The Origin and Early Differentiation of the Germ Layers.—The outer germ layer has been already noted (p. 431) in the early gastrula as a compact cell mass of comparatively uniform thickness. Its subsequent growth has

also been reviewed in the table and figures of pp. 433–434. By the time of the blastopore's closure it has given rise to the sagittal thickening out of which the central nervous system is shortly to be formed. This, in its early characters, suggests closely the neuron of the Teleost (Pl. 32, figs. 34 and [transverse section] 36). Its deep keel-like thickening resembles closely as well the earliest conditions of Lepidosteus, while diverging from the type of Acipenser. To the conditions in this form, however, an apparent similarity exists in the stage already noted in Pl. 30, fig. 16, where a slight axial groove is for a short time present; this condition, of interest, accordingly, from its sturgeon-like feature, is further illustrated in the transverse section of Pl. 32, fig. 37; the axial groove is never deeper than here figured, and shortly passes away, flattening as the neuron increases in size and depth. The lumen which the neuron later acquires takes its definite origin in the dissociation of cells in the vertical plane of its axis (Pl. 32, fig. 39). This condition is clearly to be compared with that of the Teleost, as described by Hoffman, v. Kupffer, H. V. Wilson, and others. It might, moreover, be regarded as confirming conclusively the position of v. Kupffer in regard to the morphological importance of the neural furrow of the Teleost, i. e. that it is homologous to the primitive neural furrow of Amniotes; and, on the other hand, it certainly removes the ground for believing that the neural axis of the Teleost was primitively solid, as Minot has maintained.[1] For there can be little doubt that Elasmobranchian characters are present in the origin of the neuron of Acipenser, and it follows, therefore, that the neural furrow of Amia, its ally, must represent, if only in a transient way, the early Amniote condition. In the Teleost, accordingly, it is reasonable to expect that abbreviated growth stages have greatly reduced the prominence of the ancestral medullary folds, while perfecting the newly acquired mode of securing a neural canal.

A further and striking similarity to Teleostean conditions is found in Amia in the early development of the optic vesicles.

[1] 'American Naturalist,' November, 1889.

Absence of neurenteric canal is, again, Teleostean. In this regard its features are notably transitional between those of Lepidosteus and the Teleost. At the closure of the blastopore, Pl. 32, fig. 35, there is no trace of the neural groove in the hinder region, the outer germ layer here fusing in a solid plug, merging below into the undifferentiated tissue, *u*; at a later stage, Pl. 32, fig. 38 (cf. H. V. Wilson, op. cit., pl. xcviii, fig. 84), the ventral lip of the blastopore has become largely merged with the growing tissue, *u*, of the tail region, and the conditions are markedly Teleostean.

The inner germ layer has already been noted (p. 431) in its intimate relationship to the yolk mass. In Pl. 32, fig. 31, the entoderm is clearly defined, extending forward from the rim of the dorsal lip to about the position of the point*, having in its texture all the characters of the similar stage in gar-pike and sturgeon. Beyond this point, however, it merges into the loose layer noted on p. 431, whose periblast-like characters have already been indicated (p. 434). The growth in extent of the entoderm may be followed in Pl. 32, figs. 31—36, 38, 39; under the ventral lip of the blastopore it appears for a short distance (fig. 35) as a separate layer, but in a later stage (fig. 39) it becomes merged with the yolk entoblast; under the blastopore's dorsal lip the darm-entoblast has separated from the yolk region (apparently by differentiation of the cells in situ) as far as the point * in fig. 33, and as far as the most anterior head region in fig. 34; at its periphery the cells become indistinguishable from those of the neighbouring yolk. The gut arises in a manner closely resembling that of Acipenser; its cavity, narrow and deep (fig. 34) tailward, flattens out broadly in the head region, as is shown in the marginal limits of the parietal zone of Pl. 30, figs. 17—20; its mode of formation, it should be noted, although of clearly marked Ganoidean character, diverges, nevertheless, toward the plan of the Teleost; it has not, however, acquired the yolk-apposed characters of the latter. The notochord arises as in the sturgeon or gar-pike (Pl. 32, fig. 38): it separates directly (i. e. delaminates) from the entoderm; in the region of the hind brain, as seen in

Pl. 32, fig. 36, it is undifferentiated from the loosely associated cells of the lower layer.

The mesoblast is notably peristomal (Pl. 32, figs. 33—36, 38, 39); it is hardly to be distingushed in the blastopore's ventral lip (cf. figs. 35, 39): its appearance is first noted in the gastrula of fig. 33. In its early growth it extends forward as a wide and flattened cell mass, thinning distally, and becoming confluent with the inner germ layer. As in the Teleosts, gastral mesoderm is absent, and the division of the middle layer into its somatic and splanchnic layers is not apparent until comparatively a late stage of development. A contrast with the conditions of the mesoblast in Acipenser and gar-pike may be made by reference to the table in Dean, op. cit., p. 47.

III. Conclusions.

The early development of Amia, as outlined in the foregoing paper, must certainly be regarded as furnishing abundant evidence of intermediate characters. To the Ganoids on the one hand, and the Teleosts on the other, these ontogenetic nearnesses become, accordingly, of the greatest interest, since they confirm the results of the structural study of recent and fossil forms upon the Amioid descent of Teleosts.

A comparison of the developmental characters of Amia with those of the gar-pike and sturgeon need be but briefly reviewed. Its type of development is in many ways curiously Lepidosteoid, as in meroblastic segmentation, relations of blastoderm to yolk, flattened segmentation cavity, late gastrulation, early neuron, and absence of neurenteric canal. It certainly resembles that of the sturgeon in some of its advancing characters, as in the mode of closure of the blastopore, decreased prominence of its ventral lip, and in the embryo's sagitally accented growth. From either of these older Ganoids the developmental type of Amia is nevertheless sufficiently different to warrant any definite conclusions as to its descent; one might, the present writer believes, safely infer that, like that of the sturgeon, it is in general Lepidosteoid.

But the early development of Amia is clearly to be recognised

as of an advancing type. It bridges over in many and important characters the gap which has always been pointed out as separating the Ganoids and the Teleosts. Its abbreviated development indicates, in short, the very stages which are most abbreviated in the cænogenesis of the latter group. Its meroblastic mode of cleavage is decidedly Teleostean, especially in the relations of its segmentation cavity and yolk nuclei, although in these regards it also closely resembles Lepidosteus. Its transitional characters are most clearly marked during gastrulation, and during the early growth of the embryo. These Teleostean features might, in conclusion, be briefly summarised.

Small area of blastoderm at the beginning of invagination (?). Flattened segmentation cavity. Early relations of inner germ layer of dorsal lip with periblast-like conditions of the yolk cells; cœlenteron is then practically confluent with the segmentation cavity. General thinness of the down-growing blastoderm, whose Randwulst corresponds to the germ-ring; close apposition of blastoderm to yolk mass. Early appearance of the embryo; and in general early differentiation of the germ layers of the blastopore's dorsal lip, attended by a corresponding lack of differentiation of the ventral lip. The mode of the closure of the blastopore; the presence of Kupffer's vesicle and the absence of neurenteric canal. The early growth of the neuron as an insunken tract thickest in the sagittal plane. The early prominence of the brain mass. The evanescent medullary groove; the solid character of the early neuron, and its secondary mode of acquiring a neural canal. The peristomal mode of origin of the mesoblast; its late differentiation; the absence of gastral mesoblast. The early mode of establishment of the embryo's outward form.

EXPLANATION OF PLATES 30—32,

Illustrating Dr. Bashford Dean's paper on "The Early Development of Amia."

PLATE 30.

Figs. 1, 2, 4, 15, 19, and 20 drawn by Bashford Dean from the living eggs, the remaining figures from eggs hardened in alcoholic (50 per cent.) picro-sulphuric mixture, by Dr. Arnold Graf. × about 50.

Fig. 1.—Living egg shortly before the appearance of the first cleavage furrow. About ½ hour after fertilisation.

Fig. 2.—First cleavage; it sharply separates the halves of the germ-disc, but extends no further marginally than its limits. 1 hour.

Fig. 3.—Second cleavage, seen from above. 2 hours.

Fig. 4.—Third cleavage; similar in marginal extension to the first and second. 3 hours.

Fig. 5.—Fourth cleavage, 4¼ hours.

Fig. 6.—Fifth cleavage, 5½ hours.

Fig. 7.—Sixth cleavage, 6¼ hours.

Fig. 8.—Seventh cleavage, 7⅓ hours.

Fig. 9.—Eighth cleavage (early blastula), 8½ hours.

Fig. 10.—Early gastrula, 46 hours.

Fig. 11.—Early gastrula, 48 hours.

Fig. 12.—Gastrula, about 50 hours.

Fig. 13.—Gastrula showing indented rim of blastopore at the dorsal lip. 54 hours.

Fig. 14.—Late gastrula, showing mode of closure of the blastopore; the embryo's appearance is faintly indicated in the light-coloured area immediately above the centre of the figure. 71 hours.

Fig. 15.—Closure of the blastopore, and the appearance of the embryo. 78 hours.

Fig. 16.—Early embryo showing neural folds; its length surrounds about 185° of the egg's circumference. About 93 hours.

Fig. 17.—Early embryo, showing head region; its length surrounds about 195° of the egg's circumference. Two somites present. 95 hours.

FIG. 18.—Early embryo; tail region of embryo of Fig. 17.

FIG. 19.—Early embryo showing head region; its length surrounds about 220° of the egg's circumference. Six somites present. 100 hours.

FIG. 20.—Early embryo; tail region of embryo of fig. 19.

PLATE 31.

Vertical sections of cleavage stages and of blastula. × about 55.

FIG. 21.—Second cleavage; sectioned parallel to a cleavage plane through the resting nuclei.

FIG. 22.—Third cleavage; sectioned near and parallel to the plane of the second cleavage.

FIG. 23.—Third cleavage; sectioned in a plane passing through the points *—* of Pl. 1, fig. 4.

FIG. 24.—Fourth cleavage; sectioned in a plane (slightly oblique) near and parallel to the first (or second) cleavage.

FIG. 25.—Fifth cleavage.

FIG. 26.—Sixth cleavage.

FIG. 27.—Seventh cleavage.

FIG. 28.—Eighth cleavage.

FIG. 29.—Early blastula. Eleven hours after first cleavage.

FIG. 30.—Blastula. Thirty-three hours after first cleavage.

a. Central blastomere. *e*. Entoblast. *e'*. Epidermic stratum of ectoblast. *gyb*. Germ yolk blastomere. *m*. Merocyte. *m'*. Blastomere arising from merocyte-bearing germ-yolk. *n*. Nucleus. *sc*. Segmentation cavity. *y*. Yolk. *yg*. Germ-yolk.

PLATE 32.

Vertical sections of gastrulation stages and of early embryos.

FIG. 31.—Early gastrula (47 hours), nearly sagittal section. × 35.

FIG. 32.—Gastrula (50 hours), nearly sagittal section.

FIG. 33.—Gastrula (55 hours), nearly sagittal section.

FIG. 34.—Late gastrula (or early embryo) (75 hours), nearly sagittal section.

FIG. 35.—Early embryo (78 hours); section slightly oblique to embryo's axis, passing through the region of the closed blastopore. × about 70.

FIG. 36.—Early embryo (78 hours); transverse section passing through the region of the hind brain. × about 70.

Fig. 37.—Early embryo (77 hours); transverse section of neuron of the hinder trunk. × about 160.

Fig. 38.—Early embryo (95 hours), transverse section; somewhat in front of the undifferentiated tissue of the tail mass.

Fig. 39.—Early embryo (95 hours), sagittal section of the region of the closed blastopore. × about 80.

Fig. 40.—Early embryo (95 hours); transverse section of neuron in a region somewhat behind the hind brain.

c. Cœlenteron. *ch.* Notochord. *dl.* Dorsal lip of blastopore. *h.* Head region (of neuron). *i.* Inner germ layer. *k.* Kupffer's vesicle. *m.* Middle germ layer. *m'.* Merocyte. *o.* Outer germ layer. *o'.* Epidermic stratum of the outer germ layer. *sc.* Segmentation cavity. *u.* Undifferentiated tissue of the lip of blastopore. *vl.* Ventral lip of blastopore. *yp.* Yolk-plug. * denotes the innermost limit of cœlenteron.

On Kynotus cingulatus, a New Species of Earthworm from Imerina in Madagascar.

By

W. Blaxland Benham, D.Sc.Lond., Hon. M.A.Oxon.,
Aldrichian Demonstrator in Comparative Anatomy in the University of Oxford.

With Plates 33 and 34.

The subject of the present paper was handed to me by Professor Bell, of the British Museum, in 1893, for identification. The delay of more than a year in completing the description of this worm, which presents points of novelty deserving earlier publication, is due to press of other work. I have once more to acknowledge my indebtedness to my friend Professor Jeffrey Bell, and to tender my thanks to the authorities of the British Museum for their generosity in permitting me to "work my will" on the specimens handed to me.

The genus Kynotus was founded by Michaelsen[1] in 1891, and in addition to the original species, three have since been described, two by himself[2] and one by Rosa.[3] All these, like the present new species, were collected in Madagascar.

One of the most striking characters of the genus is the great number and small size of the segments composing the body, quite apart from the total size of the worm. The species of this genus are of considerable length, though none are of any

[1] 'Arch. f. Naturgesch.,' 1891 (K. madagascariensis).

[2] 'Jahrb. Hamburg. Wiss. Anst.,' ix, 1891 (K. longus); 'Arch. f. Naturgesch.,' 1892 (K. kelleri).

[3] K. michaelsenii, 'Boll. Mus. Zool. Torino,' vii, 1892.

great thickness,—being, in fact, relatively thin. A second feature, and one that leads to some difficulty in assigning the organs to their proper position, is the amount of secondary annulation presented by the segments in the anterior part of the body, and in the assumption by these annuli of the appearance of true segments; so great, indeed, is the resemblance, and so deep are the interannular grooves, that an external examination alone is absolutely insufficient to enable one to tell what are "annuli" and what are "segments." This secondary ringing of the primary segments deceived Michaelsen in his descriptions of the two earlier species, K. madagascariensis and K. longus; and he attributed to certain internal organs a position so different from that occupied by these organs in all known earthworms, that I was led to suggest that he had mistaken "annuli" for "segments" or "somites."[1] Almost at the same time Rosa showed, from an examination of a new species, K. michaelsenii, that Michaelsen had indeed fallen into this error; and Michaelsen himself, in describing a fourth species, acknowledged that this had been the case. But, as we shall see below, we are even now in some doubt as to the extent and limits of this "annulation of the segments"—at any rate, in four out of the five species—so far as the first few segments of the body are concerned.

In the bottle sent to me were three portions, each about nine inches (225 mm.) in length; each is the anterior part of a worm of much greater length, probably at least eighteen or twenty inches. Each piece consists of some three hundred or more segments.

One of these three pieces is of especial interest, as it is genitally mature, and is provided with a clitellum—a structure observed, hitherto, only in K. michaelsenii, and there of much less extent than in the present species, the specific name of which refers to the great extent of this organ. This specimen, and one of the other two, also possessed a large everted copulatory organ of relatively enormous dimensions—a

[1] Benham, "Description of Three New Species of Earthworms," 'Proc. Zool. Soc.,' 1892, p. 149.

fact, too, which is of interest, as adding to our knowledge of the varied modes of copulation amongst earthworms.

External Anatomy.—The details refer to the specimen provided with a clitellum, and preserved in the British Museum, which I have not opened or otherwise injured.

This worm contains three hundred and fifty-six rings,[1] most of which are true segments, though some of the anterior rings are only "annuli." Its length is 225 mm., its diameter about 12 mm. in front of, but scarcely half this behind the clitellum. The posterior end was truncated, and the worm had evidently been cut through, probably at about half its length. The pre-clitellar region measures about 37 mm. (one and a half inches); the clitellum itself is 50 mm. (two inches) long.

After the first dozen rings the body narrows slightly, then at the twenty-third ring gradually widens again, whilst at the thirty-first ring the diameter increases suddenly; this diameter is retained throughout the clitellum, behind which, at the fifty-seventh ring, the body suddenly diminishes in diameter.

The body is cylindrical and strongly contracted, so as to feel quite hard in the anterior region.

The general colour of the worms, in spirit, is dull buff; the dorsal surface behind the clitellum is black, and similar black pigment occurs on alternate rings in front of this organ.

I have been unable to detect a prostomium; there is no trace of it externally, and on slitting open the buccal cavity of one of the specimens I saw no trace of it in a retracted condition. This is the more curious, since K. longus is provided with a large prostomium, and Rosa mentions it in his species.

The first two rings and part of the third are marked by longitudinal grooves, as in other species (Pl. 33, fig. 1). The first ring is obscurely divided into two by a furrow, which in one specimen was so distinct as to cause me on first counting to reckon this ring as two rings.

The fourth and following rings are marked by a distinct ridge round nearly their middle, dividing each ring into two.

[1] I use the term "ring" to indicate the apparent segment: that is, what anyone would at first sight regard as a "segment."

This secondary ringing becomes more marked as we pass backwards, and the thirteenth ring is marked by two slight grooves, dividing it into three well-marked secondary rings. This same phenomenon is presented by the following rings up to about the twenty-second, after which it becomes less and less distinct, till it is scarcely recognisable on the thirtieth ring. These rings, up to about the twenty-fifth, are all of nearly the same size. The rings of the clitellum are not thus marked, but the post-clitellar rings are biannulate dorsally. Any zoologist, even one familiar with earthworms, examining these rings would regard them, I do not doubt, as "segments;" but such is not the case in the anterior part of the body. The twenty-third ring and every subsequent ring is a "segment" (a somite), as is shown by the internal anatomy—septa, nephridia, blood-vessels, as well as by the chætæ,—but anteriorly to this most of the rings are "annuli," or secondary ringings of the body, two of which go to form a "segment" (fig. 1). This, again, is shown by internal anatomical arrangements, and the only doubtful rings are the most anterior three or four. From the position of the nephridiopores, I believe that the first (sometimes annulated) ring is a segment; the second and third rings make up the second segment. The segment III is also biannulate, but the fourth segment is not annulated; each of the following segments, V to XII inclusive, are biannulate, behind which every segment consists of only one ring or annulus (see fig. 6).

Rosa construes the anterior rings rather differently. He believes the first two rings constitute the first segment, and that each of the next two rings is a segment, and that the fourth segment is biannulate. Beyond this point we are in accord.

The point of difference, then, is that we have six rings at the anterior end to fit into four segments. Now, as we shall see, the most anterior nephridium opens at the anterior margin of the second ring, the second nephridium between rings 3 and 4, the third between rings 5 and 6. At this point also is the first septum.

Proceeding now with the description of the external anatomy of K. cingulatus. As in three of the other species, there

are no chætæ in the anterior segments of the body; the first chætæ to be recognisable are those on the twenty-sixth ring (segment XVI), though in sections I have recognised them in the preceding ring. On segments XIII, XIV, XV, the ventral couples are replaced by long penial chætæ, which do not project in the preserved specimen, but the apertures of the penial sacs are seen (fig. 2). The chætæ are all lateral; the individuals of a couple are very close together. The two couples of one side are separated by a space of 3 mm. on the clitellum, and in front of this space is only 2 mm.; behind it the ventral space, separating the right and left inner or lower couples, is 11 mm.[1]; the dorsal space, separating the right and left outer or upper couples, is rather less.

These chætæ are relatively small, measuring only 0·84 mm. (fig. 10); they have the usual shape, and possess a swelling or node nearer the free end. (In K. longus and K. kelleri, to which my species presents several points of similarity, the swelling is absent and the chætæ are smaller.) These chætæ are ornamented, as in some of the other species, by very inconspicuous transverse groups of short lines (fig. 11).[2] Is this ornamentation here and in some other cases, where it is so simple and ill-defined, a mere wearing away of the surface of the chæta, showing the broken ends of groups of fibres of which the chæta is composed? In some worms, as in Rhinodrilus and Trichochæta, the markings on the chætæ are much more distinct than in the present instance. Here the "ornamentation" has to be carefully looked for; and as the tip of the chæta is greatly worn down, it is just possible that the "ornamentation" may be the result of wear and tear. On the other hand, a similar ornamentation occurs in the penial chætæ, which presumably would not be worn down. On the clitellum, too, each couple lies in a pit in the epidermis, the chætæ themselves not reaching the surface.

[1] These measurements were taken when the body-wall (of one specimen) had been flattened out.

[2] The lithographer has emphasised these markings: they should have been very much fainter and at the same time more regular.

The clitellum occupies twenty-six segments; namely, XXI to XLVI inclusive (i. e. rings 31—56) (fig. 1). There is not the slightest doubt that these rings are here true segments and not annuli; for the chætæ and nephridiopores are perfectly visible—indeed, better marked than on ordinary segments,—and the intersegmental grooves are deep. This clitellum is complete; its anterior and posterior boundaries are very well marked, and there are no lateral ventral ridges or markings analogous to tubercula pubertatis. The glandular thickening is continued right across the ventral surface, as in Diachæta and Pontoscolex.

The extraordinary length of the clitellum is approached only by Allolobophora gigas (Dugés), where it occupies segments XXX to LI inclusive, i. e. covers twenty-two segments. Amongst the Geoscolicidæ (Rosa) the clitellum is usually pretty extensive, covering some eight to ten segments, and in Diachæta thirteen segments. The great length of this organ in the present worm is the more noteworthy since in the only other member of the genus in which it has been observed, viz. in K. michaelsenii, it only covers seven segments, XIX to XXV (rings 27—33). Rosa states that it is quite evident, but finds that the inter-segmental grooves are reduced to simple lines, and are not as deep as in the non-clitellar segments. Again, in this species the longitudinal margins are evanescent, and do not pass beyond the ventral chætæ, i. e. it is "incomplete." The clitellum in Rosa's species appears, then, to differ from that in mine—a difference that is not difficult to explain, as it is possible that the worm was not quite mature.

We must wait till we can obtain a more abundant supply of these worms before we can settle this point; for although four species have hitherto been described, each species is represented by only a single specimen, and of my three specimens only one has the clitellum fully developed. In a second I can detect traces of it in the smoother surface of a number of segments corresponding to the area occupied by the organ in the specimen just described, whilst in the third I can detect no trace of it. It is possible that, as in Moniligaster, which

was for so long believed to be without a clitellum, till Bourne described it in M. sapphirinaoides, it is a very temporary structure in Kynotus.

Generative and other Pores.—As in the other species, there are four pairs of openings leading into more or less extensive internal sacs (figs. 1, 2). Of these the largest lies in segment xv (ring 25) in line with the ventral chætæ; this is an "eye-like" opening, with the margins marked by radial grooves, and presenting, within, a rounded papilla, sunk below the general surface, and not visible in all the three specimens. Immediately in front of this pore (which Rosa and Michaelsen describe as the "male pore") lies a second on segment xiv (ring 24) of smaller size, and having rather a slit-like shape. In front again, on segment xiii (ring 33), another of the same appearance, but lying rather more laterally than the two posterior pores. The most anterior pair of pores lies on ring 21, the anterior annulus of segment xii; these have the same appearance as the others, but lie very much nearer the ventral mid-line than they do. In arrangement these pores agree with what has been described by previous authors.

Of these four pairs of pores the three anterior pairs are the openings of "prostates," or "sphermiducal glands," as Beddard has recently proposed to term these structures, and of the sacs containing penial chætæ. The fourth and largest pair gives exit to large copulatory organs of very characteristic appearance and of relatively enormous size, through which the sperm-duct opens. These organs I shall speak of as "claspers," for they are evidently not capable of insertion into any sac during copulation: they are not penes in the ordinary sense of the word.

In two of my specimens the organ on the left side was protruded—in one case fully, in the other only partially. The fully protruded clasper is represented from below in fig. 2, and from above in fig. 3. It is somewhat circular in outline, flat, or even slightly concave dorsally or outwardly, convex ventrally, i. e. medially. On the latter face is a "semicircular ridge" (fig. 2, *1* and *3*) near the free margin, from which it is separated by a groove. From nearly the middle of its course

this ridge sends down a branch (*2*) crossing the convex surface and ending abruptly, like the main ridge, close to the body-wall. This ridge throughout its extent is traversed by a very narrow furrow.

The histological structure of this peculiar organ, so far as it can be determined on my specimens, is fairly simple; it is a solid mass of muscle, covered by an epithelium of a single layer of cells (fig. 17), as will be more fully described below.

Presumably this pair of organs serves to hold two animals together during copulation, the organs of each clasping the sides of the other, somewhat in the same manner, no doubt, as the peculiar "penial appendages," or, as I would call them, claspers of Siphonogaster (Alma); the chief differences between the two organs being the presence of chætæ and the absence of any power of withdrawal of the organ into the body in the case of the latter.

We know little of the mode of copulation even in our native earthworms, but we can distinguish at least four kinds of apparatus for holding the two worms together:—

1. The penis-like terminal duct of the spermiducal gland of Perichæta, Acanthodrilus, and other worms, which appears to be capable of pleurecbolic eversion, and is presumably received by the copulatory sac, a portion of the spermatheca; to such an apparatus the term "penis" appears applicable.

2. "Suckers," such as I have described in Microchæta papillata; and under this head we must include probably the terminal "atrium" of the sperm-duct of Criodrilus, and perhaps of Geoscolex.

3. The "claspers" of Kynotus and of Siphonogaster, and perhaps of the Eudrilidæ.

4. The tubercula pubertatis of the Lumbricidæ, Sparganophilus, Rhinodrilus, &c., which secrete a fluid and help to "stick" the two worms together.[1]

In the case of the first three, specialised chætæ, copulatory

[1] The external muscular organ of Stuhlmannia variabilis is very exceptional, and it is not quite clear in which group we should place it; possibly in the first.

or penial chætæ may be associated directly or indirectly with the various organs, and aid materially in holding the two worms together during copulation.

To return to Kynotus, the second of my three specimens indicates the manner in which this clasper is protruded, for here the organ is protruded to just half of its extent (fig. 4, *5*). The first portion to make its appearance on protrusion is the hinder border (fig. 4, *1*), and in this case the dorsal or outer surface is strongly curved so as to be much more concave than when fully protruded (see fig. 5).

I was unable to observe the oviducal or the spermathecal pores on external examination.

The nephridiopores become visible only on the twenty-sixth and following rings; they exist anteriorly to this, but owing to the great amount of contraction of the body-wall they are invisible. They lie nearly midway between the couples of chætæ on each side, and are especially distinct on the clitellum (fig. 1, *np.*).

There are no dorsal pores.

The Internal Anatomy.—The position of the eight thick septa (*a*—*h*) which exist in the present species is a most important point to determine, and is not quite so easy as it would appear (fig. 6). The most anterior septum is quite thin, and lies behind the pharynx; it is inserted between the fifth and sixth rings.

The first thick septum, *a*, is inserted between rings 6/7.
The thick septum *b* " " " 8/9.
" " *c* " " " 10/11.
" " *d* " " " 12/13.
" " *e* " near the hinder margin of 14th ring.
" " *f* " " " 16th "
" " *g* " in the 18th "
" " *h*, somewhat thinner than the preceding seven, near the hinder margin of the 20th ring.
The next, i. e. tenth septum, is inserted near the hinder margin of the 22nd ring.
The eleventh, between 23/24 rings.

The following septa are inserted behind every successive ring in the normal manner. My diagram shows the position of these septa in relation to rings and to segments.

In Rosa's species the strong septa are seven in number, and are fixed at the segments v/vi to xi/xii, corresponding to those in the present species labelled *b* to *h*.

In K. madagascariensis, too, the septa *b*—*h* (Michaelsen's septa ii to viii) agree with those in mine, but the most anterior one, which should correspond with *a*, is placed between rings 7/8.

In K. longus (if we make the allowance suggested by Michaelsen himself, that he counted a portion of the everted buccal region as the first ring, and therefore subtract one from all his numbers) there is a perfect agreement with mine.

We may, then, use these strong septa as characteristic of the genus. Both in number and position, seven of them agree in four of the known species.[1]

The alimentary canal is provided with a gizzard lying in segment iv, thence the œsophagus passes back as a narrow tube without diverticula, merging into the sacculated intestine behind the genitalia in about the twenty-fifth segment; there is no typhlosole. That part of the gut which passes through the segments xi—xvi is particularly narrow,—not much wider, indeed, than the dorsal blood-vessel.

With regard to the vascular system, I only noted the following points:—The dorsal vessel is very distinctly moniliform in this anterior region; there is a supra-œsophageal vessel passing through segments v to x, which appears, however, to unite with the dorsal vessel at each septum, and from these points of union the hearts are given off. Of these there are six pairs lying in the segments just mentioned, the first one being smaller than the rest.

The nephridia commence far forwards; there are three

[1] K. kelleri appears to be exceptional, for Michaelsen states that the eight septa begin at segments vi/vii, and end at xiii/xiv, and places the first prostate in segment xiv. It is just possible that some mistake, so easily made, has occurred in reckoning the "rings" in the anterior part of the body.

pairs lying in front of the first thick septum (*a*). Of these, the first forms a large "peptonephridium" lying below the pharynx; its duct was traced to the body-wall, which it penetrates in front of the second ring, i. e. segment II (see fig. 6, *np.*). The second nephridium is also of considerable size; its duct opens between rings 3/4, i. e. at the anterior margin of segment III. The third nephridium lies behind the thin septum in segment IV, and its duct was traced to its opening between rings 5/6.

From these facts I conclude, as I have stated above, that the second segment is two-ringed; the third is two-ringed; whilst the fourth consists of one ring only (the sixth).

The following nephridia are all of fairly large size, and quite of the Geoscolicid pattern (fig. 23); the more posterior ones behind the segment XV are provided with a "cæcum" (fig. 24), those more anterior are not. The tubule of the latter is spirally coiled, forming corkscrew-like bunches of peculiar character.

The generative system is but insufficiently known in the genus. Rosa, who has contributed most to our information on the subject, found the testes in segments X and XI, in which were masses of spermatozoa free in the cœlom, and he traced the male duct to the organ in segment XV; I can confirm him in both these statements.

The copulatory organs, whose external features have been described above, lie in segments XII, XIII, XIV, and XV (fig. 7). In each of the first three segments are paired spermiducal glands or prostates (*pr.*) in connection with sacs containing penial chætæ (*p. ch.*) The gland is a convoluted white tube of considerable size, and in well-developed worms extends backwards into the neighbouring segments. Each gland is enveloped in a thin sheath of muscle (see fig. 15, *sh.*); its muscular duct receives, just before joining the body-wall, the neck of the chætigerous sac or "chætophore." This is of considerable size, and has a thick muscular wall; it contains three (or four) chætæ, one of which is much longer than the others which appear to be reserve chætæ, and

have same shape as the first. The fully developed penial chæta (fig. 8) is 4·9 mm. in length, measuring as accurately as possible along the curve; the inner end is sharply bent and somewhat enlarged: the free end (fig. 9) is quite similar to that described by Rosa and Michaelsen. Its tip projects freely into the duct of the gland, the rest of it lying in a sac of its own with thick bundles of longitudinal muscles forming its walls (figs. 12—14). Each of the reserve chætæ lies in its own sac (figs. 12, 13, ch^2., ch^3.), but their pointed ends are enveloped in this sac, and do not project into the lumen of the duct. This seems to indicate that the penial chætæ are lost from time to time.

These features can be well seen in following serial sections. A section somewhere about the middle of the chætophore shows three chætæ, each surrounded by a coat of circular muscles, with a certain amount of longitudinal muscle. Passing towards the body-wall, first one and then the second reserve chæta disappears as the section passes beyond their points, while the functional chæta still persists. Now the wall of its follicle presents a more distinct epithelium, and approaches the duct of the prostate; it becomes wrapped in the same coat of circular muscles as the duct (fig. 12); and further onwards (figs. 13, 14) the lumen of the chætophore communicates with the duct of the prostate.

The penial chætæ when mounted in glycerine appear to be hollow, and the transverse lines figured by Michaelsen seem to me to be confined to the inner surface of the apparent wall (fig. 9, *r.*), and are not of the nature of "ornament." The pointed end of the chæta is, however, ornamented in the same kind of way as the ordinary chæta. In transverse sections it is seen that the axis of each penial chæta is of a different character from the cortical zone: the latter is yellow, and has the usual appearance of a chæta (fig. 14, *cor.*); it is evidently brittle, for it exhibits cracks across it, and is frequently torn away in sectionising. But the "medulla" (*med.*) stains pink in borax carmine, is homogeneous, and evidently softer than the cortex.

The penial chæta of Kynotus, in fact, resembles the chætæ of several Polychæta in this respect, and I am not aware that this feature has been noted previously in Oligochæta.

With regard to the prostate, or spermiducal gland, it has the structure so frequently described for many earthworms; its lumen is surrounded by a layer of narrow columnar cells: this is surrounded by a very thin coat of circular muscle, which is traversed by the narrow necks of the great club-shaped gland-cells (fig. 16). In a transverse section of the entire gland (fig. 15), the limits of the various loops of the coil are not by any means well defined; one sees two or three ducts apparently embedded in a mass of glandular tissue, and the whole surrounded by a sheath of muscle, in which run blood-vessels. Closer examination generally enables one to trace the outline of the groups of gland cells belonging to each section of the duct.

The clasper (figs. 17—20).—I have already described the appearance of this peculiar organ when protruded.

In the worm which was dissected, in which the "clasper" was entirely retracted, there was seen lying in segment xv (the 25th ring) an irregularly oval body, convex upwards, with a very irregular surface; its long axis is parallel with that of the worm's body (*S.*, fig. 7). This is the structure which Michaelsen terms "bursa propulsoria." What appearance it would present from within when completely everted I am unable to say: whether the whole structure is capable of being protruded or not is at present unknown.

This "bursa propulsoria," or retracted "clasper," is larger than the segment to which it belongs, and pushes apart the septa in front and behind. Its external opening is of course invisible from within, as it lies below the organ.

From its outer side there arises a muscle (fig. 7, *m.*) which passes forwards, and becomes continuous with the longitudinal muscles of segment xiv. This is called by Rosa the "retractor muscle." At the hinder end of this organ there arises from its ventral surface, a gland (fig. 7, pr^1.), exactly like those

of the preceding segments except that it is much larger, and extends through some five or six segments. Rosa terms this the "pseudo-prostate."

The minute structure of the clasper has been described by Rosa, and my observations agree very closely with his, though I cannot distinguish so definitely as he does the divison of the enclosed chamber into two by an imperfect horizontal septum. Nevertheless two regions of the cavity are readily distinguished in transverse sections (figs. 17, 18); one portion of the lumen (*C*) is lined by close-set columnar cells, the other (*A*) by gland cells intermixed with ordinary cells, some of which are empty, and others filled with secretion (fig. 19, *gl.*).

The rest of the organ is muscular, with more or less abundant blood-vessels distributed through it. When the cavities are traced out it is found that the portion *C* is continuous with that surface of the protruded organ which is directed inwards (ventral), whilst the epithelium lining *A* covers the outer surface of the organ. The epithelium is everywhere one cell deep; there is no basement membrane, and the blood-vessels (fig. 19) pass up between the cells. In the section through a part of the protruded chamber the part labelled *B* (fig. 17) is, of course, the prominent organ seen in figs. 4 and 5, which was the specimen sectionised, and corresponds to Rosa's "grande scudo ovale," which projects downwards from the roof of the upper chamber.

The duct of the gland enters that region of the lumen marked *C*; its secretion, therefore, is discharged on the inner, ventrally directed face of the organ which is presumably used to grasp the other worm during copulation. Deeper in the organ the two cavities become continuous as in fig. 18.

The two sperm-ducts of one side, after passing backwards along the body-wall on the medial side of the prostates, reach the "bursa," and pass along its medial border. They then bend round it posteriorly, and enter the muscle surrounding the neck of the gland (pr^4.); here they turn forwards and

enter the duct, before the latter communicates with the "bursa-propulsoria."

Of the female organs I have only observed the spermathecæ (fig. 7, *spth.*). These lie, as in other species, in Segments XIII, XIV, XV (rings 23, 24, 25), opening along the anterior margin, though I was unable to detect the pores on external examination. The spermathecæ are somewhat pear-shaped sacs, variable in size and in number. In one specimen dissected there are, on the right side, three in a row in each of the above-mentioned segments; on the left side the numbers were one, two, and two in these segments.

A second specimen gave the following numbers:—on the right side none in the segment XIII, three in XIV, and two in XV; on the left side two sacs in each of the three segments. The number is evidently variable in this species. We have no information, of course, as to the extent of variability in the other species.

There are one or two peculiarities in the structure of the body-wall worthy of mention. Below the epidermis is a layer of connective tissue, especially thick on the ventral surface, where it has the appearance of a homogeneous matrix, with spindle-shaped nuclei embedded in it (fig. 22, *bt.*), which recalls the "basement tissue" of Nemertines. Deeper down the longitudinal muscles are separated into blocks by incursive fibrous connective tissue, which forms a fairly thick layer internal to the longitudinal muscles (fig. 21, *ct.*) A similar but much thicker connective tissue exists also in Brachydrilus. Each "block" of muscle is made up of several bundles, each of which is probably derived from a single cell, as Vejdovský has shown to be the case in Lumbricus. Further, the connective tissue between the blocks appears to pass through the circular layer of muscles and to terminate in the basement tissue (fig. 21).

The blood-vessels in this region are very well developed, and enter the epidermis, between the cells of which they ramify (fig. 22), as in Moniligaster, Perichæta, Criodrilus, and other earthworms.

Affinities.—The present worm, which I have regarded as a new species, presents certain resemblances to K. longus and to K. kelleri; but from the former it differs in the number and arrangement of the spermathecæ, which are there in two groups of eight on each side, belonging to the rings 25 and 26 (probably to segments XIV and XV). It has, further, an elongated prostomium, the bursa propulsoria is "disc-like," and flattened in the longitudinal direction, and the prostates are pear-shaped ("birnformige").

From K. kelleri the prostate of the bursa (Rosa's "pseudo-prostate") is "zipfelformige;" those of the penial chætophores are "birnformige." As mentioned above, the ordinary chætæ of these two species are without a "node."

From K. madagascariensis the present worm differs in that this species has numerous small spermathecæ in three rows on each side, as well as in other matters of detail, such as the form of the prostates, &c.; whilst K. michaelsenii is evidently a different species, for it only has two pairs of penial chætophores and prostates.

Postscript.—Since this paper was written, two new species of Kynotus have been described by Michaelsen ("Zur Kenntniss der Oligochaeten," in 'Abhandl. aus dem Gebiete d. Naturwissenschaften,' Bd. xiii), in which he makes some further observations on the peculiar annulation of the body wall. The species are K. oswaldi and K. distichotheca, both of which differ in various points from the above-described form.

K. oswaldi presents a clitellum of considerable length, occupying eighteen segments, viz. XIX to XXXVII. In both, the male pore is on the 26th ring; but from observations on the relation of nephridia and septa he believes this to be the sixteenth segment, whilst Rosa finds it on the fifteenth (ring 23) in K. michaelsenii, which he has re-examined. His enumeration of the segments is different from that given by me for K. cingulatus. The first strong septum lies between the rings 7 and 8, and in front of it are three pairs of nephridia, the first pore being in front of the 3rd ring; he therefore considers

each of the first three rings to be a segment, so that there are five segments (instead of four, as in my species) in front of the first strong septum. At first sight one might be inclined to suggest that Michaelsen had overlooked the first nephridiopore, but as he stripped the cuticle from the worm there can be no mistake in the matter; the first nephridium of K. cingulatus is therefore absent in these two species. It is still possible thus to regard two of these rings as forming one segment, as I have described for the present species. He finds the nephridiopores to lie in front of the rings 3, 4, 6, 8, 10, 12, &c.; whereas I find them in front of 2, 4, 6, and so on. It is just possible that I have made an error in observation, for I did not strip the cuticle for my specimen, which was too hardened for this manipulation, but I traced the duct to its pore.

Nevertheless he shows that K. michaelsenii differs in the amount of annulation from K. longus and others in that the segment III is biannulate, whilst in other forms the biannulation commences on the next segment, and that it ceases at segment X; whereas in K. longus it ceases at segment XIII, and in K. oswaldi, as in my species, at segment XII.

This annulation, like the number of thick septa, appears to be a specific character, as well as the segment on which the chætæ commence.

EXPLANATION OF PLATES 33 and 34,

Illustrating Dr. Blaxland Benham's paper on "Kynotus cingulatus, a new species of Earthworm from Imerina, in Madagascar."

(Throughout the figures "annuli" are indicated by Arabic, and "segments" by Roman numerals).

FIG. 1.—Ventral surface of Kynotus cingulatus (× 2). The annuli are marked with Arabic numbers mostly on the right side. The Roman numerals

on the left side indicate the true segments. The worm is represented as if the hinder part were slightly twisted round its long axis, so as to bring the side into view. *B.* The protruded clasper; the pores of the four pairs of male organs are shown (see also Fig. 2). *m.* Mouth. *np.* Nephridiopore *i. ch.* Inner couple of chætæ. *o. ch.* Outer couple of chætæ.

Fig. 2.—A portion of Fig. 1 enlarged (× 4), showing annuli and tertiary ringing of the segments. Annuli and true segments are indicated as before. *B.* The clasper. *1, 2, 3.* The curved ridge on its ventral face. *b.* The pore on the left of the figure through which this organ is protruded. p^1, p^2, p^3. The three pores of the prostates or spermiducal glands and penial chætophores. *i. ch.* Inner couple of chætæ.

Fig. 3.—The clasper of the same specimen seen from the outer surface, rather dorsally; the annuli are marked. *o. ch.* Outer chætæ.

Fig. 4.—View of the clasper of another specimen in a partially protruded condition; the animal is seen partly from the side. *1, 2,* indicate those parts of the ridge similarly numbered in Fig. 2. *b.* The pore through which the clasper is protruding. *np.* Nephridiopore. *i. ch., o. ch.,* as before. The annuli are here numbered.

Fig. 5.—The same partially protruded clasper seen antero-laterally; a view nearly corresponding to Fig. 3.

Fig. 6.—Diagrammatic representation of the relations of annuli, segments, and septa. The annuli are marked by Arabic numerals on the left; the segments by Roman numerals on the right; the septa by thick lines; and the interannular furrows by fainter lines. The eight strong septa are indicated by small letters, *a—h.* The three anterior nephridiopores are represented as dots (*np.*) on the right side. The characteristic copulatory organs are represented in their true position on both sides.

Fig. 7.—The characteristic copulatory organs of the right side considerably enlarged. The annuli are indicated on the right, the segments on the left side. Pr^1, Pr^2, Pr^3. The three spermiducal glands, each a convoluted tube enveloped in a muscular sheath. *p'. ch'.* Penial chætophore connected with the first prostate. *S.* The "bursa propulsoria," or sac containing the clasper. pr^4. Its gland. *m.* Retractor muscle. *spth.* Spermathecæ. *ner.* Position of the nerve-cord.

Fig. 8.—The fully developed penial chæta (× 35). Camera drawing.

Fig. 9.—The free end of the penial chæta (× 180). Camera drawing. *c.* The denser cortical portion. *m.* The medulla. *r.* The ring-like markings on the inner surface of the cortex. N.B.—The outline has suffered in reduction of the drawing.

Fig. 10.—An ordinary chæta (× 35).

Fig. 11.—The tip of an ordinary chæta greatly magnified. The markings are represented too strongly.

Fig. 12.—Transverse section of the penial chætophore near its junction with the duct of the spermiducal gland. *d. pr.* Duct of gland. *ch*^{1}. Sac of the functional penial chæta. *cir.* Coat of circular muscles enveloping these. *lg.* Longitudinal muscles. *sh.* Sheath. *ch*^{2}, *ch*^{3}. Reserve chætæ.

Fig. 13.—Transverse section of the penial chætophore lower down, where the lumen has opened into the duct of the gland. Letters as in Fig. 12.

Fig. 14.—The common duct enlarged. *Ep.* Epithelium. *Cu.* Cuticle. *Cir.* Circular muscles. *ch*^{1}. The penial chæta. *Cor.* Its cortical, and *med.* its medullary portion.

Fig. 15.—Transverse section of a prostate. The lumen (*l. l.*) is cut through four times, and appears embedded in a mass of gland-cells (*pr.*). *ep.* Epithelium. *sh.* Muscular sheath surrounding the whole gland. *b. v.* Blood-vessels.

Fig. 16.—A portion of the preceding, enlarged. *ep.* Epithelium. *gl.* Gland-cells. *m.* Muscular coat round the epithelium.

Fig. 17.—Transverse section of the bursa propulsoria and partially protruded clasper (Figs. 4 and 5). The section does not go through the most projecting region, but rather obliquely through the side (× 35). *B.* The protruding portion of the clasper. *A. C.* The two regions of the bursa; the former lined by gland-cells, the latter by columnar epithelium. *ep.* Epidermis of body-wall. *cir.* Circular coat of muscles. *lg.* Longitudinal muscles. *m.* Muscular substance of the clasper. *r.* Retractor muscle.

Fig. 18.—Another section through the same further backwards, so as not to involve the pore (× 35). The regions *A. C.* are here continuous. Other letters as before.

Fig. 19.—A portion of the epithelium lining the region *A.* of the bursa. *gl.* Gland-cells. *b. v.* Blood-vessels. *m.* Muscle-fibres.

Fig. 20.—A portion of the epidermis of region *C.*

Fig. 21.—Portion of the body-wall in the ventral region. *Ep.* Epidermis. *b.t.* Thick basement tissue. *cir.* Circular muscles. *ct.* Connective tissue separating the longitudinal muscles (*lg.*) into bundles. *b. v.* Blood-vessel.

Fig. 22.—Portion of the above sections more highly magnified, to show the peculiar basement tissue (*b.t.*) with nuclei (*n.*) embedded in it, and the vascularity of the epidermis.

Fig. 23.—One of the anterior nephridia, showing its peculiar spirally-coiled tubule (*t.*) and long duct (*d.*).

Fig. 24.—One of the posterior nephridia with a cæcum (*c*) to the duct, and a different coil to the tubule.

Notes on the Ciliation of the Ectoderm of the Amphibian Embryo.

By

Richard Assheton, M.A.

With Plate 35.

The fact that the embryo and larva of Amphibia possess a ciliated ectoderm has been frequently noticed, but since, as far as I know, a description of the distribution of the cilia, and of the currents of water over the surface of the body, which result from the action of the cilia, has not been hitherto published, my notes upon the ciliation of the tadpoles of Rana temporaria and Triton cristatus may perhaps be of interest.

Quite recently the fact of the existence of a ciliated embryo among craniate Vertebrates seems to have been doubted or overlooked. Osborne (10), in his preface to Willey's "Amphioxus and the Ancestry of the Vertebrates," with respect to the "real invertebrate and vertebrate affinities" of Amphioxus, writes thus :—"For example, among the real invertebrate ties of the Protochordates are the ciliated embryos of Balanoglossus and Amphioxus, the Tornaria larva and ciliated ectoderm of Balanoglossus."

Willey (17), in the text of the book, page 113, says, "The fact that Amphioxus has a free-swimming, ciliated embryo is important as providing a general connecting link between the Vertebrates and the Invertebrates, since the possession of a ciliated ectoderm is very common among invertebrate embryos, but entirely unknown among the craniate Vertebrates."

Again, a little further on, the same author expresses his views still more strongly. On page 175 he writes: "The ciliation of the ectoderm in the larva of Amphioxus continuing, as it does, long after the muscles have been fully differentiated, and when the cilia are therefore no longer required for purposes of locomotion, should be especially noted as evidence of a very archaic organisation. We shall find in the last chapter that the possession of a ciliated ectoderm is a prime characteristic of Balanoglossus and many of the lower worms (e.g. Nemertines). In none of the craniate Vertebrates is the ectoderm at any time ciliated."

Eycleshymer (4), discussing the question of the cause of the continuous rotatory movement of the vertebrate ovum, writes, on page 355: "Clarke states that in Amblystoma punctatum the surface of the body is covered with cilia at the time the neural folds close, by means of which it keeps up its rotatory motion. I have endeavoured to detect cilia by teasing in normal saline solution also by osmic acid fixation, but without success."

Clarke's (3) actual words are—

"The entire surface of the body is now" (i.e. when both the anal or caudal and cephalic ends are becoming more definitely indicated as they grow away and stretch out from the body of the embryo) "covered with cilia, by aid of which it keeps up a horizontal rotatory motion upon its axis."

Balfour (1), on page 141, says of the newt that "the skin is ciliated, and the cilia cause a rotation in the egg."

Again, of the frog the same author says, page 127: "The outer layer of epiblast-cells becomes ciliated after the close of the segmentation, but the cilia gradually disappear on the formation of the internal gills. The cilia cause a slow rotatory movement of the embryo within the egg, and probably assist in the respiration after it is hatched. They are especially developed on the external gills."

Marshall and Bles (7) also notice the fact, page 42: "The whole surface of the tadpole is, as in the earlier stages, ciliated;

the cilia working from head to tail, causing the animal when perfectly quiet to move forwards slowly in the water."

Preyer (12) and Carriere (2) also describe the presence of cilia upon the external gills of Salamander and Siredon respectively.

Stöhr (15), in a very brief account of the "sogen Haftorgane" of the Anura, describes the action of the cilia in connection with these organs, to which I must refer again.

The ciliated ectoderm of Rana temporaria.

The first signs of the presence of cilia in the frog embryo occur shortly before the closure of the neural folds. At the time when the folds are first visible, and even when they are commencing to fold, there is still no trace of ciliation.

Fig. 1, Pl. 35, represents the earliest stage at which I have detected any ciliary movement. I have not at this stage seen the cilia, but have observed a streaming of carmine granules along certain regions.

It will be seen that the edges of the neural plate are raised up as prominent ridges, but as yet they have not met at any point. The anterior or cranial portion is marked by its great lateral expansions, which do not become infolded, and which give rise to the ganglia of certain of the cranial nerves. It is upon these lateral expansions and the actual edges of the neural plate that the cilia are first developed. The arrows in fig. 1 indicate the direction of flow of the current set up by the action of the cilia upon the surrounding fluids.

Fig. 2 shows the extent and course of the current when seen from the side a few hours later. The neural folds have not as yet met.

As the neural folds bend upwards the current becomes more marked and extends somewhat further back, and also rather further ventral-wards.

Just before the neural folds close—when they have closed anteriorly, but are sufficiently open along the back to allow a view of the neural groove—a distinct motion is visible upon the anterior ventral surface of the embryo,—that is, over the area

in the centre of which the mouth occurs subsequently. The direction of the flow here is, as in the case of the dorsal current, from before backwards (vide fig. 3).

I have tried most carefully to find any indication of a ciliated ectoderm upon that portion of the neural plate which is folded up and becomes the neural tube. I can find no indication of a ciliation.

The cavities of the brain and spinal cord of the adult are said to be lined by a ciliated epithelium. Although doubted by some authors, this has been satisfactorily demonstrated by others (vide Wightman, 'Studies of the Johns Hopkins University,' vol. iv). At what stage the cells that bound these cavities became ciliated I cannot say, but of every specimen which I have examined I can say that at the time of closure of the neural folds there is no motion of suspended particles at any spot along the neural groove, although a rapid current is produced along the external portion of the edges of the neural plate.

Klein (6) has described a ciliation of the neural groove while still open in the chick, in embryos with about seventeen protovertebræ.

By the time that the neural folds have closed, except at quite the posterior end, the whole of the dorsal surface is ciliated, including the groove formed by the junction of the neural folds. By far the most rapid movement of particles takes place along the line indicated by the large arrows in fig. 4 (*N.B.C.*), which is along the extreme outer limit of the neural plate, or rather over that portion of the epiblast which subsequently gives rise to the sensory ganglia of the 5, 7, 8, 9, 10 cranial and the spinal nerves.

The ciliation now spreads very rapidly, and by the time the embryo with seven or eight mesoblastic somites measures about 3 mm. in length, which is about the time of the perforation of the anus, the whole surface of the embryo is ciliated, but the currents vary very much in intensity.

Fig. 5 is a diagram of the currents produced by the cilia as seen from the side.

The action of the cilia is strongest at the anterior end, and causes the water to be driven backwards as from a centre, the centre being the most anterior end of the embryo; in fact, just as would occur if the embryo was swimming rapidly forward.

The cilia on the anterior surface can at this stage be seen distinctly with a Zeiss D objective. There are, however, certain tracks along which special currents of water flow, which we may take to mean that along these paths the cilia are especially large, more numerous, or more active.

A very well-defined and strong current (fig. 5, *N.B.C.*) passes over the bases of the developing branchial arches, which would seem to correspond to that marked by the large arrows in fig. 4. Along the ventral surface the motion is extremely slack—it is rather a series of eddies. The currents at the hinder end are interesting. The action of the cilia of the whole of the hinder region is to tend to cause a current of water to flow towards the blastopore and anus. The most rapid current is that along the back.

At one time—when we may presume both blastopore and anus are open—there is a strong exhalent current from the positions of both of the openings. The water brought by the action of the cilia may be seen to curl over the edges of the blastopore and anus, and apparently is immediately shot out again with considerable violence (fig. 6).

Whether this indicates any interchange of fluid between that within the archenteron and the medium in which the embryo lies I cannot say. The anal current becomes stronger while the blastoporic becomes rapidly weaker, and soon ceases with the final closure of the blastopore.

Fig. 7 illustrates the direction of the currents of water over the posterior end a few hours later, at which time the tail has begun to grow out.

Special Currents along the Ventral Surface.

Until about now the ventral and antero-ventral currents have been quite simple; there is a general flow along the

ventral surface from before backward, although it is very much more rapid around the region of the future mouth.

About the time the tadpole measures 4 mm. two curious areas, known variously as "suckers," "Haftorgane," "crochet de Rusconi," &c., below the region of mouth (which is now represented by a slight depression), have become prominent features, and in connection with them a considerable modification in the ciliation of that region occurs.

The term "sucker" is hardly suitable, for it seems doubtful whether attachment is ever in Anura effected by true suction.

With regard to the functions of these organs, which perhaps might be more rightly called cement glands, Marshall and Bles, in their paper upon the development of the blood-vessels of the frog, made a suggestion which seems to me to be entirely unsupported by evidence.

They wrote thus on page 215 :—"Though the mouth is not yet open, the tadpole shows a distinct increase in bulk as compared with the 8 mm. stage. It has occurred to us as possible that the suckers may be used for absorbing food, and that in this way the increase may be explained; . . . and sections of the sucker show that the greatly elongated and columnar cells of the sensory layer of the epiblast covering them are often produced at their free ends into protoplasmic processes, that would seem well fitted for absorbing the jelly."

The "increase in bulk" noticed by them between the $6\frac{1}{2}$ mm. stage and 5 mm. stage may be as well accounted for by the large increase of the meshes of the mesoblast which occurs at this period. To effect this conversion of a compact mesoderm into a network of finely drawn-out stellate cells, no doubt it is necessary that water should be absorbed. It seems, however, hardly likely that absorption by the skin should take place at the spots where the epiblast appears four to eight times thicker than anywhere else.

The true function of these organs has been correctly stated by Stöhr (15) in these words:

"Sie bestehen aus langgestreckten einzelligen Drüsen (bei Bufo cinereus) die sich durch starke Pigmentirung auszeichnen

und ihr Klebriges Sekret in einem Hohlraum ergiessen, aus welchem dasselbe durch Flimmerharre nach Aussen befördert wird."

Fig. 8 is a semi-diagrammatic section across these organs of a tadpole of Rana temporaria 8 mm. long, at which time they reach their greatest development.

They are essentially of the nature of mucous glands, bordered by strongly ciliated ridges.

They secrete a very sticky substance, by means of which the tadpole can anchor itself to any convenient object. The "protoplasmic processes" alluded to by Marshall and Bles are probably masses of exuded mucous or cement, and are thus the product of the cells rather than processes of the cells themselves.

Both the ciliated cells and the mucous cells are developed from the outer or epidermic layer of epiblast. The nervous layer of epiblast forms a single layer of cells as elsewhere.

Fig. 9 is a semi-diagrammatic figure of a section taken rather further back than that represented in fig. 8. In this the ridges are more prominent. The long cement-secreting cells, *C.C.*, really lie with their bases much further forward than their necks and openings.

Fig. 10 shows the ventral surface of a tadpole of $3\frac{1}{4}$ mm. The cement-secreting cells are in the depressions *M*.

The sides of the ridges surrounding the glands are completely covered with long cilia.

The cilia here are the most conspicuous of all upon the body.

These cause very violent currents of water, as indicated in the figure. The central current passes over the stomodæal depression (*S*) along the groove leading from the mouth between the two glands.

In an older stage, $6\frac{1}{2}$ mm., fig. 11, the glands have so increased in size as now to be practically joined across the mid-ventral line. This necessitates an alteration of the course of the central current, which is now thrown outwards, and is then caught by the strong lateral currents and passes round the

ridges. A small portion passes straight on, making a stream which becomes very prominent a little later.

When the tadpole measures about 8 mm. the cement-glands are at their greatest development. Fig. 13 is a ventral view of this stage.

The two glands appear as though raised upon a horseshoe-shaped platform. Each gland, which is elongated in an antero-posterior direction, is bordered on both sides by a high ridge richly ciliated (fig. 9). The shape of these ridges and direction of action of the cilia upon them is such as to produce the following currents in the adjoining water, as indicated by the arrows in fig. 13.

(i) Externally, strong currents, *C.C.*, which skirt round the ridge towards the mid-ventral line.

(ii) Centrally, a strong stream, *S.C.* (the deeper portion of which passes first into the stomodæal cavity, in which particles may be seen to revolve in eddies for some minutes), runs up the groove from the stomodæal depression, and turns over the hinder border of the inner ridges of the glands, and drives the water in a strong stream partly straight outwards and away from the larva, and partly along the ventral surface (vide fig. 14); the effect being to sweep clean the hinder part of the glands.

(iii) The cilia on the crests of the ridges and on the sides of the ridges facing the glandular cells cause water to be drawn into the glands, which sweeps any secretion from the gland backwards until it comes into the very strong stream described last. These currents are indicated by the small arrows in fig. 13.

When the tadpole first leaves the egg membrane, and wriggles or is driven outwards from the jelly mass by the action of its cilia, it may perhaps be said to adhere to the jelly by suction, for the jelly is sufficiently fluid to be drawn into the hollow of the glands by the currents of water over the ridges, and too viscid to pass easily through and out again. But suction plays no part at all in the mode of adhesion to any object of a more rigid nature, such as a plant leaf, piece of root or stick. The tadpole is seen to be anchored by the sticky

mucous secretion, which, produced by the glands, is driven out by the system of water-currents described above.

Special Currents upon the Dorsal and Lateral Surfaces.

Although I cannot speak with certainty with regard to the earliest stages, yet as far as I have been able to determine I have never found a stage in which every cell of the epidermic epiblast bears cilia.

In 6 mm. tadpoles ciliated cells are scattered thickly over the whole surface, but amongst them are other cells which bear no cilia. On certain regions the ciliated cells are more numerous, and the cilia on certain spots are longer.

I described above a very well-defined current, marked *N.B.C.* in fig. 5. There is still in older tadpoles a current which is swifter than any other (excepting those connected with the glands described above), and very well indicated, which seems to be identical with that marked *N.B.C.* in fig. 5. I have similarly named it *N.B.C.* in fig. 12, of which I am now writing.

This current dips into the nasal depression which is provided with long cilia, and flows rapidly over the developing external gill-filaments.

This conspicuous current exists until the posterior nares are formed and the gill-filaments are covered over by the operculum, whereupon the current is no longer distinguishable as a special stream, but is merged in the general much-reduced flow of water which sweeps slowly from before backwards over the whole body. This is an interesting current, for it will be noticed that the greater part of the water which washes the external gill-filaments until the tadpole is about 9 mm. long has previously passed over the developing olfactory epithelium. Whether this epithelium at this stage plays any part in testing the water before it reaches the gills must be left to conjecture.

Fig. 15 is drawn from a horizontal section of a 6 mm. tadpole. It represents the ectoderm along the path of the current just described, and is between the olfactory pit and the base of the external gills. The ciliated cells are here very numerous,

being only very slightly less abundant than those which do not bear cilia.

Ciliated cells occur more sparsely over all the rest of the sides, back, and ventral surfaces, and cause a steady flow of water, which is rather more rapid over the tail.

The gill-filaments are provided with about one ciliated to every two non-ciliated cells (fig. 16).

The ciliation after about the 7 to 8 mm. stage begins to become less effective. A tadpole of 6 or 7 mm. will progress, if placed upon its side in water along the bottom of a flat glass vessel, at the rate of one millimetre in from four to seven seconds.

The Ciliation of the Later Stages of Larval Life.

In tadpoles of 12 mm. in length, ciliated cells are still to be found on all parts of the body. The general flow is from before backwards. The motion is, however, much less rapid, and there are no longer any special currents.

For instance, the streams connected with the cement-glands are now hardly distinguishable from the general flow. Areas wherein at an earlier stage every cell was ciliated, now contain many cells without cilia.

The cement-glands have become much reduced. They are mere circular bosses. The high lateral ridges have entirely disappeared. Nor is the flow of secretion nearly as copious, and the tadpole makes but little use of it.

The action of the cilia now seems to be no more rapid along the dorsal surface than ventrally.

The mouth and posterior nares are open, and the tadpole draws the water by muscular as well as by ciliary action into the pharynx by all three apertures. The exhalent flow by the opercular spout is extremely rapid and quite regular, and exhibits no signs of a muscular expulsion.

At 18 mm. there is still a flow of water over the whole surface of the tadpole, but there are now regions which do not bear cilia. For instance, the fringe of tentacles which have grown round the lips of the mouth is quite destitute of cilia.

There are no cilia upon the extreme dorsal and ventral edges of the tail. There are none on the eyes.

The motion of water over the body becomes slower, and in a 20-mm. tadpole it can hardly be termed a flow. The ciliated cells are now so few and far apart, and so feeble, that a series of eddies takes the place of a regular streaming. This, however, is not the case with the tail. Scattered all over the sides of the tail are cells which in surface view appear elongated (fig. 18, *C.*), and bear long and very active cilia, which work in the direction of the shortest axis of the cell.

The general result of the action of these cells is the production of a rapid flow of water from about the level of the notochord in a diagonal direction, both dorsally and ventrally. A granule of carmine may be seen to be dashed from one cell to another like a shuttlecock, and made to take a zigzag course across the tail fins, as indicated in fig. 17.

As the tail expands these cells become more and more separated, and so become less numerous to a given area where the tail expands most, and remain more numerous towards the base (fig. 17).

Fig. 18 is a camera drawing of the surface of the tail of a tadpole 19 mm., showing the ciliated cells, which are at this stage more deeply pigmented than the majority of the other surface cells. On the development of the hind limbs and diminution of the tail the cilia disappear from the tail, as from the rest of the epidermis.

The Newt (Triton cristatus).

I have not observed any cilia or currents of water presumably due to cilia before the complete closure of the neural folds in Triton cristatus. I do not, however, wish to assert that there is never any ciliation prior to that time, as my observations have been few in number. Clarke (3), as quoted above, also seems not to have noticed the cilia until after the closure of the neural folds. As the head and tail become distinct the whole of the anterior end of the embryo is richly ciliated, and

certain distinct currents are produced thereby. Posteriorly, however, the ciliation is very scattered and feeble, and produces no distinct flowing.

Figs. 19 and 20 represent the lateral and ventral views of such an embryo.

Over the dorsal and lateral surfaces there is a steady flow along the longitudinal axis of the embryo. Ventrally there is an especially rapid stream from the pre-oral region into the stomodæal pit, whence the water passes over the ventral wall of the embryo. The water from the lateral parts of the pre-oral area also flows towards the stomodæal pit, and then passes outwards and upwards towards the locality of the branchial arches. This current is more markedly present in a 6-mm. newt, as shown in fig. 22.

In fig. 21 a lateral view is given, showing how the action of the cilia at this stage is to bring as much water as possible into one stream, which may be said to start about the position of the olfactory epithelium and skirt below the "balancers" (*M.*), and pass very rapidly over the spots where the external gill-filaments are about to develop. Water from the dorsal region is also swept down into this same stream.

Over the posterior end of the embryo the ciliation is very slight. After the complete development of the external gills the ciliation over the greater part of the body becomes less active, and by the time the newt tadpole measures 16 mm. it has entirely disappeared excepting upon the gill-filaments themselves.

The special ciliated region which is present in the frog behind the stomodæum in connection with the cement-glands is absent in the newt. Ciliated cells occur here as elsewhere, but no special streams are produced. The balancers, which are placed much further from the mid-ventral line than are the cement-glands of the frog, are similar to the cement-glands in that at their extreme points there are cells which secrete a similar sticky cement by which the young newt can attach itself to weeds. They differ in that the cement-glands, which are very much smaller, are borne upon processes of the body-

wall which contain blood-vessels and connective tissue. In the frog they are formed of the two layers of epiblast only.

Clarke describes these organs, which he terms balancers, as being used to support the larva when it hatches and falls into the mud. This may be so, but they are certainly also used for the suspension of the larva from weeds. Their walls are not ciliated.

Comparison of the Ciliation of the Frog and Newt.

In both animals the anterior and dorsal regions are more richly ciliated than the ventral and posterior.

In both animals the stomodæal pit and area immediately surrounding the stomodæum are especially rich in ciliated cells, which cause a strong flow of water into the stomodæum, where particles suspended in the water may be seen whirling in eddies for a time and then passing out over the posterior lip.

In both animals a very distinct stream of water is produced, which passes first over the olfactory epithelium, and subsequently washes rapidly over the developing external gills.

In the newt this stream appears to be the main flood, into which tributary currents flow from both the dorsal and ventral regions (vide figs. 21 and 22).

In the frog this stream is in the main parallel with other currents, but is distinguishable by its very much greater rapidity (vide figs. 12 and 14).

In the frog currents are set up by the ciliary action at an earlier stage, and are maintained to a later stage than in the newt. In the newt the tail soon loses its ciliation, whereas in the frog it remains active almost up to the time of the metamorphosis.

If a ciliated ectoderm really is "evidence of a very archaic organisation," a consideration of the exact distribution of the cilia, and the determination of any special areas or bands of cilia at the several stages of development of the embryo, may be of great morphological interest.

Although I am inclined to think that at no time is every

cell of the ectoderm of the frog's tadpole ciliated, yet for a certain period ciliated cells are so numerous as to render it legitimate to speak of the whole surface as being ciliated, e.g. tadpoles 3 mm. to 10 mm. This, however, is not the case from the beginning.

The first sign of ciliation is along the edges of the neural plate. This is followed by a ciliated patch on the antero-ventral surface, in the centre of which there arises later the stomodæal depression. Shortly afterwards the whole surface of the embryo becomes ciliated, but the above-mentioned areas remain recognisable by reason of the greater intensity of the ciliation upon them.

If for the moment we omit the consideration of the function of these specially ciliated tracts, it is possible to draw a comparison between the condition just described and the distribution of the cilia upon the free-swimming larvæ of certain Echinoderms.

The ciliated edges of the neural plate (which tract is subsequently to be recognised as the cause of the naso-branchial current of water) might be compared with the longitudinal ciliated band, while the antero-ventral patch of the stomodæal region might be compared with the adoral ciliated band of the Auricularia larva of Synapta digitata described by Semon (13). The comparison between the edge of the neural plate of Vertebrates and the longitudinal ciliated band of the Auricularia and Tornaria has been made by Garstang (5), and the fact that the edges of the neural folds are ciliated in at any rate one Craniate certainly supports his suggestion.

Garstang concluded that the longitudinal band of cilia were "practically homologous with the medullary folds of the Vertebrata." I am not sure what he means exactly by the "medullary folds." In Rana temporaria, up to the time of the closure of the neural folds, it is only the edges—that is to say, that part of the neural plate which does not actually form part of the tubular nervous system—that are ciliated. The actual neural plate is not ciliated until later.

If, therefore, the ciliated ridges of the frog embryo may be

regarded as homologous with the longitudinal ciliated band of Auricularia, it is the ectodermal space between the bands of the latter which may be regarded as the homologue of the neural plate of Vertebrata, and the longitudinal ciliated bands would perhaps be more exactly represented in the craniate embryo by the system of branchial sense-organs. The possibility of a connection between the Chordate and Echinoderm phyla has been very often suggested; and in view of the remarkable agreement of the actual ontogenetic fate of the blastopore in both these groups the idea of a possible relationship between the phyla would be much more favorably considered were it not for the very general belief in the concrescence theory of vertebrate development. It is only by some form of concrescence in development that it is in any way possible to bring embryological evidence in favour of the theory of vertebrate descent from a gastrula with an elongated mouth, one end of which gave rise to the present vertebrate mouth, the other the vertebrate anus.

It should of course be remembered that cilia are of very general occurrence in the animal kingdom, that they are found in the simplest as well as the most highly differentiated types, and that they appear at the later as well as the earliest stages of development.

These facts lead one to suppose that cilia may be with great ease acquired by an organism. The ciliation of the Amphibian may be only an ontogenetic adaptation. If the ciliation of the tadpole is a purely cœnogenetic feature, what purpose does it subserve?

There can be very little doubt that, on the whole, it is respiratory. As regards the three special systems described in this paper, one, that connected with the cement-gland, has a very obvious use, and may very probably be a recent modification of a general ciliation of the ectoderm.

The second, that connected with nasal epithelium and gill-filaments, has also a very obvious use in producing a very rapid flow over the gill-filaments, and very possibly its connection with the olfactory epithelium may be advantageous.

This system might very well also be a comparatively recent adaptation; but, on the other hand, its very early development in the embryo, and its appearance in connection with the edges of the neural epithelium before there is any sign of gill-filaments, suggest a much more archaic origin.

The third system, that which produces a flowing into the stomodæal pit, has certainly no obvious use to the embryo, although, in a small free-swimming form with open mouth and complete alimentary canal, such a current of water would have had an important function.

Although I do not lose sight of the possibility of the whole ciliation of the amphibian tadpole being cœnogenetic, yet the occurrence of ciliated tracts, which may be compared in position and relation to such important morphological features as the blastopore and mouth, with certain ciliated tracts of Tornaria and Echinoderm larvæ is, at any rate, worthy of notice. A ciliated ectoderm has never been described, as far as I know, for any Elasmobranch. I have examined living specimens of one member of this group, Scyllium canicula, at various ages during the first four months of development, and have never found any trace of ciliation. Although presumably a more ancient type than the Amphibians, the great difference in the condition of the egg and surrounding fluids may be sufficient to account for the disappearance of a primitive ciliation; for of what use could a ciliation be immersed in the very viscid, almost jelly-like albuminous fluid surrounding the ovum in an Elasmobranch egg-capsule?

It is interesting to note that in default of a ciliation to produce a constant flow of water over the gill-filaments and skin, the Elasmobranch embryo maintains an incessant undulating movement of its body from the time it is sufficiently folded off from the yolk until the time of hatching.

Similar conditions at first present the same objection to the occurrence of a ciliated ectoderm in the Amniota, in whose case a special organ of respiration is subsequently developed.

In none of these cases is it surprising to find a ciliation absent. In the case, however, of embryos developing from holoblastic

eggs under conditions similar to those of amphibian development, one would certainly expect to find a ciliated ectoderm. I do not know whether this is the case in Petromyzon, in Ganoids, or Ceratodus.

In connection with Sedgwick's theory of the respiratory nature of the neural groove, it is interesting to find that the neural groove is not ciliated as long as it remains open to the exterior. As stated above, I have not determined at what time it becomes ciliated. Certainly I have never succeeded in seeing cilia in sections of the neural tube at any stage of tadpole life.

CAMBRIDGE;
June, 1895.

List of Literature referred to.

1. BALFOUR, F. M.—'A Treatise on Comparative Embryology,' second edition, 1885.
2. CARRIERE, J.—"Die postembryonale Entwickelung der Epidermis des Siredon pisciformis," 'Arch. f. mikros. Anat.,' vol. xxiv.
3. CLARKE, S. F.—"Development of Amblystoma punctatum," 'Studies from the Biological Laboratory of the Johns Hopkins University,' vol. ii, 1880.
4. EYCLESHYMER, A. C.—"The Early Development of Amblystoma, with Observations on some other Vertebrates," 'Journal of Morphology,' vol. x.
5. GARSTANG, W.—"Preliminary Note on a New Theory of the Phylogeny of the Chordata," 'Zoologischer Anzeiger,' vol. xvii.
6. KLEIN, E.—"Histological Notes," 'Quart. Journ. Micr. Sci.,' vol. xx.
7. MARSHALL, A. M., and BLES, E. J.—"The Development of the Blood-vessels in the Frog," 'Studies from the Biological Laboratories of the Owens College,' vol. ii.
8. MORGAN, T. H.—"On the Amphibian Blastopore," 'Studies from the Biological Laboratory of the Johns Hopkins University,' vol. iv.
9. MORGAN, T. H.—"The Growth and Metamorphosis of Tornaria," 'Journal of Morphology,' vol. v.
10. OSBORN, H. F.—Preface to 'Amphioxus and the Ancestry of the Vertebrates,' by Willey.

11. Pfitzner, W.—"Die Epidermis der Amphibien," 'Morphologisches Jahrbuch,' vol. vi.

12. Preyer, W.—"Verlangerung der Embryonalzeit bei Wirbelthieren," 'Jen. Zeit. f. Naturwiss.,' 1881.

13. Semon, R.—"Die Entwickelung der Synapta digitata," 'Jen. Zeit. f. Naturwiss.,' vol. xxii.

14. Sedgwick, A.—"The Original Function of the Canal of the Central Nervous System of Vertebrata," 'Studies from the Morphological Laboratory in the University of Cambridge,' vol. ii.

15. Stohr, Ph.—"Ueber die Haftorgane der Anurenlarven," 'Sitzungsber. phys.-med. Ges. Wurzburg,' 1881, No. 8, p. 118.

16. Wightman, A. C.—"On the Ventricular Epithelium of the Frog's Brain," 'Studies from the Biological Laboratories of the Johns Hopkins University,' vol. iv.

17. Willey, A.—'Amphioxus and the Ancestry of the Vertebrates,' 1894.

EXPLANATION OF PLATE 35,

Illustrating Mr. Richard Assheton's paper, "Notes on the Ciliation of the Ectoderm of the Amphibian Embryo."

List of Reference Letters.

A. Anterior end of embryo. *AN.* Anus. *BP.* Blastopore. *C.* Ciliated cell. *C.C.* Stream of water in connection with the mucous or cement gland. *C.GL.* Mucous or cement-gland cells. *C.GL'.* The necks of the mucous or cement cells. *DG.* Dorsal groove formed by the junction of the edges of the neural plate. *EP.E.* Epidermic layer of epiblast. *EP.N.* Nervous layer of epiblast. *N.* Nasal depression. *N.B.C.* Naso-branchial stream. *N.G.* Neural groove. *M.* Mucous or cement gland (sucker). *P.* Posterior end of embryo. *P.S.* Primitive streak. *R.G.* Rudiment of Gasserian and other ganglia of the cranial nerves. *RM.* Ridges, richly ciliated, bounding the cement-glands. *S.* Stomodæum. *SC.* Stomodæal stream.

Fig. 1.—Surface view of the embryo of Rana temporaria. The neural plate is folding upwards. The arrows indicate the only area upon which cilia are at this time developed, and the direction of the flow of water produced by their action.

Fig. 2.—A few hours later than Fig. 1. The cross indicates the furthest point backwards upon which cilia may be present.

Fig. 3.—A slightly older stage. In this a second area of ciliation has appeared, as indicated by the arrows, along the antero-ventral surface.

Fig. 4.—Embryo of frog, showing current along the dorsal groove formed by the junction of the neural folds.

Fig. 5.—Embryo of frog, about 3 mm. long. The whole surface is now ciliated, although ventrally the ciliation is very scanty. Three well-marked streams are indicated by the arrows *N.BC.*, *SC.*, and *CC.*, respectively.

Fig. 6.—Diagram showing the currents of water produced by the ciliation about the region of the blastopore and anus.

Fig. 7.—Diagram showing the currents of water produced by the ciliation at the posterior end of the embryo, after the closure of the blastopore and growth of the tail.

Fig. 8.—A semi-diagrammatic figure of a transverse section across the so-called sucker of the frog embryo of about 6½—7 mm. The epidermic layer of epiblast, *EP.E.*, is a layer of one cell in thickness, which cells at two points, *C.GL.*, become enormously lengthened, and secrete a very sticky kind of mucus. The neighbouring cells bear cilia. Those upon the walls of the ridges nearest the cement or mucus-secreting glands bear very short cilia; those more remote, and especially those between the two bases, bear very long cilia. Each long mucous gland-cell is broad at its base, contains a large nucleus surrounded by "granular" protoplasm at that part, and narrows into a long neck as it reaches the surface, filled with the sticky secretion, which stains slightly with most stains, but with plain hæmatoxylin it stains deeply.

Fig. 9.—A semi-diagrammatic figure of the "sucker" at a rather later stage, namely, a tadpole 8—9 mm. in length. In this the ridges bounding the glands are much more prominent. In reality the gland-cells lie diagonally from before backwards, so that their necks and openings are much more posteriorly situated than their protoplasmic bases.

Fig. 10.—Ventral view of a 3¼-mm. tadpole, showing the currents of water produced by the ciliation of the ectoderm.

Fig. 11.—Ventral view of a 6-mm. tadpole. The arrows indicate the general flow of water and the special streams on the ventral surface.

Fig. 12.—Lateral view of the same embryo as preceding.

Fig. 13.—Ventral view of anterior end of an 8-mm. tadpole. The arrows indicate the main currents produced by the special ciliation connected with the stomodæum, the mucous glands and olfactory epithelium, and branchial filaments.

Fig. 14.—Side view of the same embryo as preceding.

Fig. 15.—Section of the epidermis of a 6-mm. tadpole, taken horizontally between the nasal depression and the developing gill-filaments.

Fig. 16.—A piece of a gill-filament of an external gill of an 8-mm. tadpole. The ciliated cells are darker, and project slightly above the remainder.

Fig. 17.—Diagram of the tail of a 12-mm. tadpole, to show the general arrangement of the long ciliated cells drawn in Fig. 18. The zigzag lines terminating in arrows show the course taken by particles as they are dashed from cell to cell across the tail fins.

Fig. 18.—This shows three of the ciliated cells (*C.*), which are oblong and darker in colour than the surrounding polygonal non-ciliated cells.

Fig. 19.—Side view of the embryo of Triton cristatus, showing the direction taken by currents of water due to the ciliation of the anterior end of the embryo. The two stars indicate a spot where the motion is very rapid—the stomodæum.

Fig. 20.—The same embryo of Triton cristatus. A ventral view.

Fig. 21.—An embryo 6 mm. in length of Triton cristatus, seen from the side. The arrows indicate the flow of water produced by the ciliation.

Fig. 22.—A ventral view of the anterior end of the same embryo.

Ontogenetic Differentiations of the Ectoderm in Necturus.

Study II.—On the Development of the Peripheral Nervous System.

By

Julia B. Platt.

With Plates 36—38.

Introduction.

I undertook the study of the development of the lateral line system in Necturus at the suggestion of Professor C. O. Whitman, and am indebted to Professor K. Brooks for access to the literature of my subject. As the questions of chief interest to me are connected with the relation of this system to vertebrate segmentation, and to the formation and distribution of the cranial nerves, I turned to the earlier stages of embryonic development to discover there, if possible, the first indications of ectodermic differentiation which might serve as the source either of cranial ganglia or of sensory specialisations in the skin. This led to the discovery that large numbers of cells, arising in the ectoderm and migrating below the surface of the body, take part neither in the formation of ganglia nor nerves. They are, however, distinguished in Necturus from the surrounding tissues by marked difference in the size of the yolk granules they contain.

I grouped together the ganglionic and connective-tissue cells which thus migrate inwards from the ectoderm under a common term, "mesectoderm," and in a former paper (32)

traced the lines of their origin and the path of their migration to the time when the nervous contingent separates from the connective-tissue residue in which later cranial cartilages form. Here my work naturally diverged in two directions, one leading to the further development of the peripheral nervous system, the other to the formation of the cranial cartilages, and I consequently closed my first study at this point, preferring to consider separately the two topics it served to introduce.

Since the lateral line system dates its origin to an earlier period of embryonic development than that with which this paper properly opens, I shall review briefly the observations recorded in my former paper in regard to those ectodermic thickenings, or ridges, which are its precursors, but in so doing shall take little notice of the connective-tissue cells that constitute part of the mesectoderm. They are the subject-matter of a following study.

1. In Review.

As the neural folds develop in Necturus, the ectoderm becomes deeper in the line that marks the uplifting of the folds from the surface of the egg, thus forming a rather wide band of deep ectoderm, which begins to be differentiated at the anterior end of the embryo and gradually extends backwards. When the neural folds close the band on each side of the trunk of the embryo is replaced by three narrow longitudinal ridges, of which the median is the deepest. These three ridges extend backward from the line of the third intersegment posterior to the ear. Anterior to this line the ectoderm at the side of the head continues deep, but becomes marked by two longitudinal ridges, the dorsal of which passes through the auditory epithelium, and is the source of the dorso-lateral proliferation of mesectoderm (v. Kupffer's lateral ganglia). This ridge continues the line of the dorsal ridge on the trunk.

The lower of the two primitive ridges on the head is the source of the epibranchial proliferation of mesectoderm. It is the continuation of the median of the three trunk ridges,

namely, of that ridge which marks the separation of the protovertebra from the nephrotome. The most ventral of the ridges on the trunk marks the line at which the nephrotome separates from the remaining parietal plate. This line is continued later on the head by a ridge which, beginning at the oral invagination, extends backwards below the gill clefts as these successively form, and finally unites with the ventral ridge of the trunk.

As the protovertebræ are cut off from one another, the median longitudinal ridge becomes differentiated by a series of intersegmental elevations which correspond to intersegmental expansions of the alimentary canal. This appears to favour Boveri's (5) theory that gill clefts once extended throughout the length of the body, for in the branchial region these intersegmental elevations on the median longitudinal ridge mark the beginning and the dorsal limit of the gill clefts, while on the trunk of the embryo they extend towards, although they do not touch, the corresponding intersegmental expansions of the alimentary canal.

The longitudinal ridges soon become connected with one another by transverse intersegmental ridges. On the head of the embryo these transverse ridges are formed from the separate elements of the line of dorso-lateral proliferation of mesectoderm, which severally unite with a corresponding epibranchial thickening of the ectoderm, which, in turn, blends with the upper limit of that transverse ridge of ectoderm which meets the endoderm prior to the formation of the gill clefts.

If the skin of an embryo at this stage be removed and viewed by transmitted light, it is seen to be divided into a series of small squares, each of which is bounded by the longitudinal and transverse ridges I have just described, and each of which corresponds to the outer surface of a protovertebra. This method of determining the position of the ectodermic ridges cannot be applied in the branchial region or in the anterior part of the head, since the rapid proliferation of mesectoderm, which is here taking place, renders it for the time impossible to separate the ectoderm accurately from the tissues beneath.

Beginning at the anterior extremity of the dorsal longitudinal ridge, we find that it gives rise to cells which join the trigeminal portion of the neural crest, and take part in the formation of the Gasserian ganglion and its anterior continuation—the ramus ophthalmicus profundus. This nerve is formed from the ectoderm in the same manner as are the cranial ganglia.

The migration of cells into the trigeminal mesectoderm extends from the anterior limit of the dorsal ridge to the intersegment between the mandibular head cavity and the anterior of the two somites which in Necturus as in Scyllium (van Wijhe, 37) lie above the hyomandibular cleft. In this intersegment the proliferation of mesectoderm extends downwards to the epibranchial ridge, which here passes over the hyomandibular cleft, and curves towards the oral invagination in conformity to the cranial flexure.

In the continuous band of deep ectoderm above the eye, I have been unable to distinguish any limited area, which in giving rise to ganglion cells on the profundus would be the homologue of what is described by Beard (3) as the "sense-organ" of the ciliary ganglion in the Elasmobranchs.

This area, which is distinct in Elasmobranchs, appears in Necturus to have fused with the dorso-lateral thickening of the ectoderm connected with the Gasserian ganglion, so that we here have but one band of deep ectoderm, which proliferates cells continuously to the Gasserian ganglion and to the profundus. I do not find that the band of deep ectoderm above the eye disappears at any time throughout its entire length, yet it becomes greatly reduced in width by the migration of cells into the trigeminal mesectoderm, and shortly previous to the stage with which this paper begins the band is interrupted by spaces, from which the cells have migrated so rapidly as to leave the ectoderm above scarcely, if any, deeper than on the surrounding surface of the head. This condition is but transitory, and other cells soon occupy the place of those that have gone, restoring the depth and continuity of the ridge.

Anterior to the hyomandibular cleft the lens arises, as described by von Kupffer (23) in Petromyzon as a specialisation

of the epibranchial ridge. It would be possible to associate that proliferation of cells to the ophthalmicus profundus, which constitutes the ciliary ganglion with the lens as dorso-lateral and epibranchial differentiations in the intersegment between the mandibular and præmandibular head-cavities. These cavities are not distinct in Necturus.

My description of the changes that take place in the ectoderm prior to the stage with which this study begins may be more easily followed by referring to the figures given with the first study (32).

Between the two somites above the hyomandibular cleft we find at an early stage two circular areas, in which the ectoderm becomes deep in both the dorso-lateral and epibranchial lines. The two areas unite with one another later in an intersegmental ridge, which meets the intersegmental proliferation of cells to the trigeminal mesectoderm above the hyomandibular cleft. Thus the hyomandibular cleft is the ventral continuation of two intersegmental lines, one connected with the trigeminus, the other with the facialis. The specialised areas connected with the facialis increase greatly in depth and extent, and at the time when the migration of cells to the facial mesectoderm is most rapid the dorsal thickening becomes continuous, on the one hand with the auditory epithelium, and on the other with the trigeminal portion of the dorso-lateral ridge.

The specialised areas of ectoderm in the next intersegment are the ear on the dorso-lateral line, and the epibranchial thickening above the hyobranchial cleft. The two areas become sharply separated from one another only as the ear becomes constricted off as a closed vesicle. From the auditory epithelium cells migrate into the auditory ganglion, and from the epibranchial thickening into both facial and glosso-pharyngeal mesectoderm.

The two following intersegmental ridges are above the first two branchial clefts, and are connected with the migration of cells into the vagus mesectoderm. In these intersegments it is not easy to distinguish the dorso-lateral from the epibranchial thickening of the ectoderm, so deep is the entire ectoderm in

this region, in preparation for the formation of the large vagus ganglia.

I may mention here that while the hyobranchial and the first two vagus clefts arise in the line of a corresponding intersegment, after the second vagus cleft has appeared the entire branchial apparatus begins to change its position in relation to surrounding structures, and the second vagus cleft in consequence pushes forwards, so that when the third cleft appears it lies in the intersegment that was originally occupied by the second cleft. Hence the primitive somatic and branchial segmentation do not entirely correspond.

The migration of cells from the ridges in the ectoderm, which I have described, is not by the wandering of cell after cell into the tissue below, but by the peeling or splitting off of the cells en masse, leaving the ectoderm above for the time no deeper than on the surrounding surface of the head. A new limiting membrane forms; and where we once found an area of deep ectoderm we now find a mass of mesectoderm cells, covered by ectoderm of no unusual depth.

The primitive longitudinal and the transverse ridges of the trunk I described in my first study (32) as transitory differentiations of the ectoderm, which disappear and leave no clue to the cause of their existence unless interpreted in the light of later events, when it is found that the three lateral lines of sense-organs occupy the position once held by the primitive longitudinal ridges.

The result of further study in regard to the primitive ridges of the trunk is recorded in this paper; the supposition, however, that the ridges indicate sensory differentiations in the ectoderm, which are subsequently to be developed in the lateral line system, is not vitiated by their temporary disappearance, for ectodermic thickenings and ridges on the head of which many of the cells are undoubtedly sensory, inasmuch as they develop into ganglia, disappear completely for a time in giving rise to cells of the mesectoderm, then reappear as the Anlagen of lateral line organs, while other similar lines on the head give rise in part of their length to sense-organs, and in part

disappear, leaving neither sense-organ nor ganglion to testify to their previous existence.

The plan of the lateral line system in Necturus, as thus early laid down, is that of three longitudinal lines on each side of the embryo, connected by intersegmental cross-lines, with special differentiations at points of intersection. To show what portions of these lines are retained in the final system, what their modifications are, and what their relations to the sensory nerves, is the purpose of this study.

2. The Embryo of Pl. 38, fig. 1.

In Pl. 38, fig. 1, I have represented an embryo in which the final lateral line system has begun to develop. The ridges or thickenings in the skin are left white in the drawing, as if raised above the surface of the embryo. Some of the ridges are actually visible on the surface, but their appearance is exaggerated in the drawing, which has further supplemented the visible ridges by others that become evident only in section.

The position of ear and eye is outlined, and the contour of the cranial ganglia is indicated by a flat shade. The posterior part of the Gasserian ganglion, which is in no way connected with the lateral line system, has been omitted to avoid confusion with the facial ganglion. The fine lines in which the ganglia terminate indicate the root of the ganglion or the small sensory nerves that have already separated from the skin.

In the primitive dorso-lateral line we find above the eye the ridge from which the supra-orbital sense-organs are about to be formed. Just below lies the Gasserian ganglion and the ramus ophthalmicus profundus, both in part composed of cells that have but now migrated from the primitive supra-orbital ridge which occupied the position of the present ridge. As in an earlier stage, this ridge still forms an unsegmented line extending backwards to the anterior of the two intersegments that meet in the hyomandibular cleft. The primitive thickening in the dorso-lateral line which belonged to the posterior

of these two intersegments has disappeared entirely in giving rise to part of the facial mesectoderm.

The ear has developed from the dorso-lateral thickening in the hyobranchial intersegment, and it will be noticed that although the ear undoubtedly belongs in the lateral line system, and is in fact the centre of that system, it is not properly a "branchial sense-organ," as Beard suggests (2), for this term cannot be accurately applied to sense-organs above the epibranchial line.

The dorso-lateral thickening of the following intersegment has for the time disappeared in giving rise to cells that now form part of the vagus mesectoderm. In the second intersegment posterior to the ear a knob-like structure at the anterior end of a longitudinal club-shaped ridge is the beginning of the most dorsal of the lateral lines of the trunk. Posterior to this intersegment the primitive dorso-lateral ridge has disappeared, and anterior to the intersegment, as has just been pointed out, two of the primitive intersegmental differentiations in the dorso-lateral line are missing, namely, those connected originally with the facial and glossopharyngeal ganglia.

It is evident that in following the later distribution of sense-organs one must look to the most dorsal of the lateral lines of the trunk for representation or omission of sensory differentiations in intersegmental lines posterior to the first intersegment back of the ear.

At its anterior extremity the ridge of the epibranchial line, about to give rise to the infra-orbital sense-organs, blends with the wide and deep nasal epithelium which has now begun to invaginate. Following the line upward, one finds that at the first of the intersegments meeting in the hyomandibular cleft the ridge fuses with that of the dorso-lateral or supra-orbital line, while above the hyomandibular cleft a round area of sensory epithelium has been cut off from the infra-orbital ridge as the primitive epibranchial sense-organ of the hyomandibular cleft.

The epibranchial thickening in the posterior of the two

intersegments above this cleft has disappeared, like the dorso-lateral, in giving rise to facial mesectoderm.

A distinct sensory area is found above the hyobranchial cleft, but posterior to this intersegment the epibranchial ridge is only interrupted where the region from which the sense-organs above the gill clefts are to be formed is separated from the club-shaped ridge that begins the main lateral line of the trunk. It will be noticed in fig. 1 that the posterior extension of the vagus ganglion lies between these two divisions of the epibranchial line. At an earlier stage this extension of the ganglion formed with the lateral line ridge and the ridge above the gill arches one deep ectodermic thickening. The two ridges here separated are consequently morphologically parts of the same ridge out of which a section has been cut to form the ganglion.

Pl. 36, fig. 4, represents a cross-section though the region just described in a younger embryo than that of fig. 1. The plane of the section is indicated in fig. 1 by a corresponding number. In fig. 4 the large vagus ganglion is being cut from the primitive epibranchial ridge (*p. ep. r.*), leaving a few deep cells above, which later increase in number and rearrange themselves to form the beginning of the lateral line of the trunk. Below the ganglion, cells are migrating from the ectoderm into the ectodermic connective tissue, but, although the whole ectoderm is here deep, that special elevation which constitutes the beginning of the sense-organs above the branchial arches has not yet appeared, the original ridge being temporarily obliterated in this region by the formation of mesectoderm.

The most ventral of the three primitive longitudinal ridges (fig. 1) begins near the oral invagination, and extends backwards touching the ventral margin of the successive gill clefts. The ridge ends at the transverse ridge in the posterior margin of the last gill cleft. It therefore extends as far as the second intersegment posterior to the ear. I have previously called attention to the fact that the branchial clefts do not correspond to the intersegments, inasmuch as the second and third vagus

clefts arise successively in the second intersegment. The posterior continuation of the ventral ridge on the trunk of the embryo has disappeared with the other longitudinal ridges of the trunk.

Of the transverse ectodermic ridges at the gill clefts but two remain, that at the posterior margin of the last cleft and the hyomandibular ridge. The other ridges were lost as the clefts formed, but although the ectodermic ridge at the hyomandibular cleft touched the wall of a corresponding pocket from the alimentary canal no cleft formed, and the endoderm afterwards receded, leaving the ridge of ectoderm in the position it originally occupied. This ridge of deep ectoderm, once the rudiment of the hyomandibular cleft, is now the Anlage of the hyomandibular line of sense-organs. I prefer with Ewart (10) the designation "hyomandibular" for this line of sense-organs, rather than "operculo-mandibular," which Allis employed to designate an homologous branch of the lateral line in Amia (1).

In fig. 1 a short ridge is seen to extend forward from the middle of the hyomandibular ridge. This is the beginning of the mandibular line of sense-organs, which thus branches off from the hyomandibular line and grows towards a point near the mid-ventral axis of the body, posterior to the oral invagination, where it fuses with the ventral longitudinal ridge. It is worthy of note that while two primitive intersegmental ridges meet above the hyomandibular cleft, below, two sensory lines form from that cleft.

Pl. 36, fig. 2, *a*, is a section through the supra-orbital ridge of an embryo at the stage of development represented in fig. 1, where the plane of the section is indicated. The cells of the supra-orbital ridge are seen to have the radial arrangement peculiar to the sense-organs of the lateral line. The section, however, does not cut a sense-organ. The cells of the sensory ridge arrange themselves radially about the long axis of the ridge before dividing into groups arranged about a point above the centre of a sense-organ. In cross-section, therefore, the sensory ridge presents the same appearance as the median plane of a sense-organ.

Fig. 2, *b*, is a cross-section through the same region as fig. 2, *a*, but in a younger embryo, in which as yet but one vagus cleft is found. The section shows the primitive ectodermic ridge connected with the trigeminal ganglion and ramus ophthalmicus profundus. Comparing the two sections *a* and *b*, one sees that in *b* the ectodermic ridge has been produced by increase in the cell layers of which the ectoderm is composed, with no tendency to radial arrangement; while in section *a* the ridge is produced by the radial arrangement and depth of a group of cells in the lower of the two layers here composing the ectoderm.

Pl. 36, figs. 3 and 4, are both from an embryo with two vagus clefts, and pass through planes given in fig. 1. But few cell outlines are drawn, since they are rendered indistinct by yolk granules, which I have omitted as unimportant in the present study. The plane of fig. 3 lies anterior to the hyomandibular cleft, and at the beginning of the facial ganglion, a few cells of which touch the ectoderm dorsally in the section, while below a few connective-tissue cells of ectodermic origin separate the ectodermic ridge from the wall of the alimentary canal. Only those mesectoderm cells which come in contact with the ridge are reproduced. The section shows a primitive ridge about to add its cells to the facial mesectoderm. In the dorsal part of the ridge may be seen a slightly radial arrangement of the cells. This appearance might indicate either the formation of a secondary sensory ridge from the primitive ectodermic thickening, or that a round mass of ganglion cells is about to be cut from the skin. Which of the two processes is actually taking place is to be discovered by comparing the next stage of development with the present. Such a comparison shows that the section lies posterior to the branchial sense-organ above the hyomandibular cleft, and illustrates the initial stage in the excision of the epibranchial part of the facial ganglion. Fig. 4, which cuts the primitive ridge that has just given rise to part of the vagus ganglion, shows a similar excision at the point of completion.

The three sections (figs. 2, 3, 4) give the relative depth of

the primitive ridges connected with the trigeminal, facial, and vagus Anlagen. These ridges are formed not alone by increase in the number of cell layers composing the ectoderm, but also by increase in the depth of the cells themselves. The latter fact would be more evident in the figures had a longer strip of ectoderm at either side of the ridge been included in the drawing. The change in the depth of the cells is a gradual one.

The long axis of a nucleus usually corresponds to that of the cell, and the long axis of an ectoderm cell lies usually either in the direction in which the cell is migrating if it be migratory, or in the direction of the transmission of energy if it be nervous. As both the path of migration and the line of transmission of energy in the posterior part of the vagus ganglion, through which fig. 4 cuts, are parallel to the long axis of the embryo, the cells composing the ganglion changed the direction of their axes on leaving the ectoderm, and the section which passes through the long axis of the cells in the deeper layers of the ectodermic ridge cuts across that of the ganglion cells.

In Pl. 38, fig. 1, a commissural connection between the glossopharyngeal and vagus ganglia is seen a short distance below their respective roots. This commissure is formed from cells of the neural crest, which at first extended as a continuous sheet from the beginning of the glossopharyngeus to the posterior limit of the vagus Anlage. The cells of the crest in migrating down the side of the brain divided into two groups, in each of which the long axis of the cells corresponds to the direction of the migration, and is consequently vertical. The two groups remain connected with one another, however, by a short commissure, which, serving possibly to transmit impressions from one ganglion to the other, is composed of cells with axes parallel to the long axis of the embryo, or at right angles to the axes of the cells composing the main vagus and glossopharyngeal ganglia.

The fusion of the vagus ganglion with the ectoderm begins immediately posterior to the commissure, and a mass of ecto-

derm cells is here cut off to form the ganglion of the lateral line. As I have mentioned above, the long axis of the cells composing this ganglion is at right angles to that of the cells in the neural part of the vagus ganglion, but in the same plane as that of the cells in the commissure. The consequence is that when the cells of the lateral line form nerve-fibres, the greater part of the fibres, following the path of least resistance, pass over the commissure and enter the brain with the root of the glossopharyngeal ganglion. Cells which lie on the inner side of the lateral line ganglion and in immediate contact with the primitive vagus ganglion send fibres to the brain by the vagus root, but the ninth nerve is the chief root of the lateral line ganglion.

Pl. 36, fig. 5, is from a horizontal section through the dorsal and median lateral lines of the trunk at the time of their beginning. The plane of the section is shown in fig. 1. One who has seen the growing lateral line plough its way through the skin, leaving a row of sense-organs in the wake, would perhaps imagine the structure at the right of the section to be a large sense-organ which the advancing line, cut longitudinally at the left of the section, had formed in its path. Such, however, is not the case. The section drawn is from the series reconstructed in Pl. 38, fig. 1, and the position of the lateral ridge in relation to the somites has been carefully determined. There is no sense-organ at a later stage in the place now occupied by the group of cells in question. The first sense-organ of the dorsal lateral line does not lie anterior to the third intersegment back of the ear. The group of cells now found at the second intersegment consequently either push their way farther, or leave the skin in giving rise to ganglion and nerve. Moreover the fate of that part of the main line which has as yet developed is not different, and the cells of this deep ridge also either advance in the skin, or give rise to ganglion or nerve, for the first sense-organ of the main lateral line does not fall anterior to the fourth intersegment. The entire ectodermic thickening represented in fig. 5 ultimately dis-

appears, leaving the skin where it now is apparently unmodified.

There is in Necturus a qualitative as well as structural difference between the primitive and secondary ectodermic ridges. The primitive ridges, formed by the multiplication of layers in the ectoderm without radial rearrangement of the cells, may disappear, leaving no apparent modification in the structure of the embryo; or they may be directly modified into secondary ridges, as is the case with the primitive ridge of the hyomandibular cleft; or, lastly, they may be the source of large additions to the mesectoderm, as in the dorso-lateral and epibranchial lines on the head. The mesectoderm thus proliferated may be either exclusively ganglionic, as when the lateral line ganglion is cut from a primitive epibranchial ridge; or may be composed of elements partly ganglionic and partly connective tissue, as in the primitive dorso-lateral proliferation connected with the trigeminus; or, finally, the proliferation may be exclusively of connective-tissue cells, as at the margin of the gill clefts below the epibranchial line.

Where the proliferation of mesectoderm is composed of both nervous and connective-tissue elements, it often appears impossible to decide whether cells not closely grouped in the body of a ganglion are to be classed with the nervous system, or are to form the basis of a branchial cartilage, until the prolongations of some of the scattered and apparently homogeneous mesectoderm cells show the fine fibrillar striation peculiarly nervous.

The secondary ridges, however, composed of cells radially arranged, are the source of nervous structures alone, and their radial arrangement may be co-ordinated with the fibrillar differentiation of nerve-cells forming part of the mesectoderm, as indicating an exclusively nervous character.

In fig. 1 it will be seen that the chief nerves of the lateral line system are beginning to develop. From the vagus-glossopharyngeal group, fibres in a dorsal bundle lose themselves in the main lateral line of the trunk at the point where fibres of the dorsal lateral line, following the growth of the knob of cells at the head of the main line, will soon appear. Another bundle

of fibres belonging to this group enters the intersegmental ridge on the posterior margin of the third vagus cleft. This is the beginning of the nerve of the ventral lateral line of the trunk.

There is a small nerve connecting the glossopharyngeal ganglion with the branchial sense-organ below the ear, which is not represented in the drawing.

Fibres of the ramus ophthalmicus superficialis facialis have just left the skin at the posterior limit of the supra-orbital ridge, while the ramus buccalis begins to be formed at the dorsal extremity of the infra-orbital ridge. A small branch not represented in the drawing connects the primitive sense-organ above the hyomandibular cleft with the facial ganglion, while a prolongation of the facial ganglion fuses with the dorsal margin of the hyomandibular ridge, although I find as yet no nerve-fibres here.

3. Comparative and Critical.

v. Wijhe (37) first called attention to the fact that the cells of the neural crest in the Selachii fuse with the ectoderm in two planes. Misses Johnson and Sheldon (21), in their "Notes on the Development of the Newt," extend this observation to the Amphibia. The first fusion occurs, they tell us, "above the level of the notochord;" and in the cases of facial and glossopharyngeal ganglia a second fusion takes place "in the dorsal wall of the corresponding gill cleft." Like v. Wijhe, they find the dorsal fusion connected with the development of the lateral line.

Froriep (13) believes the ventral fusion found in the Selachii to be the homologue of a similar union of ectoderm and ganglion which he discovered in the Mammalia, and first associated with the gill clefts as "Kiemenpaltenorgan."

v. Kupffer (22, 23) shows that the neural Anlagen in Petromyzon also fuse with the skin in both dorsal (lateral) and ventral (epibranchial) lines, receiving at each place of fusion large ganglionic additions from the ectoderm. These fusions between ectoderm and ganglion, which v. Kupffer finds

in Petromyzon, I believe to be homologous with those I have described in Necturus.

The more dorsal of the two fusions, however, which v. Wijhe and Misses Johnson and Sheldon mention, are not homologous with those found on the dorso-lateral line in Necturus; for, as fig. 1 shows, the primitive sense-organs connected with the glossopharyngeal and vagus ganglia lie in the epibranchial line, while in those segments immediately posterior to the ear the primitive dorso-lateral ridge is entirely reduced by the formation of mesectoderm, i. e. the vagus and glosso-pharyngeal ganglia with associated connective tissue, and can consequently not be directly associated with the formation of the lateral line. The same conclusions hold in regard to the facial segments.

The section given in Pl. 36, fig. 5, chances to pass through several cells (*a*, *a'*, *b*, *c*) in the act of dividing; and as these cells surely take part in the formation of the nervous system, it is worthy of note that their planes of division lie in each of the three dimensions of space. We consequently have here not merely presumptive but positive evidence against Mall's (27) statement that "the primitive growing point of all vertebrate nerves is in the layer of cells on the outermost side of the ectoderm, and the axis of division is parallel with the ectoderm." Neither is the primitive growing point of the lateral line nerve in the layer of cells on the outermost side of the ectoderm, nor is the axis of division always parallel to the surface of the ectoderm. Yet Mall studied Necturus!

The series of sections (Pl. 36, figs. 2—5) serve also to demonstrate the inaccuracy of Beard's (4) statement that the ganglion splits off from the deeper layers of the ectoderm, leaving an external sense-organ. This does not happen in Necturus. The large dorso-lateral and epibranchial ganglia are formed from cells which split off en masse, leaving the ectoderm external to them for the time thin. A sensory ridge may appear later in the exact place where the ganglion arose, as happens in the supra-orbital line, or sense-organs may form at either side of the ganglion Anlage, as in the vagus region,

or again, as on the path of the lateral line of the trunk, cells may migrate individually from the sensory ridge into the ganglion; but just that relation of ganglion and sense-organ which Beard describes I have never found in Necturus.

In designating the several divisions of the lateral line system I shall adopt the plan of Ewart (**10**) rather than that of Allis (**1**), as I also think it wise to associate each division of the system with the particular nerve it supplies. The infra-orbital line is thus limited to that part of the system which gives rise to the ramus buccalis. For the organs that develop from the sensory epithelium above the hyomandibular cleft Ewart suggests the appellation "otic," and "glossopharyngeal" or "temporal" for those developing from the primitive sensory epithelium in connection with the glossopharyngeal nerve.

The line of organs formed from the longitudinal ridge external to the vagus ganglion I shall call the epibranchial line, as they give rise to an independent nerve in Necturus, and do not form part of one of the sensory lines of the trunk.

I must take exception, however, to Ewart's statement that, from the contact of the buccal and superficial ophthalmic ganglia at their proximal ends, "it might be inferred that there has been a splitting of the original epidermic thickening above the spiracular cleft, the splitting resulting not only in the formation of two ganglia, but also of two sensory canals—the supra-orbital above and the infra-orbital below the eyeball." The development of these lines in Necturus does not support this view. The supra-orbital line of sense-organs traces its origin to the anterior part of the primitive dorso-lateral ridge, which developed in approximately its present length when the cells of the neural crest were still connected with the mid-dorsal wall of the brain, at a period consequently long preceding that in which the proper lateral line system begins. The cells of the ramus ophthalmicus profundus trigemini are connected with the anterior part of the primitive ridge at the time when the posterior part of the ridge begins to assume a radial structure, and to give rise to fibres of the ramus ophthalmicus superficialis facialis.

The original epibranchial ridge to which the infra-orbital line dates its origin is hardly less primitive. In fact, the otic part of the system which Ewart views as the primitive, undivided part of the severed infra- and supra-orbital lines, is, in Necturus, itself cut off from the infra-orbital ridge very shortly before the stage represented in fig. 1.

The description given by Mitrophanow[1] (29) of the development of the supra- and infra-orbital lines in Acanthias accords better with Ewart's prognostication, inasmuch as Mitrophanow finds that these lines grow in directions nearly at right angles to one another from a thickening of the ectoderm above and anterior to the hyomandibular cleft; but Mitrophanow also finds that the thickening connected with the otic nerve takes its rise from the infra-orbital line. It consequently cannot be viewed as representing in Acanthias the undivided rudiment from which the supra- and infra-orbital lines have parted.

Mitrophanow does not mention the early connection of the primitive supra-orbital ridge with the trigeminal ganglion and ramus ophthalmicus profundus, nor does he lay stress on the extensive additions which the peripheral nervous system receives from the skin along the lines of ectodermic thickening. In fact, instead of viewing the skin as the source of the sensory nerves, the author speaks of the branchial sense-organs above the first and second branchial clefts as following in their development the small supra-branchial branches of the glossopharyngeal and vagus nerves; and again, in stating his conclusions (loc. cit., p. 211) Mitrophanow says of the several branchial sense-organs that "leur formation est simultanée avec le développement des petites branches nerveuses supra-branchiales." Evidently the author does not recognise the precedence of the sensory ridge.

Since Mitrophanow claims as the result of his study that the segmentation of the lateral line system is entirely secondary, I shall be interested to discover when I again have my Acanthias material with me whether traces of the primitive segmentation

[1] I regret that the Russian publications of this author are inaccessible to me.

so evident in Necturus cannot also there be found, for it is difficult to believe that the great similarity which exists in the position and direction of the main lines of sense-organs in Necturus and Acanthias should not be the result of a similar course of development.

Noticing that in Amia the lateral line nerve innervates a continuous canal beginning with the sensory differentiation above the first vagus cleft, Ewart (loc. cit.) infers that the embryonic sense-organ found here gave rise to the pre-commissural, commissural, and trunk portions of the canal, "with or without involving the branchial sense-organs lying above the second, third, and fourth vagus clefts," which probably assisted in forming the several vagus ganglia, but have taken little or no part in forming the lateral line.

In Necturus the sensory ridge above the vagus clefts is not formed, as are the lateral lines of the trunk, by the prolongation of a ridge developing from a given point, but is formed by the direct modification of a band of deep ectoderm that lies below the ganglion cut from the centre of the primitive epibranchial ridge. This sensory line is therefore to be homologised serially with the sensory differentiations above the hyobranchial and hyomandibular clefts, and its anterior extremity is not the beginning of the lateral line of the trunk.

Where, then, are the dorsal nerves which in Amia and Læmargus should innervate the separate branchial organs above each vagus cleft? For, as v. Wijhe (37) tells us, every typical head segment should contain on each side, besides its somite, a dorsal and a ventral nerve-root. Yes, but it is also true that every typical vertebrate segment should include a musculature supplied by a motor nerve and a sensitive outer covering giving rise to a sensory nerve. The two nerves in a typical segment should undoubtedly be connected with that part of the central nervous system which their segment includes.

Consider how far the segments of the vertebrate head have departed from their type.

Beginning with the præmandibular segment, we find ganglion. covered by a sensory epithelium which sends its fibres to auditory-

ramus ophthalmicus profundus to that segment of the brain which supplies motor fibres to the mandibular somite. Part of the sensory skin covering the mandibular somite sends its fibres to the brain by the same root.

At a slightly later stage in the development of the embryo, a band of sensory epithelium on both præmandibular and mandibular segments, which first sent its fibres to the brain in the second (mandibular) segment, now sends them by the ramus ophthalmicus superficialis facialis to that segment of the brain which supplies the motor fibres of the third and fourth somites.

Again, the intersegmental sensory ridge above the hyobranchial cleft gives rise to two sense-organs lying primarily in the same transverse line. One of these organs, the ear, sends its sensory fibres to the brain segment which supplies the motor fibres of the third and fourth somites, while the lower sense-organ in the transverse line sends fibres to the brain in the segment supplying motor fibres to the fifth, glossopharyngeal, somite.

The sensory epithelia on the vagus segments send a large part of their fibres to that segment of the brain which gives rise to the motor root of the glossopharyngeus; part of the fibres, however, are distributed to the brain in the first vagus segment.

Behold the heterogeneous mixture, which omitted facts would still further complicate; and yet we are told that there is a segmental value in the dorsal nerve-root!

Each primitive ganglion may be, indeed, a segmental structure, so also is the motor nerve, but the value of that analytical division of the several cranial nerves which ascribes a separate segmental root to each sensory branch is not apparent.

The "root" of the ophthalmicus profundus is primarily supplied by part of the skin covering two or more segments—the mandibular, præmandibular, and other anterior segments, [illegible] there be. The "root" of the ophthalmicus superficialis is supplied by fibres from the same sensory area

[1] I regret

covering the same segments. The root of the buccalis derives its fibres from a more ventral band of sensory tissue on these very segments. The otic branch of the facialis contains fibres arising originally from the median part of the anterior of the two intersegmental ridges meeting in the hyomandibular cleft. The sensory fibres in the hyomandibular root of the facialis come from the ventral portions of the two intersegmental ridges here united. The following primitive intersegmental line of sensory epithelium supplies from its dorsal region branches to the root of the auditory nerve, from its median region fibres to the so-called "dorsal root" of the glossopharyngeus; and from the incomplete account of the innervation of sense-organs on the gular plate given by Allis (1) in his description of the lateral line in Amia, we must conclude that the ventral portion of the intersegmental ridge at the margin of either the hyobranchial or first branchial cleft is also represented in the "root" of the glossopharyngeal nerve. A band of tissue on the successive vagus segments sends its fibres to the "root" of the lateral line nerve in the glossopharyngeal segment, but also sends fibres to the "roots" of the vagus nerve. I will go no further than to add that, as far as the lateral line organs are concerned, their fibres choose the nearest and most direct path to the auditory centres in the brain, which seem to be also the centres of the entire lateral line system, yet both development and comparative anatomy tend to show that it is a matter of little moment whether these fibres enter the brain by one nerve-root or another.

4. The Development of the Spinal Nerves, and the Relation of the Vagus Ganglion to its Myotome.

In Pl. 38, fig. 1, I have indicated the position of the anterior spinal ganglia. There is in Necturus one ganglion for each segment of the head and trunk, if one regards the auditory-facial group as composed of two primitive ganglia. As I have already mentioned, the præmandibular segment is not distinct from the mandibular which contains the Gasserian ganglion. To the following two segments would belong the auditory-

facial ganglia. The first protovertebra posterior to the ear lay below the Anlage of the glossopharyngeal ganglion, but gives rise to no myotome, being apparently crushed out of existence by the growth of the ear. The second segment posterior to the ear gives rise to the first myotome, and contains the vagus ganglion. The third segment is that of the first spinal ganglion, &c.

The two anterior spinal ganglia possess no dorsal root, but consist each of a small group of cells at the base of the motor nerve. It was of interest to know from what source these ganglia came, and with the solution of this problem in view I turn to earlier stages in the development of the peripheral nervous system of the trunk.

The posterior division of the neural crest begins with the facial Anlage, and from it successively the neural portions of the facial, glossopharyngeal, and vagus mesectoderm are separated. The facial and glossopharyngeal portions of the crest lie above protovertebræ that develop no myotomes, but immediately disappear in giving rise to mesenchyme over which the cells of the neural Anlage migrate beneath the skin to the epibranchial ridge.

The vagus segment, however, develops its proper myotome, and when the cells of the neural crest migrate downwards only the anterior part passes over the dorsal wall of the protovertebra. The posterior part of the Anlage lies, as in the spinal region, between the brain and the protovertebra. When the protovertebra begins to extend dorsally as its muscle-plate forms, the growth of its anterior part is checked by the vagus Anlage, which passes over the protovertebra from the brain to the epibranchial ridge. Therefore only the posterior part of the myotome can grow upwards. This it does, and then extending forwards replaces the missing dorsal part of the anterior half of the myotome. In consequence the anterior part of the vagus ganglion appears at this stage of development to cut half through the myotome.

Pl. 36, figs. 6 and 7, illustrate this relation. Fig. 6 is anterior to fig. 7 by one third of the width of a myotome, and

shows the dorsal and ventral portions of the myotome parted by the vagus ganglion, which originally lay entirely dorsal to the protovertebra. Fig. 7 shows the posterior part of the vagus Anlage, the neural cells of which have migrated from their original position above the dorsal wall of the brain to their present position between the brain and muscle plate, where they finally become attached to the brain by a nerve-root. The difference in relation to the muscle plate between the position occupied by cells of the neural crest in the head, and that occupied by cells of the neural crest in the trunk, has been regarded as one of the essential distinctions between cranial and spinal ganglia. It therefore seemed of interest to note that a ganglion on the border line between head and trunk develops in its anterior part like other cranial ganglia, and in its posterior part like the ganglia of the trunk. The position of the neural outgrowth, therefore, seems to me of little value in distinguishing the two groups of ganglia.

There is another peculiarity connected with the development of the vagus segment to which I would call attention. The protovertebral divisions posterior to the ear are at first of about the same size; but as the third vagus cleft forms in the intersegment that bounds the vagus segment posteriorly, the protovertebra of this segment increases in width, and, when the muscle plate develops, a vertical division of the protovertebra occurs in a plane corresponding to the present position of the second vagus cleft, which, as will be remembered, has pushed forwards from its original intersegmental position, now occupied by the third cleft, and consequently lies beneath the vagus protovertebra. The two parts of the protovertebra thus severed are each smaller than the following protovertebræ, and it is through the anterior of the two that the vagus root apparently cuts its way in the manner I have above described.

To find a dorsal as well as ventral segmentation interpolated in a region where one looks for reduction and consolidation was unexpected.

From the vagus segment the neural Anlage continues backwards, becoming gradually reduced in size, until it consists in

cross-section of but two or three cells above the dorsal wall of the spinal cord (figs. 8, 10, 11, 13). These cells appear to arise in the angle where the spinal cord separated from the skin, and a thickening in the ectoderm which forms the slight dorso-lateral ridge of the trunk seems occasionally about to add its deeper cell to the cells that have already migrated into the neural Anlage. Should this really take place, the dorso-lateral ridge of the trunk would be not potentially, but actually the homologue of the primitive dorso-lateral line of ectodermic proliferation on the head, of which it is the posterior continuation. Fig. 11 passes through such a cell above the fifth permanent (i. e. myotome-forming) protovertebra.

The dorsal ridge is very slight and soon disappears, but the median longitudinal ridge is well marked and continues comparatively long. It is most conspicuous in those sections which pass through or near an intersegment, where the median longitudinal ridge unites with an intersegmental ridge. Figs. 9 and 12 show the median longitudinal ridge between the fourth and fifth permanent protovertebræ at two stages of development. Fig. 9 is from the younger embryo. It is seen that at an early stage the median ridge may be three cells deep near an intersegment, while the surrounding ectoderm is composed of but two layers. In the later stage, given in fig. 12, the ridge is seen to extend to a point that reaches far in between the muscle plate and the pronephros.

The appearance of the ridge as represented in figs. 9 and 12, though typical, is by no means constant. Neither is the ridge three cells deep at every intersegment in the younger embryo, nor does it extend so far into the lower tissues at each intersegment of the older embryo.

The boundary of the ectoderm, elsewhere true as if formed by a limiting membrane, often appears frayed on the ridge as if cells had just pulled away. Had they done so, however, it would be difficult to obtain positive evidence of the fact, for the reason that at this very time cells begin to wander towards the notochord from the dorsal extremity of the lateral plates, forming a scattered mesenchyme in which cells wandering from the

ectodermic ridge would be lost. In the head, cells that migrated from the neural crest or thickened ectoderm were easily distinguished from surrounding tissues by difference in the size of the yolk granules they contain; but in the trunk, at this early stage, cells of the skin and spinal cord are not more free from yolk than those of the protovertebra, nephrotome, or lateral plate.

Fig. 11 gives the comparative distribution of yolk granules through the tissues of the trunk. The first cell differentiation occasioned by reduction of the yolk granules occurs in the ventral part of the spinal cord, and gradually extends through the cord dorsalwards. Some of the cells from the neural crest which lie in the loose mesenchyme at the side of the spinal cord also soon become clearer than the more ventral mesenchyme cells that have possibly come from the lateral plates, but there are always intermediate cells in regard to whose origin the yolk granules furnish no clue. Therefore, should cells from the median ridge join those migrating at the same time from the lateral plates, I know of no means by which the ectodermic cells may be identified.

On reviewing my sections, however, I find that in the region where the intersegmental ridge joins the median longitudinal ridge, and where as yet no mesenchyme cells from below have wandered, cells may be observed to migrate from the ectoderm. In Pl. 36, fig. 8, I have represented such a cell. The section is from the same series as fig. 9, but its plane is two segments anterior to that of fig. 9. The migration of cells from the neural crest above the second permanent protovertebra (fig. 8) has not extended as far as the fourth protovertebra represented in fig. 9. The intersegmental ridge is a dorsal extension from the median longitudinal ridge, and being deepest where it meets the median ridge, gradually fades away as it rises between the protovertebræ. As development proceeds, however, the longitudinal ridges of the trunk become less distinct, while the intersegmental ridges gain in prominence, thus repeating in the trunk the sequence of the ectodermic ridges occurring in the head. The cell migration actually observed in favor-

able positions confirms the evidence given by the depth of the median ridge (fig. 9), by its far-reaching prolongation into the underlying tissues (fig. 12), and its frayed edge, making it probable that the median and transverse ridges of the trunk throughout their length are the source of cell proliferation from the ectoderm, and of addition to the mesectoderm.

When the neural Anlage has extended over several segments of the trunk, and lies above the spinal cord as a band of tissue five or six cells wide if measured from one lateral margin to the other, the even surface of the spinal cord is interrupted at the point where the motor nerve is to be formed by the outgrowth of a fine protoplasmic prolongation, which soon after its appearance becomes attached to one or more of the neighbouring mesenchyme cells. These cells, moreover, send prolongations to meet the spinal outgrowth, as seen in fig. 10, which passes through the root of the third spinal nerve.

Somewhat later, but before the cells of the neural crest in their downward course touch the root of the motor nerve, a condition represented in figs. 16 and 17 is found. There is as yet no indication of the formation of a "Randschleier," and in both sections cells are seen to migrate from the spinal cord. Fig. 17 passes through the root of the third spinal nerve in the younger embryo, and fig. 16 through that of the tenth in an older embryo. Fig. 13 shows a further stage in the development of the motor nerve in which its main path is already established by the bipolar prolongations of a medullary cell. The section passes through the root of the fifth nerve in the same series from which figs. 14, 15, and 16 are taken.

Figs. 14 and 15 cut respectively the right and left roots of the fourth spinal nerve, and show that the cells which migrated from the spinal cord have now taken on the fibrillar striation that belongs to nerves. The nucleus of the cell which lies in the nerve path is entirely enclosed by the striated protoplasm of the nerve, in the threads of which the yolk granules seem entangled. Although, in this later stage, the cells of the neural mesectoderm have come in contact with the

mesenchyme cells below, and form with them a loose connective tissue, in which I am unable to distinguish the cells of one source from those of another, yet comparison of sections 14 and 15 with sections 13, 16, and 17, in which the neural Anlage has not as yet reached the level of the motor root, shows most clearly that the first nuclei found in the motor nerve have migrated from the spinal cord through the motor nerve-root. Ganglion cells have frequently been observed on motor nerves, and although His (**20**) affirms that no medullary cell migrates permanently from the cord into a motor nerve, Dohrn (**6**), claiming to have observed the passage of such cells from the spinal cord to the motor nerves of the trunk, supports this view by observations recorded in a paper on the origin and development of the eye-muscle nerves in the Selachii (**7**). The description there given of the development of the trochlearis is at variance with observations simultaneously published by Froriep (**13**) and myself (**30**). Dohrn's account of the origin of the oculo-motorius differs no less from the account I gave (loc. cit.), which has since been confirmed by Mitrophanow (**29**) and Sedgwick (**34**). Froriep, Mitrophanow, Sedgwick, and the author find that the first ganglion cells of the trochlearis or oculo-motorius in the Selachii are of peripheral, and not central origin. It is therefore with the greatest pleasure that I now confirm Dohrn's observations in regard to the origin of the ganglion cells on the motor nerves of the trunk, and add this evidence in support of his view that an actual and permanent migration of medullary cells takes place.

The cells of the neural crest do not immediately take part in forming the spinal ganglia, bnt wander in a continuous sheet down the sides of the spinal cord, and are there lost in a loose connective tissue, of which at first only those cells that come directly in contact with the motor nerve appear to develop nervous properties. Shall we then say with Goronowitsch (**17, 18**) that the cells of the neural crest have become "mesoderm"? By no means. I do not for a moment imagine the actual disparity of the cells dependent on my ability to distinguish them.

In the anterior segments of the trunk the cells of the neural Anlage form two layers at its ventral edge before coming in contact with the mesenchyme below. As the Anlage, in this early stage, lies between the sharply bounded spinal cord on the one hand, and no less distinct myotome on the other, it is easy to determine the number of cells composing it by counting the nuclei on successive sections. The result shows that there is no early accumulation of cells in definite regions pointing to the formation of ganglia, and consequently no primitive segmentation. Comparing segment with segment, one finds a gradual diminution of the Anlage as one approaches the tail. My purpose in counting the cells was chiefly to determine the number of neural cells taking part in the formation of the connective tissue in the posterior portion of the vagus segment which contains no spinal ganglion, and in the following segment where the ganglion, which develops at a relatively late stage, consists merely of a small group of cells at the root of the motor nerve.

Counting the neural cells between the spinal cord and the first myotome, before the neural Anlage meets the mesenchyme, I find on the left side of the embryo 166 cells. The order on successive sections is—10, 8, 8, 7, 5, 5, 5, 7, 4, 6, 9, 6, 4, 4, 5, 6, 4, 5, 5, 9, 6, 4, 5, 4, 6, 4, 5, 4, 6. In the following segment there were 126 cells, scattered quite as irregularly. In the third segment 123, and in the fourth 125. The slight increase in the number of cells in the fourth segment is probably due to preparation for the large brachial ganglion that lies in this segment. The following segments show rapid reduction in the number of neural cells.

I then counted all of the connective-tissue cells above the level of the motor nerve-root in the first trunk segments of the series from which figs. 13, 14, 15, 16 are taken, where the neural crest of the anterior segments has united with the mesenchyme, and forms a loose tissue of two layers at each side of the spinal cord.

The result gives for the posterior part of the vagus segment on the left side of the embryo 150 cells. Their order in section

is—7, 9, 6, 10, 6, 7, 6, 4, 3, 11, 6, 5, 5, 4, 5, 3, 6, 2, 7, 7, 6, 8, 7, 4, 6. The sections in the two series compared are of the same thickness, although it takes twenty-nine sections to pass through a segment in the younger embryo, which twenty-five sections cover in the older embryo. For the second segment, I found above the level of the motor nerve 138 cells, an increase of only twelve cells. The third segment gave 137 cells, an increase of fourteen cells. In the fourth segment I found 186 cells, showing rapid increase, probably due, as above mentioned, to the formation of a large brachial ganglion, although as yet no grouping of the cells into a ganglionic mass is found.

These figures are merely of relative value, as the number of neural crest cells varies in different embryos at about the same stage of development, and varies also on opposite sides of the same embryo.

Comparing the number of cells in the first segment of the two series, we find that the neural cells of the younger embryo, even had there been no increase by division, which is improbable, more than suffice to account for all of the connective tissue above the level of the motor nerve-root in the older embryo. I have not extended the enumeration, as the figures given demonstrate the continuity and extent of the tissue of ectodermic origin at the side of the brain in segments in which either no spinal ganglion or but a small one is formed. Part of the neural cells, however, that lie in the posterior division of the vagus segment form the posterior vagus root, the two vagus roots thus corresponding to the divisions of the primitive segment.

There is not only a rapid multiplication of neural cells to form the spinal ganglia, but also an additional migration of cells from the spinal cord through the dorsal nerve-root. The neural crest cells of the trunk resemble those of the head in having at first no special connection with one another, or with the central nervous system, and only on reaching the level at which the spinal ganglion is to develop do they send prolongations in two directions, one constituting the peripheral nerve,

the other the dorsal root. The cells that secondarily migrate into the spinal ganglia are, however, like the primitive cells of the motor nerve, from the first bipolar. Thus the cells of the neural crest are but potentially nervous, while the cells migrating into the peripheral nervous system through a dorsal or ventral root are from the first differentiated nerve cells.

The ganglia at the base of the first motor nerves in Necturus are, therefore, composed in part of cells that have migrated from the spinal cord through the motor nerve-root, and in part of neural crest cells that have come in contact with the motor nerve on their downward path. There is, however, little increase in the neural cells of the first two segments, such as helps to form the following spinal ganglia, and no secondary additions from the cord through a dorsal nerve-root.

5. The Embryo of Pl. 38, fig. 18.

In fig. 18 I give a second stage in the development of the lateral line system. The supra-orbital ridge has become wider, and the cells of which it is composed begin to arrange themselves about two parallel lines in anticipation of the double row of sense-organs about to form. The infra-orbital ridge has become distinctly separate from the nasal epithelium. The hyomandibular ridge is little changed. The sensory thickening above the hyobranchial cleft has elongated vertically, and from the anterior extremity of the epibranchial ridge above the vagus clefts a bit of sensory epithelium, that has parted from the rest, has also elongated dorsally. Thus two sensory ridges now replace portions of two intersegmental ridges that were lost in giving rise to ganglia, and to the ear.

The posterior part of the ventral ridge below the gill clefts begins to disappear, but the vertical ridge at the margin of the last cleft shows a slight ventral extension which is the rudiment of the ventral trunk line of sense-organs that is about to grow backwards from this point. Both the dorsal and median trunk lines have lengthened, and it will be noticed that no ridge is now found (fig. 18) where the rudiment of the median and dorsal trunk ridges is seen in fig. 1.

The growth of the lateral line of the trunk is chiefly through division and migration of cells that originally formed the anterior extremity of the line, and were in contact with the vagus ganglion. The cells that compose the growing lateral line are much more free from yolk than are the cells of the skin through which the line ploughs its course. The sensory ridge is thus sharply distinguished from neighbouring tissue. I believe, however, that the ridge is not exclusively composed of cells foreign to the segments through which it passes, but that a few cells at each side of the ridge join those that have advanced from anterior segments.

Pl. 36, fig. 19, from an embryo at the stage of fig. 1, on which the plane of the section is indicated, shows the relative amount of yolk in the cells of the lateral line ridge and in those of the adjacent skin. Fig. 20 is a cross-section through the skin in a plane given on fig. 18, and shows the present appearance of the skin where in the earlier stage (fig. 1) a deep lateral ridge was found. I would call attention to two facts: first, that the lateral nerve (fig. 20, *l. n.*), consisting in cross-section of one nucleus and a small bundle of fibres, is far too small to account for the disappearance of a ridge once as deep as that of fig. 19; secondly, the even distribution of yolk granules in fig. 20 shows that the deeper cells of the ridge, which were free from granules, have not remained in the skin after ceasing to form a sensory ridge.

What, then, became of these cells? The answer is given in fig. 5, which illustrates one of the most peculiar phenomena in vertebrate development with which I am acquainted. Those lateral line cells that find themselves in a position with which for some reason they are dissatisfied, leave that position, and making their way over the heads of their neighbours, between the outer and inner layers of the skin, crowd themselves down into a front place in the advancing line, with a self-seeking independence that is almost human.

In describing the development of the sea-bass, Wilson (**39**, p. 239) also calls attention to the strangely independent action of individual cells, which are evidently under no common

pressure. The circumstance which gives occasion for this comment is connected with the folding off of the alimentary canal, and Wilson ascribes the apparently independent action of the cells to inherited tendency to follow ancestral lines of migration. The action of cells, however, in the path of the lateral line in Necturus seems the more peculiar since we cannot ascribe it altogether to heredity, because of the irregularity with which the sense-organs are formed. In a segment on one side of the embryo a sense-organ often appears that is omitted on the other side. Now one, now two segments are omitted. Here two, there three sense-organs are allotted to a given segment. The inherited tendency is evidently one that allows wide range to individual variation, and this fact renders the independent action of individual cells most striking.

I am convinced that we shall never have even approximately accurate knowledge of the course of vertebrate development until we are by some means enabled to follow the migration of individual cells. We recognise the advancing mass, or the elongating cord, but shut our eyes to the fact that cell after cell moves on its independent mission, wandering alone—who knows how far? or in what direction?

Merkel (**28**), in commenting on Mall's (**27**) statement that the direction of the transmission of an impulse is already determined by the position of the cell in the ectoderm, the receptive pole being that originally on the surface of the body, says (loc. cit., pp. 299, 300) that it would be interesting to know if this view be really of general validity, for Merkel finds it conceivable that the direction of a nerve-current might change; at least "such a possibility must be first excluded before one can speak with certainty even in regard to the retina." The migration of cells in the lateral line, which I have just described, seems to me to demonstrate that the law Mall states is not generally applicable, for the impulse which induces three of six cells lying in a continuous line, and equally exposed to the surrounding water, to migrate while the rest remain, must surely be received from within. A transmitting pole has therefore become a receiving pole, and

the universality of the law of polarity, as Mall states it, is destroyed.

6. The Embryo of Pl. 38, fig. 21.

Fig. 21 shows the nerves and lateral line system of an embryo in which pigment has begun to appear in the skin, and when the stem of the external gills has become distinctly visible. The length of the embryo is 15 mm., but the variation in length at the same stage of development is frequently as much as two millimetres.

On the supra- and infra-orbital lines sense-organs can now be distinguished in the skin, when removed and studied by transmitted light, although sections still show a continuous deep ridge, as in fig. 22, which passes through the infra-orbital line. The supra-orbital line has extended ventrally, and now passes around the nasal epithelium. The dorsal part of the line has given rise to a double row of sense-organs, as has also the median part of the infra-orbital line, where this line crosses the space between the eye and nose. The number of sense-organs is not constant, and gradually increases as the embryo grows older. Moreover where the lines are double a continuous area of deep sensory ectoderm connects the two rows of organs, in which further sense-organs may develop later between the rows now found.

On the hyomandibular lines distinct sensory spots have not yet appeared. The dorsal part of the primitive ridge has become double, and has also extended in an anterior direction, as indicated by the two small branches that run forward from the hyomandibular nerve. The mandibular ridge now meets the anterior extremity of the ventral longitudinal ridge near the median plane of the embryo, and posterior to the mouth. At the corner of the mouth the mandibular line passes from a direction nearly horizontal to one nearly vertical. The line, however, curves on to the ventral surface of the embryo, and thus approaches again the horizontal plane, although in a direction at right angles to that of the dorsal part of the line. The posterior or hyoid part of the hyomandibular line is little

changed, but has annexed to itself, as the innervation shows, the anterior part of the ventral longitudinal line. That part of the original ventral ridge, which bounded the branchial clefts ventrally, has now disappeared, and the ridge can be followed but little beyond the plane of the hyomandibular ridge.

The glossopharyngeal ridge has divided into two sensory areas. The long axis of the upper, like that of the undivided ridge, is vertical, while the long axis of the lower division is now longitudinal. In the interval between the two areas the deep sensory cells of the original ridge have entirely disappeared from the ectoderm.

The posterior intersegmental ridge is little changed. The nerve which supplies it appears at this stage of development to enter the vagus ganglion by a root distinct from that of the nerve supplying the epibranchial or horizontal part of the ridge. The two nerves, however, enter the ganglion by a common root in a slightly older embryo.

The ridge at the posterior margin of the last branchial cleft no longer exists. The slight ventral extension of the ridge seen in fig. 18 has grown backwards as the ventral trunk line which is now about to pass beneath the arm, and has consequently reached the region in which the anterior sense-organs of this line are to form. No trace of the ventral ridge is found in the space through which it has just passed from the posterior margin of the gill cleft to its present position. The anterior cells of this ridge have migrated as did those of the dorsal and median trunk ridges.

Four distinct organs lie in the path of the dorsal line, and, as fig. 21 shows, their position is not strictly segmental. There is, however, a strong tendency in the dorsal trunk line to form a sense-organ at each intersegment, and the anterior organ of the line most frequently lies between the third and fourth myotomes (no account being taken of the division of the vagus myotome, the anterior part of which is rudimentary). In the embryo represented in fig. 21 the anterior sense-organ of the dorsal trunk line lies above the fourth myotome.

A long row of closely and irregularly scattered sense-organs mark the path of the median trunk line—one, two, or three organs falling apparently as chance determines to each segment.

In fig. 21 the facial and vagus nerves are coloured red, the trigeminal and glosso-pharyngeal black. The eye muscle nerves are not represented. I have been unable to find the trochlearis in these early stages. The abducens may be easily followed in a slightly older embryo, and possibly already exists. The oculo-motorius passes from the floor of the mid-brain to the ramus profundus, which it meets at a point near the most dorsal of the branches which fig. 21 represents as leaving the profundus. The ganglia are represented in a flat shade as in the preceding reconstructions, and the position of ear, eye, and nose is indicated. The embryo from which the lateral line organs are reconstructed is the embryo drawn. The nerves, however, in this and in the following reconstruction have been traced from transverse, horizontal, and sagittal sections through embryos at the same stage of development as the embryo drawn, but killed and stained with formic acid and gold chloride, which I have found satisfactory and reliable reagents for the topography of the nervous system, Lee (**25**, p. 143) to the contrary notwithstanding.

From the Gasserian ganglion three branches arise; the ramus ophthalmicus profundus, the ramus mandibularis, and a branch which almost immediately unites with the ramus buccalis facialis. The anterior part of the ganglion forms the posterior part of the ramus profundus, and lies close to the median wall of the optic vesicle. Several small branches, of which the two larger have been drawn, pass upward between the eye and the brain, connecting the profundus with the sensory ectoderm of the supra-orbital ridge, which, it will be remembered, originally contributed to the origin of the nerve. Between the eye and nose the profundus sends a branch directly outwards, and then divides into its two chief branches, which closely embrace the thick-walled olfactory vesicle. The anterior of these branches anastomoses with the

olfactory nerve, which is not represented, and with the ramus ophthalmicus superficialis facialis.

The homologue of that branch of the trigeminus which fuses with the ramus buccalis is described by Dohrn (8) in the Selachii as the ramus "infra-maxillaris," by v. Wijhe (38) in the Ganoidei as the ramus "maxillaris superior," while Strong (36) mentions the nerve in the tadpole as an accessory branch of the trigeminus.

In Necturus this nerve is one of the chief primitive branches of the trigeminus, although fig. 22 shows its entire present length from the Gasserian ganglion above to the point where the nerve is lost in the ramus buccalis, which lies immediately below the ectodermic ridge of the infra-orbital line. The stage represented is but a transitory one in the splitting off of the nerve, which takes place throughout the length of the common Anlage. In a slightly older embryo one finds two distinct nerves side by side, one belonging to the facialis, the other to the trigeminus. The relation of these nerves to one another seems to me of interest, because so closely resembling that of the ramus ophthalmicus superficialis to the profundus. In the one case the supra-orbital ridge gives rise successively to two distinct nerves; in the other the infra-orbital ridge gives rise to a nerve from which the inner part splits off as a trigeminal branch, while the outer part remains as a branch of the facialis. The difference in the manner of formation is therefore chiefly one of time. A longer interval separates the two nerves formed from the supra-orbital ridge than that separating those formed from the infra-orbital; but the close similarity of origin suggests that this branch of the trigeminus might well be called the "buccalis profundus," and so I have ventured to name it in Necturus under the shelter of dissident authorities.

Dohrn (8, p. 267) says of the "N. infra-maxillaris" in Pristiurus, that many branches pass to it from the infra-orbital canal, that these branches have a much more oblique and a longer course than those of the buccalis, "und,—was noch auffallender ist—sie gehören einem Nervenstamm an, der von

Hause aus vor den Facialiscomponenten liegt, während die Zweige doch aus Schleimcanälen herstammen, die hinter dem Buccalis-Schleimcanalsystem liegen. Diese auffallende Verbindung hinterer Ectodermpartien mit vorderen Nerven machte mich argwöhnish ob überhaupt eine Regel in diesen Verbindungen zu erkennen sei; desshalb verfolgte ich sehr sorgfältig die beginnende Zweigbildung all' dieser Schleimcanalnerven. Ich konnte dabei constatiren, dass aus derselben Schleimcanalanlage Zweige an verschiedene Nervenstämme abgegeben werden, und dass derselbe Nerv Zweige aus verschiedenen Schleimcanälen empfängt. Dies scheint darauf zu deuten, dass ausser den Zweigen, welche von vorn herein bei dem Auseinanderweichen der Nerven und der zugehörigen Ectodermpartien als Brücken zwischen beiden bestehen bleiben und sich allmählich in die Länge ziehen, noch andere Zweige selbständig vom Ectoderm gegen das Innere zu wachsen und sich mit denjenigen Nerven secundär verbinden, welche sie auf ihrem Wege finden."

V. Wijhe (loc. cit., p. 312) holds it one of the important results of his study to have established the independence of the two nerves, "maxillaris superior" trigemini and buccalis facialis, since these nerves lie so near to one another in many of the Ganoidei as to have been mistaken by earlier investigators for a single nerve. Hence neither Dohrn nor v. Wijhe reached the conclusion towards which the development of the nerves in Necturus points, namely, that each represents part of a single nerve, originally a branch of the trigeminus, from which the lateral line component separated, making use of the more direct path offered by the contact of facial and trigeminal mesectoderm to send the sensory impressions received from the organs of the lateral line through the root of the facialis to the cranial centres of that system.

The ramus mandibularis breaks up into several branches on the mandibular muscle, which I can follow into the muscle, but not far beyond.

The ramus ophthalmicus superficialis facialis still lies throughout its length immediately below the sensory ectoderm.

A branch supplying the short dorsal row of sense-organs may be distinguished in the nerve-plexus which here underlies the skin. The ramus buccalis has been mentioned. A small branch, the ramus oticus, connects the solitary supra-branchial sense-organ of the hyomandibular cleft directly with the facial ganglion.

The ventral branches of the facial, glossopharyngeal, and first vagus ganglia closely resemble one another. Each ganglion rests upon the dorsal wall of the corresponding branchial cleft, and sends a large branch downwards, from the proximal end of which another branch runs backwards and outwards into that branchial arch on the anterior wall of which the main nerve lies. A third and smaller branch extends from the ganglion inwards and forwards on the dorsal wall of the branchial cavity. In the facial group this anterior branch, the ramus palatinus, is already fairly developed, but the fibres running forwards from the glossopharyngeal and vagus ganglia can hardly as yet be called nerves. They resemble a bush of tiny fibres, leaving the ganglion in a bundle, but almost immediately scattered and lost on the adjacent pharyngeal wall.

The vertical branch of the facialis, the ramus hyomandibularis, begins like the following branchial nerves close to the posterior wall of the branchial pocket, but almost immediately crosses the dorsal corner of the pocket, and comes in contact with the skin at the point where the endoderm of the hyomandibular pocket last touches the ectoderm. Here for a short distance the nerve is bounded within by the endoderm, without by the ectoderm; but as the branchial pocket recedes from the surface of the embryo the nerve clings to the ectoderm, and divides into its two sensory branches, which closely underlie the corresponding sensory ridges.

The vertical branchial nerves of the glossopharyngeus and vagus begin at relatively the same point as the hyomandibularis, but continue as they begin close to the endodermic wall of the gill cleft, which they follow downwards. Then, turning inwards with the gill cleft, the distal part of the nerve occupies

a nearly horizontal position, passing through the mesectoderm, with the cartilage Anlage above and the branchial wall below, as seen in fig. 23, Pl. 36. This figure shows the distal part of the glossopharyngeal nerve. Near the median plane of the embryo the nerve ends in small branches. Fig. 21 does not represent the distal part of these glossopharyngeal and vagus nerves.

The lateral branchial nerves of the glossopharyngeus and vagus, which run backwards and outwards, and which appear to be serially homologous with the ramus hyoideus facialis, are chiefly distributed to the branchial muscles which lie against the anterior wall of the posterior cleft, although branches also go to the skin or are lost on the walls of the blood-vessels. As the hyoid muscle is very large, and fills the posterior half of the hyoid arch, its nerve is correspondingly large, and to reach the muscle measures but half the width of the arch, while the following smaller homologous nerves reach their respective muscles near the posterior wall of the arch.

The branchial nerves of the second vagus arch arise at this stage from the root of the nerve of the first arch, and only later do these nerves acquire independent connection with the ganglion. The course of the nerves in the posterior arch is similar to that of those in the first vagus arch. One nerve passes downwards against the posterior wall of the branchial cleft, the other passes into the posterior arch supplying its lateral musculature and the external gill. The secondarily acquired independence of the nerves of the posterior vagus arch seem to me significant in connection with the manner in which the third vagus cleft forms and the primitive vagus myotome divides.

Two small branches, that enter the ganglion by a common root, supply the two divisions of the glossopharyngeal sensory ridge. The nerves connecting the following intersegmental (vagus commissural) and epibranchial ridges with the vagus ganglion appear to enter the ganglion at this stage by separate roots. I may be mistaken in this, however, for in an embryo but little older the nerves can be traced to a common stem.

The proximal parts of the nerves of the dorsal and median trunk lines now lie some distance below the surface. The median parts of the nerves closely underlie the skin, and the distal parts run for some distance in the ectoderm. Near the base of the nerve of the ventral trunk line several large branches are given off, and soon lost in surrounding muscular tissue.

The first spinal nerve sends its chief branch immediately downwards to the ventral wall of the muscle plate, along which the nerve runs for a short distance backwards, thus avoiding the branchial region; then taking again a vertical direction, it meets the second spinal nerve, which passes downwards near the anterior wall of the pronephros. The two nerves here unite with one another and with the ventral lateral line nerve. Beyond the point of union the spinal nerves may still be followed forwards for a short distance. The next three spinal nerves form the brachial plexus. From each of the spinal nerves dorsal and lateral branches arise, which are not represented in the reconstruction, and the discussion of which I shall postpone.

Goette (16) tells us that in the "Unke" the first spinal nerve arises from the second trunk segment, and passes over the sterno-hyoideus muscle to the genio-hyoideus, and that the second and third spinal nerves form the brachial plexus. Hence the first spinal nerve in Necturus has apparently no homologue in Bombinator.

Ecker and Wiedersheim (9) speak of the hypoglossus as represented in the Amphibia in general by the first spinal nerve, which in the frog arises by two roots, an anterior larger root, and a posterior smaller root, supplied with a small ganglion. The nerve follows a course similar to that of the two pre-brachial nerves in Necturus, which therefore probably together represent the hypoglossus of other forms.

I was interested to find that the first two spinal nerves in the Ganoidei, according to Stannius (35) and v. Wijhe (loc. cit.), have only anterior roots. V. Wijhe regards these nerves as lower vagus roots, a supposition which is not supported by the development of the nerves, if homologous, in Necturus.

7. The embryo of Pl. 38, figs. 31, 32, 33.

The embryo represented in figs. 31, 32, 33, is 19 mm. long. The lines of the lateral line system are complete, and with this stage the present study closes. The number of sense-organs gradually increases as the embryo grows, but the main lines are not different in the oldest embryos I have—at eight months, or 40 mm.,—and from the superficial examination of the adult, made near the collecting ground, I believe the lines to be those of the full-grown Amphibian.

When the skin of larger embryos is examined, it is found that the sense-organs open to the surface by a slit-like scissure in the outer layer of the ectoderm. The long axis of the opening lies in different planes on the several sensory lines. Thus, on the main lateral line of the trunk the slit is horizontal, on the dorsal line of the trunk vertical, and on the ventral line the opening is often round, or may be elongated in any direction. In the groups of organs at the end of the snout the direction of the elongation also varies. In the supra-orbital, infra-orbital, and mandibular lines it is usually parallel to the direction of these sensory lines. In the ventral or anterior part of the double hyomandibular line the long axes of the openings in the outer row of sense-organs are at right angles to those of the inner row. These differences in the direction of the long axes of the openings to the sense-organs seem to be in no way related to their development, and are possibly co-ordinated with the direction in which currents of water flow as the animal moves. At the stage with which this study closes slit-like openings to the sense-organs are not found.

The histology of the sense-organ resembles that of the lateral line organs in fishes. At the present stage of development, however, the difference between supporting and sensory cells is not sharply marked, although the external cells of the organ are in general somewhat flattened against the internal pear-shaped cells.

At each side of a mid-dorsal fold in the skin a row of mucous glands is found, composed of a few cells invaginated from the deeper layer of the ectoderm, and now lying below the surface, tiny balls of cells surrounding a central cavity that opens to the surface by a small pore. Similar glands are found on the ventral surface of the body between the fore-limbs, and on the tail. Although these glands are about the size of sense-organs, nothing in their structure or in the manner of their development suggests that genetic relation of sense-organ and mucous gland on which Leydig (**26**) insists.

Pl. 38, fig. 33, represents the ventro-lateral surface of the head, showing the position of the anterior sense-organs and the distribution of pigment, which, as the figure demonstrates, makes the position of the dorsal sense-organs less apparent. I have omitted the pigment in fig. 32, which shows the dorsal sense-organs of the head and the sense-organs of the three trunk lines. The pigment cells are so grouped that a light band with irregular outline extends on each side of the embryo throughout the length of the body. Its position and relative width are shown in the small area over which, in fig. 32, the pigmentation is reproduced. The anterior sense-organs are also outlined in fig. 31, which gives their innervation.

Comparing the sensory differentiations of the ectoderm at this stage with those of the younger embryo represented in fig. 21, one finds that a cluster of sense-organs has developed on the antero-ventral surface of the snout at the anterior extremity of the supra-orbital line. The two groups on opposite sides of the head lie near to one another, but do not meet. At the anterior extremity of the infra-orbital line a similar cluster of sense-organs is found, resulting from the continued multiplication of sense-organs between the eye and nose, which had already begun in the younger embryo. The organs at the posterior extremity of the supra-orbital line have extended in a postero-dorsal direction beyond the point where this line meets the infra-orbital, and the angle at which the two lines diverge is now more acute.

The change in the relative position of the hyomandibular

and mandibular lines is considerable; and without the intermediate stage given in fig. 21, one would hardly identify these lines with the primitive ridges seen in figs. 1 and 18, from which they have developed. The changes, however, are chiefly connected with the forward growth of the mandible and the conversion of a head originally deeper than wide into a broad flat head. The dorsal part of the hyomandibular line has given rise to a double row of sense-organs; and although the line was originally nearly vertical, the dorsal part of the line is now horizontal, the change of direction begun in fig. 21 having further increased. The anterior (once dorsal) extremity of the line, moreover, now meets the infra-orbital line back of the eye.

The mandibular line, which originally made an obtuse angle with the dorsal part of the hyomandibular line, now leaves that line at an acute angle, and at the corner of the mouth closely approaches the base of the hyomandibular line. Here one or two sense-organs lie between the mandibular and infra-orbital lines, but anterior to this point no sense-organs, save those of the supra- and infra-orbital lines, are found on the upper lip. The mandibular line then bends on to the lower lip, near the middle point of which it ends in close proximity to the mandibular line of the opposite side. The two lines are distinct, however.

Although the median lines of sense-organs on the lower jaw do not primarily belong to the hyomandibular ridge, but to the ventral longitudinal, as the organs are innervated by a nerve that directly continues the main hyomandibularis, I prefer to speak of them as the anterior part of the hyomandibular line rather than as independent lines. Pl. 38, fig. 33, shows that each of these two median lines has now given rise to a double row of sense-organs, and although they are no longer united at their anterior extremity with the outer, mandibular, lines, as an index of the former union, seen in fig. 21, the innervation of the inner row of sense-organs at the anterior extremity of the mandibular line comes from the nerve supplying the hyomandibular line (fig. 31). From

the point where the primitive hyomandibular line meets the ventral longitudinal part, one, or sometimes two sense-organs extend towards the median surface of the throat.

The otic sense-organ has not changed.

From the more dorsal of the divisions of the glossopharyngeal ridge, seen in fig. 21, two or three sense-organs have developed. They do not usually lie in a vertical line, but lie often at an angle with one another, and may even be in a horizontal line, parallel to that occupied by the two sense-organs formed from the lower division of the glossopharyngeal ridge.

Since the vertical line connected with the vagus is "commissural" in many fishes, I retain this designation, and would call attention to two sense-organs seen in fig. 32 posterior to the dorsal extremity of the line. These organs appear here exceptionally, for the vagus commissural line usually consists of a vertical row of organs, as in fig. 31. I consider the two organs just mentioned of interest, because they suggest a tendency to continue the dorsal lateral line of the trunk on to the head. The epibranchial line is now represented by four to six sense-organs.

Not only is the number of sense-organs inconstant, but their position on both head and trunk is often unsymmetrical, and not infrequently an organ is found at some little distance from the line in which it properly belongs.

The dorsal line of the trunk (fig. 32) does not extend beyond the anterior part of the tail. At the point where this line ends the median line leaves its position opposite the notochord, and as it grows backwards assumes a position similar to that occupied on the segments of the trunk by the dorsal line. The median line continues to the end of the tail. The ventral trunk line ends below the hind limb with a few sense-organs that lie much nearer to one another than do the organs in the median path of the line. The position of the organs on the dorsal line is often intersegmental, although an intersegment is occasionally omitted, or occasionally an organ lies above the myotome.

To avoid confusion, in fig. 31, which shows the innervation

of the lateral line system, merely the proximal part of several nerves not connected with that system has been outlined. In the trigeminal group the peripheral distribution of the ophthalmicus and buccalis profundus is shown, while the mandibularis is cut short, and its maxillary and mandibular branches are represented as arising much nearer the root of the main stem than is actually the case. The ophthalmicus profundus is seen to send several branches to the skin, which are lost in the vicinity of the supra-orbital line, thus connecting the nerve with the ectoderm of its origin. As in the younger embryo, the ophthalmicus profundus divides distally into three main branches; one, of which only the beginning is represented in fig. 31, extends directly outwards between the nose and eye. The two remaining branches enclose the nasal epithelium, and are finally distributed to the skin of the snout. The anterior of these branches anastomoses with the ramus ophthalmicus superficialis, the posterior branch with the ramus buccalis facialis. The buccalis profundus is now distinct from the buccalis facialis throughout its entire length, and its branches are lost in the skin posterior to the infra-orbital line, as described by Dohrn (loc. cit.) in the Selachii.

The two dorsal branches of the ophthalmicus superficialis facialis, which are seen in fig. 31 to supply the posterior and dorsal sense-organs of the supra-orbital lines, are of constant occurrence. At the end of the snout the superficialis breaks up into terminal branches, supplying the anterior cluster of sense-organs.

In connection with the ramus buccalis facialis I would call attention to a dorsal branch which I have marked by a star in fig. 31. This branch supplies four sense-organs that probably belong genetically to the hyomandibular line. The dorsal part of the infra-orbital sensory ridge gives rise to a single line of sense-organs, and the nerve supplying organs in the same region as the four in question has been traced in another embryo to the ramus hyomandibularis. Thus the irregularity in the position of the sense-organs in Necturus is apparently correlated with irregularity in their innervation, which is especially

liable to occur where, as in the present case, one sensory line meets another.

I have traced the nerve twigs to each one of the terminal cluster of organs on the infra-orbital line, and find that four of the organs, which I have marked in the reconstruction, are supplied by nerve twigs composed in equal parts of fibres coming from the buccalis facialis, and from the ophthalmicus profundus. These fibres unite in a common twig that goes directly to the heart of the sense-organ.

Dohrn says (**8**, p. 274) that he has observed in the Selachii no mingling of the fibres of the ophthalmicus superficialis with those of the profundus, but considers such a union not impossible; while Strong (**36**, p. 179) speaks more positively of "the fact that the trigeminus proper does not participate in the innervation of the lateral line system," as "brought out by Allis (Amia), by Ewart (Læmargus and Raja), and by the writer (tadpole)."

In this connection, the section given in Pl. 36, fig. 26, is significant. The figure shows one of the dorsal branches of the profundus, seen in the reconstruction (fig. 31), as it passes directly through the ramus ophthalmicus superficialis to the supra-orbital line, a sense-organ of which the section cuts tangentially. The following section shows the diameter of the ophthalmicus superficialis to be as great in the plane traversed by the profundus branch as it is in this section just posterior to that plane. In other words, a few fibres of the ophthalmicus superficialis lie outside of the branch from the ophthalmicus profundus, and, as the section shows, fibres from the superficialis join those from the profundus on their way to the sensory ectoderm.

When one considers that the facialis invades a territory originally trigeminal, one is not surprised to find at every hand indications of the usurpation, showing that the separation of the specialised sensory tract, that finds its co-ordinating centre through the root of the facialis, is incomplete. I do not, for this reason, include the trigeminus among the lateral line nerves, but should nevertheless hesitate to say that the "tri-

geminus proper does not participate in the innervation of the lateral line system."

The innervation of the hyomandibular line shows that the surface covered by the sense-organs of this line has extended in a posterior as well as anterior direction, and that the lengthening of the main stem of the hyomandibularis has not kept pace with the growth of the sensory line. While in fig. 21 we find that the hyomandibularis closely underlies its sensory ridge, in fig. 31 the sense-organs that have developed from the ridge are seen to be connected with the main nerve by long slender stems that unite into four or five branches before reaching the common trunk. The present innervation of the mandibular line is not less misleading in its interpretation of development, and one would hardly fancy from the distribution of the nerve that the mandibularis primarily came in contact with its sensory line, not near the corner of the mouth, but at the posterior limit of the mandibular line, where this line meets the hyomandibular. The point where the main nerve now forks in two directions, once lay midway on the path of the undivided nerve, as shown by comparison with fig. 21. In the reconstruction, the nerves which collect the dorsal and ventral branches of the hyomandibular line, and the mandibular branch of the facialis are not united. In fact, however, the ventral branch of the hyomandibularis unites with the mandibularis, and then with the dorsal branch of the hyomandibularis, and the three nerves enter the ventro-lateral surface of the ganglion by a common root. From the point where the reconstruction leaves the nerves their course is inwards, and was difficult to represent in surface view.

I have already called attention to the fact that the inner row of mandibular sense-organs at the margin of the lower lip is innervated by the hyomandibularis (mandibularis internus), thus bearing testimony to the earlier union of the two sensory lines, hyomandibular and mandibular (fig. 21). The nerve of the posterior half of the mandibular line anastomoses with a branch from the hyomandibularis, so that these posterior organs now appear to receive their innervation from both mandibular and hyomandibular nerves.

Merely the trunk of the hyoid nerve is outlined, which soon divides into three main branches that are distributed to the hyoid muscle, and supply in general the lateral innervation of the hyoid region. Strong (**36**, p. 128) says that in the tadpole the ramus hyoideus is composed of fibres from two nerves, the facialis and glossopharyngeus; and that "while it is difficult to distinguish the two sets of fibres in the ramus hyoideus," it is probable that the fibres from the facialis are those that innervate the muscle, and that the cutaneous component is derived from the glossopharyngeal vagus complex. It is therefore of interest to note that the ramus hyoideus in Necturus sends fibres to the skin when the nerve is not united with the glossopharyngeus. Hence the difficulty which Strong acknowledges, in distinguishing the two sets of fibres in the tadpole, opens the way to doubt if the cutaneus fibres of the hyoideus in Rana may not be in part derived from the facialis.

Between the mandibularis facialis and the ventral part of the hyomandibularis a nerve is outlined which distributes its fibres on the dorso-lateral surface of the mouth. The nerve enters the ganglion near the root of the hyomandibularis, and I have called it an external palatine. It may possibly be the homologue of one of the palatine nerves described by v. Wijhe (**38**) in the Ganoidei as belonging to the trigeminus. The main (internal) palatine nerve is cut short in the reconstruction. It enters the ganglion at a deeper level than that at which the hyomandibularis arises, and is distributed to the roof of the mouth. The nerve is both larger and longer than the external palatine.

The glossopharyngeal nerves arise from the ganglion at the same point. The pharyngeal branch is cut short in the reconstruction. The dorsal branch explains itself. The main branchial nerve is post-trematic, and the distal part runs inwards as well as forwards, ending near the axis of the embryo. The lateral branchial nerve, which, as in the younger embryo, extends backwards and outwards, now fuses with a prætrematic branch from the first vagus nerve. This is the only prætrematic branch as yet found in Necturus. The two

nerves after their fusion supply the lateral musculature, the vascular system, and the skin, including the external gill. The distribution of the two serially homologous branchial branches of the first vagus arch is similar. The dorsal vagus nerve sends branches to the upper branchial muscle, through which it passes, before dividing into branches of the commissural and epibranchial lines. The main branchial nerve of the posterior vagus arch arises from the ganglion near the origin of the nerve of the anterior arch. The two pharyngeal nerves (cut off in the reconstruction) that arise from the vagus are distributed by numerous branches on the pharynx in their immediate neighbourhood.

In regard to the dorsal and median trunk lines I have nothing to add. From the ventral nerve of the lateral line a few large but short motor branches arise, and a longer branch, which supplies the ventral branchial muscles, is evidently serially homologous with the three preceding post-trematic nerves.

In regard to the morphological value of the facial nerves there is great difference of opinion. I believe, however, that the development of the ramus ophthalmicus superficialis and ramus buccalis in Necturus demonstrates that neither nerve can be considered segmental. Of the ventral facial nerves, v. Wijhe tells us (38, pp. 313, 314) that the ramus mandibularis (my hyomandibularis) always divides into an external and internal branch, and evidently does not belong to the hyoid arch, as does the ramus hyoideus, but to an anterior visceral arch. Therefore, if the ramus hyoideus represents a posterior branch (post-trematic), one would be inclined to regard the ramus mandibularis as an anterior branch; but for this it would be necessary that its course should lie on the anterior wall of a gill cleft, which is not the case, as the spiracle is found anterior to the nerve. Two possibilities exist; either the ramus mandibularis is still a ramus anterior, and the gill cleft before which it should lie has aborted, or this is not the case, and the nerve is a secondary outgrowth. If a gill cleft has aborted between the ramus mandibularis and ramus hyoideus, it probably was situated between the parts of the hyoid arch,

which contradicts the evidence given by Gegenbaur (15) for viewing the hyoid as equivalent to a single visceral arch.

To the two possibilities thus suggested by v. Wijhe the embryology of Necturus adds a third, for the hyoideus appears in Necturus, not as a post-trematic nerve, but as a lateral branch of the hyomandibularis similar to those supplying the external gills and lateral walls of the following arches. The true post-trematic nerve of the hyoid arch is the hyomandibularis and its ventral continuation, the "internal mandibular." Nor do I regard the external mandibular nerve as the prætrematic nerve of the group, but because of its relation to the mouth would homologise it also with the post-trematic nerves, the mouth being in my opinion (31) formed by the fusion of the ventral parts of one or more pairs of gill clefts. The missing cleft in which, like v. Wijhe, I also believe is therefore to be sought in the mandibular arch rather than the hyoid—a view that is supported by the endodermic origin of the cells forming the mandibular musculature in Necturus (32), and by v. Kupffer's (24) discovery that the cavity which gives rise to the mandibular muscles in Petromyzon is in fact a pocket of the alimentary canal.

Of the homologies of the "chorda tympani" I know nothing, but was much surprised to find that Strong, in agreement with Pollard (33), affirms that the nerve is represented by the ramus mandibularis internus, adding that Froriep (12) may possibly have "had the correct nerve, but was mistaken in assigning it to the lateral line system" (p. 187). A letter from Dr. Strong makes it probable, however, that the branch he designates mandibularis internus corresponds to the branch I have called "external palatine." This nerve is not a branch of the hyomandibularis in Necturus, and is certainly not the homologue of the nerve Froriep calls the external mandibular. Hence the confusion.

8. Cell and Fibre.

In regard to the formation of the nerves, much that v. Kupffer (22, 23) affirms for Petromyzon, and Dohrn (6, 8) for

the Selachii, is equally applicable to Necturus. Nevertheless we frequently hear that nerves are not primarily cellular in their structure, but arise always as fibrous outgrowths from the peripheral ganglion or central nervous system. In reply to Dohrn, His tells us (**20**, p. 342) that no thoughtful investigator will assume that sharks can differ from other Vertebrates fundamentally in the manner in which their nerves are formed; and that when it can be shown that in higher Vertebrates, such as Amphibia and bony fishes, the motor nerve-fibre arises as a thread-like outgrowth from particular spinal cells, the statement must (?) be valid for ray and shark.

It may, therefore, not be out of place to add a few words in regard to the formation of the nerves in a primitive Amphibian; for if the Cyclostomata, Selachii, and Amphibia are shown to agree in the cellular structure of their nerves, motor as well as sensory, the "thoughtful investigator" might perhaps conclude that the development of the human nervous system differs from that of the shark and tadpole.

All of the nerves which I have described in Necturus, whether motor or sensory, cranial or spinal, are formed by the continued migration of modified ectoderm cells, either from the neural crest, the closed medullary tube, the peripheral ganglia, or the superficial ectoderm. The manner of their formation, however, is not always the same, and nerves of different orders vary greatly in the number of cellular elements they contain.

In the development of the embryo we may distinguish two factors, known to philosophy as efficient cause and final cause. Efficient cause leads to that unfolding of the embryo by which each stage grows out of the preceding one in the sequence of its original development—its phylogeny modified by the present changed surroundings. Final cause, however, stamps mysteriously on the younger stage the image of a later form; and thus we find throughout development rudiments of later structures, which can be neither of immediate use to the developing embryo nor yet associated with the ancestral type its present form repeats. To disentangle these factors is the

problem of the embryologist, and we are wont to forget that in the growth we study each cause plays its part.

Observing that the complicated nervous system of the adult may be traced to the embryonic neural plate, we forget to note in this the expression of final cause, and to consider that meantime, before the neural plate enters upon its co-ordinating functions, efficient cause must find in the egg a material medium of co-ordination.

The living protoplasm of every cell is the first nervous system of the egg. Step by step that specialised structure which we recognise as a nervous system usurps the power that belongs originally to each cell. What wonder, then, that primitive nerves are cellular?

In a recent publication Sedgwick (**34**) calls attention to the fact that embryonic tissues are from the first connected by a reticulum of fine protoplasmic threads. These tiny strands of protoplasm connecting cell with cell are also evident in Necturus. They are, as development shows, associated with the first steps in the formation of the nervous system. When Sedgwick adds, however, that the cell theory is a myth, and that the reticulum forms a common medium into which the nuclei migrate, instancing the formation of the neural crest, I cannot agree with him. The ontogenetic changes that appear in Necturus seem to me to refute such a supposition.

At an early period of development the superficial ectoderm lies directly on the neural tube, and, as Sedgwick states, fine protoplasmic threads connect the two, otherwise sharply bounded, tissues. Later mesenchyme appears between the skin and brain, or, as Sedgwick tells us, nuclei migrate from below into the existing reticulum. The protoplasmic threads, however, that at first connect the brain and superficial ectoderm in Necturus contain no yolk granules, while the nuclei that come from below advance in a surrounding reticulum heavy with enormous yolk granules. Evidently they did not migrate into the reticulum they found, but brought their own reticulum with them. We have now a reticulum between the brain and skin, composed in part, as before, of delicate fibres that arise

from the skin and neural tube, but chiefly formed by the protoplasmic prolongations of the yolk-laden reticulum of the mesenchyme. Into this reticulum the nuclei of the neural crest, as Sedgwick tells us, migrate. But in the head of Necturus the protoplasm of the neural crest is filled with small yolk granules, and here again the nuclei take their surrounding protoplasm with them. A common reticulum, therefore, does not exist.

Is the cell, then, a separate unit, or merely the node of a reticulum? In Necturus many cells of the neural crest migrate from the dorsal wall of the brain to the branchial arches, while cells arising in the wall of the archenteron migrate upwards until they lie between the dorsal wall of the brain and the skin. I have not used microscopic methods that enable me to state from observation that a fusion of the protoplasmic threads passing from cell to cell does or does not take place. But if such a fusion exists, it is continually renewed to be immediately interrupted, since the nuclei in migrating past one another take their "nodes" with them. That they do this is most clearly evinced in the formation of the aortic arches, where individual cells of endodermic origin migrate with their large yolk granules through surrounding mesectodermic tissue from which the yolk granules have nearly disappeared. This constant association of a particular bit of protoplasm with a particular nucleus makes the existence of the separate and distinct cell highly probable, despite the reticular structure of the mesenchyme.

I must also dissent from Sedgwick's statement that cells of the neural crest give rise to the walls of the vascular system and to muscular tissues. It is, however, true that in Necturus cells of the mesectoderm are converted into blood-corpuscles. While the majority of the blood-corpuscles are derived from the endoderm, the mesectoderm of the branchial arches is also a source of their formation.

The path of the ophthalmicus profundus is originally occupied by cells that, like those of the cranial ganglion, result from the fusion of the neural crest with the ectoderm. The

connection of these cells with one another is at first merely by indifferent protoplasmic prolongations, such as connect the primitive neural crest cells with one another and with the brain. The cells on the path of the profundus develop nerve-fibres at about the same time as the anterior cranial ganglia. The remaining nerves that supply the skin are formed in part by fibrous prolongations from the cells of the ganglion with which they are connected, in part by the migration of bipolar cells from the ganglion, also by the distal addition of fibrous prolongations from the deeper layer of the superficial ectoderm, and finally by the direct migration of cells from this layer into the nerve. These last elements contribute also to the formation of the profundus. The difference between the formation of this nerve and that of the remaining sensory nerves consists chiefly in the fact that cells of the neural crest which take part in their formation are first gathered into a mass which we recognise as the Anlage of a ganglion, while neural crest cells participate in the formation of the profundus throughout its length. Even this difference is not absolute, for some of the scattered mesectoderm cells lying near the ganglion, but not included in its mass, contribute also to the formation of the sensory branches of the facialis.

I speak of the rudiment of the ganglion as if already composed of ganglion cells, although in fact I doubt if one completely developed ganglion cell exists in Necturus at the time this study closes. At present the cells of the ganglion resemble cells on the path of the nerve whose function it probably is to contribute to co-ordination merely by conduction, and not by modification of the impressions received.

As the nerve-cell becomes fibrillar, the entire ectoderm of the sensory ridges of the head gives rise to a multitude of tiny fibres which cover the inner surface of the sensory area like fur, and appear finally to be swept together into bundles as if by currents of conduction. Pl. 36, fig. 24, shows the tiny threads that line the sensory ectoderm of the ventral hyomandibular, inner (mandibular) line as the nerve begins to form. Fig. 25 shows the general reticulum that connects the

skin with the underlying tissues in a region not specially sensory.

Fig. 30 shows a later stage in the development of the hyomandibular nerve, when the forest of tiny fibres seen in fig. 24 has been swept into bundles connected with the nerve.

On the lateral lines of the trunk the fibres formed in the deeper protoplasm of the ectoderm do not at first penetrate the limiting membrane that bounds the inner surface of the skin, but become gathered into a nerve before leaving the ectoderm, thus giving rise to the nerve in the manner described by Dohrn (**8**).

The branchial, pharyngeal, and palatine nerves have relatively fewer cells, and are chiefly formed by outgrowths from the ganglion, yet cells from the ganglion also migrate into these nerves. Pl. 36, fig. 23, shows the distal part of the glosso-pharyngeus, and, for the relative number of cells, may be compared with the ophthalmicus superficialis (fig. 26), the buccalis (fig. 22), or the distal hyomandibularis (Pl. 37, fig. 30).

The beginning of the motor spinal nerve has been described. The distribution of its branches to the muscle plate is peculiar. As has been described in other Amphibia, the motor fibres of the spinal nerves pass directly through the ganglion. Fig. 27 gives a section through the fourth spinal, second brachial, ganglion. The muscle plate is cut above and below as it curves over the outer surface of the ganglion, and the section shows six motor nerves on their way to the muscle plate. These are not the only or even the main motor nerves of the segment, but the section serves to illustrate the irregularities that occur in the distribution of the spinal motor nerves.

Figs. 28 and 29 show two sensory spinal nerves that underlie the skin on the dorsal surface of the embryo. The structure of the nerves suggests the formation of nerves in the tail of the frog as described by Hensen (**19**). The nerves run longitudinally, and are connected with the ganglion by a nerve that passes through the dorsal part of the muscle plate to the surface of the embryo. The cells forming these dorsal nerves do

not resemble the bipolar cells that migrate from a ganglion, but look like connective-tissue cells changed in situ into nerve cells. The cells may have migrated from the skin, but I have no evidence that they do so, and incline to believe them lost cells of the neural crest, since a few scattered cells of the neural crest remain in this dorsal region. That they are in fact nerve cells is proved by their fibrillar striation and deep stain when treated with gold chloride.

The existence of nerves such as these, where the separate cell elements can be followed to their terminal fibrillæ, lends support to the view that the cells composing any nerve are not fused but separate, though indistinguishably so when united in a nerve-cord wherein the fibrillæ lie parallel to one another throughout their length.

The sections (figs. 28 and 29) speak more positively against the view that each nerve-fibre extends from the ganglion to the sensory surface, for these nerves are evidently cell chains. The frequent use of shorter paths offered by anastomosing branches shows, moreover, that the attachment of the superficial receptive cell to one fibre of transmission is not constant. The shorter path when offered is at once accepted.

This study, therefore, leads to the conclusion that it is of little moment whether the motor and sensory fibres belonging to the primitive nerves of any segment enter the brain by one root, by two roots, or by several, the position of the nerve-root being in great measure an expression of the co-ordinate relations which the central nervous system subserves. The morphological value of the nerve comes from without, and "the metameric arrangement of the peripheral nerves is probably not primary, but occurs in adaptation to the segmentation of the structures they supply" (Froriep, **14**, p. 590).

9. Summary.

(1) An early differentiation of the ectoderm into three longitudinal ridges on each side of the embryo, connected by intersegmental transverse ridges, forms the basis from which the lateral line system develops.

(2) The supra-orbital line of sense-organs and the ear form by direct modification of the primitive ridge of the dorso-lateral line. Sense-organs connected with the dorsal branches of the glossopharyngeus and vagus form from ridges that secondarily grow upwards from the epibranchial ridge after the primitive ridges of the corresponding intersegments have disappeared in giving rise to mesectoderm. The infra-orbital line of sense-organs, the otic sense-organ, the lower organs supplied by the glossopharyngeal nerve, and the organs of the epibranchial vagus line develop by the modification of the primitive epibranchial ridge. The primitive intersegmental thickening of the ectoderm where it touches the hyomandibular pocket in the line of the gill cleft is directly modified into the hyomandibular line of sense-organs. The mandibular line develops as a secondary outgrowth from the hyomandibular line. The primitive ridge of ectoderm at the third vagus cleft is the beginning from which the ventral trunk line develops. The anterior part of the primitive ventral longitudinal ridge is retained in the ventral part of the hyomandibular line of sense-organs (the internal mandibular). The lateral lines of the trunk grow from the head backwards through indifferent ectoderm, and do not result from the direct modification of primitive ridges.

(3) The primitive ridges differ functionally as well as structurally from those of the lateral line. The former are the source of both nervous and connective tissues, the latter of nervous tissues alone.

(4) The primitive ridges of the trunk, like those of the head, are sources of addition to the mesectoderm.

(5) Few of the cells that disappear from the secondary sensory ridges of the trunk, as the sense-organs arise, enter the lateral nerve. The majority of these cells migrate between the two layers of the ectoderm towards the terminal point of growth in the advancing line.

(6) The third vagus cleft forms in the intersegmental plane once occupied by the second vagus cleft, and the primitive vagus myotome divides in the plane secondarily occupied by the second vagus cleft, while the nerves of the second vagus

arch arise from the root of those of the first arch. These facts suggest that a vagus segment is interpolated in the original metamerism.

(7) While some fibres from the lateral line ganglion enter the vagus root, most of the fibres enter the brain through the root of the glosso-pharyngeus, using for this purpose the neural crest cells which bridge the space between the vagus and glosso-pharyngeal ganglia.

(8) Many cells of the neural crest of the trunk do not take part in the formation of spinal ganglia, but form part of the connective tissue at the side of the spinal cord.

(9) The motor nerves of the trunk appear before the spinal ganglia, and are formed by the migration of bipolar cells from the spinal cord.

(10) The nerve which underlies the infra-orbital sensory line divides throughout its length into two nerves connected respectively with the Gasserian and facial ganglia, thus repeating the relation of the nerves derived from the supra-orbital ridge, the ophthalmicus profundus, and superficialis.

(11) The ramus hyomandibularis and its direct continuation, the mandibularis internus, appear in Necturus as the post-trematic branch of the hyoid arch, while the hyoid nerve resembles the lateral nerves of the posterior arches.

(12) Cells of ectodermic origin contribute to the formation of blood-corpuscles in the branchial region.

(13) Although delicate protoplasmic prolongations connecting cell with cell initiate the specialised co-ordination of the nervous system, a common reticulum, such as Sedgwick describes, into which nuclei migrate, does not exist in Necturus.

(14) The root of the sensory nerve is no index to the segmental value of the nerve.

Baltimore; April 8th, 1895.

Literature References.

1. Allis, E. P.—"The Anatomy and Development of the Lateral Line System in Amia calva," 'Journal of Morphology,' vol. ii, 1889.
2. Beard, J.—"On the Segmental Sense-organs of the Lateral Line, and on the Morphology of the Vertebrate Auditory Organ," 'Zool. Anzeiger,' 1884.
3. Beard, J.—"The Ciliary or Motoroculi Ganglion and the Ganglion of the Ophthalmicus Profundus in Sharks," 'Anat. Anzeiger,' Bd. ii, 1887.
4. Beard, J.—"Morphological Studies: II. The Development of the Peripheral Nervous System of Vertebrates," 'Quart. Journ. Micr. Science,' vol. xxix, 1888.
5. Boveri, Theodor.—"Die Nierencanälchen des Amphioxus," 'Zool. Jahrbücher,' Abth. f. Anat. u. Ontog. d. Thiere, Bd. v, 1892.
6. Dohrn, Anton.—Studie 14: "Ueber die erste Anlage und Entwickelung der motorischen Rückenmarksnerven bei den Selachiern," 'Mittheilungen a. d. Zoologischen Station zu Neapel,' Bd. viii, 1888.
7. Dohrn, Anton.—Studie 16: "Ueber die erste Anlage und Entwickelung der Augenmuskelnerven bei Selachiern und das Einwandern von Medullarzellen in die motorischen Nerven," 'Mittheilungen a. d. Zoologischen Station zu Neapel,' Bd. x, 1891.
8. Dohrn, Anton.—Studie 17: "Nervenfaser und Ganglienzelle," 'Mittheilungen a. d. Zoologischen Station zu Neapel,' Bd. x, 1891.
9. Ecker, A., und Wiedersheim, R.—'Die Anatomie des Frosches,' Braunschweig, 1881.
10. Ewart, J. C.—"The Lateral Sense-organs of Elasmobranchs: I. The Sensory Canals of Læmargus," 'Trans. Roy. Soc. of Edinburgh,' vol. xxxvii, 1893.
11. Froriep, A.—"Ueber Anlagen von Sinnesorganen am Facialis, Glossopharyngeus, und Vagus," 'Archiv f. Anatomie u. Entwickelungsgeschichte,' 1885.
12. Froriep, A.—"Ueber das Homologon der Chorda tympani bei niederen Wirbelthieren," 'Anat. Anzeiger,' Bd. ii, 1887.
13. Froriep, A.—"Zur Entwickelungsgeschichte der Kopfnerven," 'Verhandlungen d. Anat. Gesellschaft,' 1891.
14. Froriep, A.—"Entwickelungsgeschichte des Kopfes," 'Ergebnisse d. Anatomie u. Entwickelungsgeschichte,' Bd. i, 1891.
15. Gegenbaur, C.—'Das Kopfskelet d. Selachier,' Leipzig, 1872.
16. Goette, A.—'Entwickelungsgeschichte der Unke,' Leipzig, 1875.

17. Goronowitsch, N.—"Die Axiale u. d. laterale Kopfmetamerie der Vogelembryonen," 'Anat. Anzeiger,' Bd. vii, 1892.

18. Goronowitsch, N.—"Untersuchungen über die Entwickelung der sogenannten 'Ganglienleisten' im Kopfe der Vogelembryonen," 'Morphologisches Jahrbuch,' Bd. xx, 1893.

19. Hensen, V.—"Ueber die Entwickelung des Gewebes und der Nerven im Schwanze der Froschlarve," 'Virchow's Archiv,' Bd. xxxi, 1864.

20. His, W.—"Die Neuroblasten und deren Entstehung im Embryonalen Mark," 'Abhandl. d. math. phys. Classe d. Königl. Sächsischen Gesellschaft d. Wissenschaften,' Bd. xv, 1889.

21. Johnson and Sheldon.—"Notes on the Development of the Newt," 'Quart. Journ. Micr. Sci.,' vol. xxvi, 1886.

22. Kupffer, C. von.—"Die Entwickelung von Petromyzon planeri," 'Archiv f. mikrosk. Anatomie,' Bd. xxxv, 1890.

23. Kupffer, C. von.—"Die Entwickelung der Kopfnerven der Vertebraten," 'Verhandlungen d. Anat. Gesellschaft,' 1891.

24. Kupffer, C. von.—'Studien zur vergleichenden Entwickelungsgeschichte des Kopfes der Kranioten,' Heft 2, München, 1894.

25. Lee, A. B.—'The Microtomist's Vade Mecum,' London, 1893.

26. Leydig, F.—"Integument und Hautsinnesorgane der Knochenfische Weitere Beiträge," 'Zool. Jahrbücher,' Abt. f. Anat. u. Ontog., Bd. viii, 1894.

27. Mall, F. P.—"Histogenesis of the Retina in Amblystoma and Necturus," 'Journal of Morphology,' vol. vii, 1893.

28. Merkel, Fr.—"Sinnesorgane," 'Ergebnisse d. Anat. u. Entwickel.,' Bd. iii, 1893.

29. Mitrophanow, P.—"Étude embryogénique sur les Sélaciens," 'Archives de zoologie expérimentale et générale,' t. xxxi, 1893.

30. Platt, Julia B.—"Contribution to the Morphology of the Vertebrate Head," 'Journal of Morphology,' vol. v, 1891.

31. Platt, Julia B.—"Further Contribution to the Morphology of the Vertebrate Head," 'Anat. Anzeiger,' Bd. vi, 1891.

32. Platt, Julia B.—"Ontogenetische Differenzirung des Ektoderms in Necturus," 'Archiv f. mikroskop. Anatomie,' Bd. xliii, 1894.

33. Pollard, H. B.—"On the Anatomy and Phylogenetic Position of Polypterus," 'Zool. Jahrbücher,' Bd. v, 1892.

34. Sedgwick, Adam.—"On the Inadequacy of the Cellular Theory of Development, and on the Early Development of Nerves, particularly of the Third Nerve and of the Sympathetic in Elasmobranchii," 'Quart. Journ. Micro. Sci.,' vol. xxxvii, 1894.

35. Stannius.—'Das peripherische Nervensystem der Fische,' Rostock, 1849.

36. Strong, O. S.—"The Cranial Nerves of Amphibia," 'Journal of Morphology,' vol. x, 1895.

37. Wijhe, J. W. van.—"Ueber die Mesodermsegmente und die Entwickelung der Nerven des Selachierkopfes,' Amsterdam, 1882.

38. Wijhe, J. W. van.—"Ueber das Visceralskelett und die Nerven des Kopfes der Ganoiden und von Ceratodus," 'Niederländisches Archiv für Zoologie,' 1882.

39. Wilson, H. V.—"The Embryology of the Sea-bass (Serranus atrarius)," 'Bulletin of the U.S. Fish Commission,' vol. ix, 1891.

EXPLANATION OF PLATES 36—38,

Illustrating Julia B. Platt's paper, "Ontogenetic Differentiations of the Ectoderm in Necturus."

Lettering.

a, a', b, c. Dividing cells. *ar.* Fore-limb. *b. hm.* Branches of hyomandibularis VII. *br*$^{1-3}$. First to third external gills. *buc.* Ramus buccalis VII. *buc. p.* Ramus buccalis profundus V. Dohrn's "N. infra-maxillaris" in Selachii (**8**, p. 267). v. Wijhe's "ramus maxillaris superior" of Ganoidei (**38**, p. 207). *d*^{1}. Dorsal branch of glosso-pharyngeus. *d*^{2}. Dorsal branch of vagus. *d. l. l.* Dorsal lateral line. *d. r.* Dorsal longitudinal ridge. *end.* Endoderm. *ep.* Epibranchial branch of vagus. *ep. r.* Epibranchial ridge. *ep. s. o.* Epibranchial sense-organs. *ex. b*$^{1-3}$. Branches of glosso-pharyngeus and vagus supplying external gills. *gl. s. o.* Glosso-pharyngeal sense-organs. *hm. r.* Hyomandibular ridge. *hm. s. o.* Hyomandibular sense-organs. *io. r.* Infra-orbital ridge. *io. s. o.* Infra-orbital sense-organs. *l. b.* Light band on each side of the body. *l. l. s. o.* Sense-organs of the lateral line. *l. n.* Lateral nerve. *m.* Mouth. *md.* Ramus mandibularis V and VII. *md. s. o.* Mandibular sense-organs. *mect.* Mesectoderm. *m. l. l.* Median lateral line. *m. n.* Motor nerve. *m. r.* Mandibular ridge. *mx.* Ramus maxillaris V. *my*$^{1-11}$. Myotomes, first to eleventh. *n.* Nose. *n. ct.* Neural crest. *oph. p.* Ramus ophthalmicus profundus V. *oph. s.* Ramus ophthalmicus superficialis VII. *ot.* Ramus oticus VII. *pal. e.* Ramus palatinus externus VII. *pal. i.* Ramus palatinus internus, chief palatine branch of the facialis. *p. ep. r.* Primitive epibranchial ridge. *ph*$^{1-3}$. Rami pharyngei IX and X. *p. tr.* Posterior transverse ridge of the branchial region. *so. r.* supra-orbital ridge. *so. s. o.* Supra-orbital sense-organs. *sp. g.* Spinal ganglia. *sp. n*$^{1, 2}$. First and second spinal nerves. *t. r.* Transverse (intersegmental) ridge. *v. hm. s. o.* Ventral hyo-

mandibular sense-organs, i.e. sense-organs of the primitive ventral longitudinal ridge. *v. l. l.* Ventral lateral line. *v. r.* Ventral longitudinal ridge. IX, X^{1-3}. Rami post-trematici glosso-pharyngei et vagi. X *g.* Lateral line ganglion. IX *s. o.*, X *s. o.* Sense-organs supplied respectively by dorsal branches of the glosso-pharyngeus and vagus. * Branch of ramus buccalis VII. ? Sense-organs on the vagus commissural line in an unusual position. 1, 2, 3, 4. Sense-organs supplied by buccalis VII and ophthalmicus profundus V.

PLATE 36.

FIG. 2.—*a.* Cross-section through the supra-orbital sensory ridge. *b.* Cross-section of the primitive supra-orbital (dorso-lateral) ridge. The planes of sections 2, 3, 4, 5, are given in Pl. 38, fig. 1.

FIG. 3.—Cross-section through the facial part of the primitive epibranchial ridge.

FIG. 4.—Cross-section through the primitive epibranchial ridge, as it gives rise to the lateral ganglion.

FIG. 5.—Horizontal section through the lateral line ridge of Pl. 38, fig. 1.

FIGS. 6—17 are from younger embryos than that reconstructed in Pl. 38, fig. 1.

FIGS. 6 and 7.—Cross-sections through the vagus myotome and ganglion. Fig. 7 is posterior to Fig. 6.

FIG. 8.—Cross-section between the second and third myotomes.

FIG. 9.—Cross-section between the fourth and fifth myotomes.

FIG. 10.—Cross-section showing the first protoplasmic prolongations at the root of the motor nerve.

FIG. 11.—Cross-section through the fifth myotome, showing the distribution of yolk granules in the tissues of the trunk at the time when the neural crest forms.

FIG. 12.—Cross-section between the fourth and fifth myotomes in an embryo older than that of Fig. 9.

FIG. 13.—Cross-section through the ventral root of the fifth spinal nerve.

FIGS. 14 and 15.—Cross-sections through the ventral roots of the fourth spinal nerves.

FIG. 16.—Cross-section through the root of the tenth spinal nerve.

FIG. 17.—Cross-section showing the beginning of the motor root of the third spinal nerve.

FIG. 19.—Section through the lateral line ridge of an embryo at the stage of development given in Pl. 38, fig. 1, where the plane of the section is marked.

FIG. 20.—Section through the skin of the embryo of Fig. 18, where the plane of the section is given.

Fig. 22.—Section through the ramus buccalis as the trigeminal and facial parts diverge.

Fig. 23.—Section through the distal part of the post-trematic branch of the glosso-pharyngeus.

Fig. 24.—Section through a sense-organ in the ventral part of the hyo-mandibular line, as the nerves begin to form.

Fig. 25.—Section through the skin above the ear.

Fig. 26.—Section showing the innervation of the supra-orbital sensory ridge by united branches from the trigeminal and facial nerves.

PLATE 37.

Fig. 27.—Section through the second brachial ganglion, showing six of the motor nerves that supply the adjacent myotome, which the section cuts above and below, as it curves around the ganglion.

Figs. 28 and 29.—Sections through dorsal sensory nerves of the trunk. The arrow in Fig. 29 shows the direction of the main stem.

Fig. 30.—Section showing the distal distribution of branches from the ramus hyomandibularis VII.

PLATE 38.

Fig. 1.—Embryo 12 mm. long, with ectodermic ridges reconstructed, and the position of the anterior ganglia indicated.

Fig. 18.—Embryo 13 mm. long, with the ectodermic ridges reconstructed, and the position of the anterior ganglia and lateral line nerves indicated.

Fig. 21.—Embryo 15 mm. long, showing the ectodermic ridges and sense-organs of the lateral line. Rudiments of the external gills have appeared, and the peripheral nerves begin to develop. Facial, vagus, and lateral line nerves are given in red; the trigeminal and glosso-pharyngeal nerves in black.

Fig. 31.—Embryo 19 mm. long, showing the anterior sense-organs of the lateral line system, and their innervation from the ventro-lateral surface. The external gills and fore-limb are removed. The nerves are coloured as in Fig. 21.

Fig. 32.—Embryo at the same stage of development as in Fig. 31. The distribution of the sense-organs on the trunk is given, and the present pigmentation of the embryo indicated.

Fig. 33.—Head of an embryo at the same stage as Figs. 31 and 32, showing the distribution of sense-organs on the head, the dorsal surface of which is now quite evenly pigmented.

INDEX TO VOL. 38,

NEW SERIES.

Amia, the early development of, by Bashford Dean, Ph.D., 413

Amphibian embryos, ciliation of the ectoderm of, by R. Assheton, 465

Assheton on the ciliation of the ectoderm of the Amphibian embryo, 465

Asterina gibbosa, development of, by E. W. Macbride, 339

Benham on Kynotus cingulatus, an earthworm from Madagascar, 445

Bidder on the collar-cells of Heterocœla, 9

Bourne, G. C., a criticism of the cell-theory, being an answer to Mr. Sedgwick's article, 137

Browne, E. T., on the variation of Haliclystus, 1

Bury, H., on the metamorphosis of Echinoderms, 45

Cells, iron compounds in, by A. B. Macallum, 175

Cells, reproductive, of Elasmobranchs, by J. E. S. Moore, 275

Cell-theory—answer to Sedgwick's article, by G. C. Bourne, 137

Cell-theory, further remarks on, with a reply to Mr. Bourne, by Adam Sedgwick, 331

Ciliation of the ectoderm of the Amphibian embryo, by Richard Assheton, 465

Collar-cells of Heterocœla, by Bidder, 9

Dean, Bashford, on the early development of Amia, 413

Earthworm from Madagascar, by Benham, 445

Echinoderms, the metamorphosis of, by H. Bury, 45

Fecundation of Sphærechinus and Phallusia, by M. D. Hill, 315

Haliclystus, variation of, by E. T. Browne, 1

Heterocœla, collar-cells of, by Bidder, 9

Hill, M. D., on the fecundation of the egg of Sphærechinus and of Phallusia, 315

Iron compounds in cells, by A. B. Macallum, 175

Kynotus cingulatus, an earthworm from Madagascar, by W. B. Benham, 445

Macallum, A. B., on assimilated iron compounds in animal and vegetable cells, 175
Macbride, E. W., on the development of Asterina gibbosa, 339
Moore, J. E. S., on the structural changes in the reproductive cells during the spermatogenesis of Elasmobranchs, 275

Necturus, differentiation of the ectoderm in, by Julia B. Platt, 485
Nervous system of Necturus, its development, by J. B. Platt, 485

Platt on the development of the peripheral nervous system in Necturus, 485

Sedgwick on the cell-theory, with a reply to Mr. Bourne, 331
Spermatogenesis of Elasmobranchs, by J. E. S. Moore, 275

PRINTED BY ADLARD AND SON,
BARTHOLOMEW CLOSE, E.C., AND 20, HANOVER SQUARE, W.

3 2044 106 275 415