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NOTICE.

Beginning with Vol. XVI., each article will have a paging of its own; and the position of an article in a volume will be indicated by the number placed at its head.

It is hoped that this arrangement, which enables us to print papers independently of one another, will ensure a more rapid publication of the material than has been possible heretofore.

All communications relating to this Journal should be addressed to the Director of the College of Science.

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JOURNAL OF THE COLLEGE OF SCIENCE, IMPERIAL UNIVERSITY
TOKYO, JAPAN.

VOL. XVII, ARTICLE 1.

Ammonium and other Imidosulphites.

By

E. Divers, and **M. Ogawa.**

The fact of the existence of ammonium imidosulphite as a product of the decomposition of the amidosulphite by very gentle heating,

$$2NH_2SO_2NH_4 = NH_3 + NH(SO_2NH_4)_2,$$

was brought before a meeting of the London Chemical Society last session (*Proc. Chem. Soc.* 1900, **16**, 113). The present paper consists of a fuller account of this salt and other imidosulphites than could then be given.

Ammonium amidosulphite, from which the imide salt is derived (this Journal, 1900, **13**, 187), is readily formed by the union of sulphur dioxide and ammonia, but is so unstable as to be largely decomposed by the unavoidable heating it suffers when these gases come together, unless cooled ether be used as their solvent. On keeping the dry salt at a temperature of about 35°, its decomposition goes on and ammonia escapes for some hours.

There is left a mottled orange mass, of waxy consistence, already described in the earlier paper (*loc. cit.*, p. 196), which is difficult to attack with solvents other than water, because it adheres very firmly to the vessel and must not be exposed to the air during treatment. By protracted digestion with successive portions of 90 per cent. spirit, aided by scraping with a pointed glass rod and strong shaking, it can however be nearly all dissolved up although only very sparingly soluble. The first portions of the solution are coloured and contain a salt the presence of which interferes with the preparation of the imidosulphite from them. The later colourless extracts yield the imidosulphite when they are evaporated, in the vacuum desiccator but not quite pure. Much better results are got by beginning the treatment with warm 95 per cent. spirit, used in successive portions, until the residue is a colourless powder, and only then resorting to the 90 per cent, spirit and carrying on the digestion at about 50°. The solution thus obtained deposits almost pure imidosulphite as it cools, and the mother-liquor can be used with advantage again and again to dissolve out more of the salt, although in that case the crystals which separate are somewhat impure. These can be purified by washing with absolute alcohol containing much ammonia to dissolve out the foreign salt (more soluble in presence of ammonia), and then dissolving up in warm 90 per cent. spirit and recrystallising. The original mother-liquor yields more imidosulphite, but impure, when it is either artificially cooled (as by ice and salt), or is evaporated in the desiccator. This impure salt can be purified in the way just described. All the ammonium imidosulphite is finally washed with ammoniacal alcohol, drained on a porous tile under close cover, and dried in a potash-desiccator. The quantity of pure salt actually obtained in this way approaches

that of a fourth only of the weight of decomposed amidosulphite taken, but the total production of imidosulphite will no doubt prove to be very much greater.

Analysis.—The salt, 0.4240 gram, distilled with potash yielded ammonia, 0.0817 gram, and then after heating for some time in a pressure-tube with hydrochloric acid and again distilling with potash, 0.03755 gram more ammonia. Simple distillation with potash of 0.4912 gram of another portion of salt gave 0.0939 gram of ammonia, and then, after oxidation by means of bromine followed by hydrochloric acid and potassium chlorate, 1.2886 grams of barium sulphate. The calculated and found precentages are :—

	Ammonia nitrogen.	Imide nitrogen.	Total nitrogen.	Sulphur.
$NH(SO_2NH_4)_2$;	15.64	7.82	23.46	35.75
Found ;	15.86	7.29	23.15	—
,,	15.74	—	—	36.02

It will be seen that the analysis establishes not only the composition of the salt to be $3NH_3,2SO_2$ but also its imide constitution.

Properties.—It occurs in minute micaceous needles. Heated very slowly in a tube it soon begins to decompose into volatile substances and a residue of sulphur, ammonium sulphate, and the $^2/_3$ normal ammonium imidosulphate $[NH(SO_3NH_4)_2]$. Even when the temperature is raised to 150°, no fusion takes place. The sublimates which form during the heating begin to appear at about 80° and consist apparently of ammonium pyrosulphite $[(NH_4)_2S_2O_5]$, and the unchanged imidosulphite $[(NH_4)_2S_2O_4NH]$. The salt is insoluble in alcohol, and in this respect is unlike ammonium amidosulphite, which is very soluble as ethyl ammonium-

sulphite and ammonia. The salt is only moderately deliquescent and, freshly prepared, is neutral to litmus.* It has a mild, unpleasant, sulphurous taste, which distinguishes it from the salt occurring with it, more freely soluble than it in 95 per cent. spirit, and already referred to.

It is freely soluble in water but slowly decomposes into thiosulphate and amidosulphate. This change beginning at once, the solution gives all the reactions of a thiosulphate. It goes on also in presence of hydrochloric acid, which when hot hastens its completion. Barium thiosulphate has been prepared from the solution, and amidosulphuric acid and ammonium amidosulphate also obtained from it. Quantitative determinations of the sulphur, sulphur dioxide, and amidosulphate, formed on boiling with hydrochloric acid, gave results in agreement with the following equation :—

$$2NH(SO_2NH_4)_2 + 2HCl = 2NH_2SO_3NH_4 + 2NH_4Cl + SO_2 + S,$$

in which the SO_2 and S represent decomposed thiosulphuric anhydride.

Potassium imidosulphite.—It has already been stated when detailing the analysis of the ammonium salt that that salt yields just two-thirds of its nitrogen as ammonia when boiled with potassium hydroxide in aqueous solution. In accordance with this fact, it has been found that potassium imidosulphite is obtained when to the ammonium salt dissolved in 70 per cent. spirit, alcoholic potash is added until the solution just renders red litmus paper permanently blue on exposure to air. The salt soon separates as minute micaceous crystals firmly adherent to the glass. After

* As first obtained by us last year and then described, the salt had an acid reaction and was exceedingly deliquescent. But as it showed a deficiency in ammonia (22.2 instead of 23.5 per cent.), and as its potassium derivative was not acid, we indicated our expectation that when obtained in a purer state the salt would prove to differ somewhat in properties from what we had then found.

repeated washing with absolute alcohol, the salt has been found to remain alkaline to litmus, quite possibly, however, because of the presence of a trace of tripotassium salt, as happens in the case of imidosulphates. The imidosulphite, unlike the corresponding potassium imidosulphate $NH(SO_3K)_2$, is very soluble in water and its solution gives the imidosulphite reactions of the ammonium salt. It has too the sulphurous taste of that salt. In the analysis, nitrogen was determined by the combustion method, sulphur by oxidation as in the analysis of the ammonium salt, and potassium by ignition with sulphuric acid, with the following results :—

	Pottasium.	Nitrogen.	Sulphur.
$NH(SO_2K)_2$;	35.29	6.33	28.96
Found ;	35.17	6.74	28.88
,,	—	6.93	—

Barium ammonium imidosulphite.—When the orange mass of decomposed amidosulphite is dissolved in water and mixed with baryta water in such quantity as to leave undecomposed some of the ammonium imidosulphite it contains, the filtered solution when concentrated in the desiccator deposits the double salt in minute micaceous crystals. Only barium and sulphur were determined. The results agree with calculation for $Ba(SO_2NHSO_2NH_4)_2$.

	Barium.	Sulphur.
$BaN_4H_{10}S_4O_8$;	29.85	27.89
Found ;	29.94	26.90

In the *Proc. Chem. Soc.*, (1900, 16, 104), the existence of this salt was indicated, but by mistake it had been taken to be a salt of the acid, $N_2H_4S_2O_3$, and was accordingly formulated as

$Ba(N_2H_3S_2O_3)_2$, $2OH_2$. It is soluble in water and its solution behaves as that of an imidosulphite, being precipitable by baryta ($N_2H_4S_2O_3$ salts are not), and besides at once gives off ammonia when moistened with potassium or barium hydroxide solution.

The treatment of the orange mass of decomposed ammonium amidosulphite with 95 per cent. spirit, as a preliminary to dissolving out the main quantity of imidosulphite with 90 per cent. spirit, yields yellow alcoholic solutions which on evaporation in the desiccator deposit crystals which are short thick prisms almost cubical in appearance and about 2 mm. across. They are thus quite unlike the minute micaceous needles of ammonium imidosulphite. They are yellow but the colour is adventitious. They can be purified and rendered white by putting them into 95 per cent. spirit and then almost saturating this with ammonia while the containing flask is kept immersed in cold water. In this alcoholic ammonia they are very sensibly soluble. The solution is decanted and the treatment repeated until only a small quantity of white powdery salt remains, principally imidosulphite The solutions left for a while in open flasks and then exposed in the desiccator over sulphuric acid lose most of the ammonia, and the crystals reform from the solution. Washed with alcohol they are left quite colourless. The mother-liquors evaporated in the desiccator yield crude yellow crystals again which can be recrystallised from alcoholic ammonia as before.

These crystals are recrystallisable without change and have also been prepared by us in two successive winters, yet they give analytical results which are closely concordant with the remarkable empirical formula, $4NH_3$, $5SO_2$ or $N_4H_{12}S_5O_{10}$.

	Nitrogen.	Sulphur.
$N_4H_{12}S_5O_{10}$;	14.43	41.24
Found in 1900;	14.11	41.14
,, ,,	14.36	40.82
,, in 1901;	14.02	41.02
,, ,,	14.40	—

These crystals are neutral and have a bitter taste, not a sulphurous one. They are freely soluble in water and very deliquescent. The solution is unstable and in some of its reactions greatly resembles that of ammonium imidosulphite. With potassium hydroxide the salt evolves ammonia at once, but analysis of potassium salts prepared from it in 70 per cent. alcoholic solution have given discordant results. A striking difference from an imidosulphite is that its fresh solution gives no barium precipitate even in presence of ammonia. Also that in freshly prepared solution, it does not decolourise iodine solution, and only slowly cold acid permanganate. It also does not give the ferric-chloride colouration of a thiosulphate which the imidosulphite does give. Its solution becomes very acid after a time and then smells much more strongly of sulphur dioxide than a similar solution of imidosulphite. The crystalline matter having nearly the composition expressed by $9NH_3$, $8SO_2$, mentioned on p. 197 of our paper on ammonium amidosulphite (this Journal, 1900, 13), we believe to have been the present salt mixed with imidosulphite and amidosulphate. On a future occasion we hope to be in a position to report upon the constitution of the body we have here described.

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VOL. XVII, ARTICLE 2.

Nitrilosulphates.

By

E. Divers and **T. Haga.**

History.—Nitrilosulphates were discovered in 1845 by Fremy, who named them sulphammonates. Their sulphonic constitution was made evident by Claus and Koch in 1869, and their nitrilic constitution by Berglund in 1875, when they received the name of nitrilosulphonates. Finally, Raschig rendered the constitution of these salts still more certain in 1887. These chemists, it should be mentioned, added nothing to the experimental facts concerning the nitrilosulphates which Fremy had made known, but Claus and Koch determined the nature of the 'sulphazidates,' salts derived from Fremy's 'neutral sulphazotates' by hydrolysis; Raschig proved the constitution of the last-named salts, which by sulphonation become the sulphammonates; and Berglund established the constitution of the 'sulphamidates' into which the sulphammonates are transformed by hydrolysis; all the experimental interconnections of these salts, here relied on, having been ascertained

by Fremy himself. The recognition of the constitution of the nitrilosulphates by Fremy was a moral impossibility, for their discovery had come, so to say, much before its time.

Name.—The reason for the substitution of the name nitrilosulphates for that of nitrilosulphonates, chosen for these substances by Berglund, has been stated on a former occasion (this Journal, 1896, 9, 220). As nitriles they are sulphates—nitrilosulphates therefore. Suitable alternative names are aminetrisulphonates and trisulphamates.

Preparation.—Ammonium nitrilosulphate, $4NH_3, 3SO_3$, cannot be obtained by the union of ammonia with sulphur trioxide, for that results in the production of the imidosulphates, which are not resolvable by heat into nitrilosulphate and ammonia, since the less ammoniated $^2/_3$-normal salt, instead of breaking up in this way-boils freely at about 355°, under reduced pressure, with very little decomposition and no production of nitrilosulphate (this Journal, 1894, 6, 55). Nitrilosulphates are only to be prepared by sulphonation of hydroximidosulphates. Fremy made known two ways in which this can be accomplished, of which however only one admits of general application. This is to treat the hydroximidosulphate with sulphur dioxide in presence of a base, a process which resolves itself in practice into treating the corresponding nitrite in this way, since the hydroximidosulphate has itself to be prepared by a similar sulphonation of the nitrite. The other way of preparing nitrilosulphates by sulphonation is to add a nitrite to an excess of solution of a pyrosulphite, when in the case of the potassium salt, the nearly insoluble nitrilosulphate soon separates. Other nitrilosulphates, being more soluble, can hardly be obtained directly in this way, but, as Fremy has shown, a solution of the ammonium salt thus prepared, very impure though it will be, can

be made to yield potassium nitrilosulphate by double decomposition.

Ammonium salt.

Nothing has been done concerning this interesting salt, $N(SO_3NH_4)_3$, $2OH_2$, since Fremy described it, but as its existence has been ignored since the time of the publication of its discovery until now, we hold it important to again introduce some account of it, derived from Fremy's memoir (*Ann. Chim. Phys.*, 1845, **15**, 408) into chemical literature. In presence of a large excess of ammonia a concentrated solution of ammonium nitrite is submitted to the action of a current of sulphur dioxide until abundant precipitation of crystals has occurred in the solution kept sufficiently cool. The washed and dried salt gave Fremy the following numbers on analysis, indicating the presence of one molecule of water of crystallisation :

	Sulphur.	Nitrogen.	Hydrogen.
$N_4H_{14}S_3O_{11}$:	29.45	17.18	4.29
Found ;	30.2	15.7	4.5

Since the potassium nitrilosulphate contains two mols. water, it is probable that the ammonium salt contains the same, and that when Fremy determined the sulphur the salt had, like the sodium salt, lost some of its water during its stay in the desiccator. We write, therefore, the formula of the salt, $N(SO_3NH_4)_3$, $2OH_2$.

Ammonium nitrilosulphate is in minute crystals which have only a slight taste, and which are somewhat sparingly soluble in water, but so much more so than the potassium salt that Fremy suggested that its solution might be used as a qualitative reagent for potassium salts. It is not volatilised by heat but decomposed into sulphate. It is a very unstable salt, being liable in the solid state to decompose (hydrolyse) suddenly with a hissing sound,

charring paper in contact with it. We have already published (this Journal, 1900, 13, 307) our success in sulphonating ammonium nitrite into hydroximidosulphate by a method, which carried farther, gave Fremy the nitrilosulphates.

Ammonium nitrilosulphate has the interest attached to it of being one more compound of ammonia with sulphur trioxide to be added to those recognised. There are six such compounds :—

(1)	$NH_3, 2SO_3$	=	$HN(SO_3H)_2$
(2)	NH_3, SO_3	=	$H_2N\ SO_3H$
(3)	$4NH_3, 3SO_3$	=	$N(SO_3NH_4)_3$
(4)	$3NH_3, 2SO_3$	=	$HN(SO_3NH_4)_2$
(5)	$4NH_3, 2SO_3$	=	$(NH_4)N(SO_3NH_4)_2$
(6)	$2NH_3, SO_3$	=	$H_2N\ SO_3NH_4$.

Of these the second is amidosulphuric acid and the sixth its ammonium salt (this Journal, 1896, 9, 219) ; the first imidosulphuric acid, known only in unstable solution (this Journal, 1894, 6, 51) ; the fourth is its $^2/_3$-normal ammonium salt (parasulphatammon of Rose) ; and the fifth, polymeric with the sixth, is its normal ammonium salt (sulphatammon of Rose ; this Journal, 1894, 6, 53) ; and the third is ammonium nitrilosulphate here described. These six compounds can all be derived the one from the other, backwards as well as forwards, except the nitrilosulphate which cannot be re-formed from the others although itself the most convenient source of them. Nitrilosulphuric acid would be the seventh of these compounds, heading the column as $NH_3, 3SO_3$, if it could exist in the free state.

Potassium salt.

The potassium salt, $N(SO_3K)_3, 2OH_2$, is familiar to those who have occupied themselves with the study of Fremy's sulphazotised salts, being one of the most insoluble of potassium salts, even

more so than the perchlorate (Fremy). It forms slender needles of pearly lustre which in crystallising fill its mother-liquor. The work of later investigators has added nothing to the account of it given by Fremy, except that the crystals are rhombic (Raschig and Fock).* His analyses gave results which agree well with calculation for the true composition of the salt, though not with his own formula for it. By preference he prepared it by passing sulphur dioxide into a solution, not too concentrated, of the nitrite and the hydroxide. With the neglect of details characteristic of his celebrated memoir, he fails however to mention the essential potassium hydroxide. Probably on account of this, later workers, have only made use of his other process, which consists in mixing solutions of the nitrite and pyrosulphite.

Sodium salt, $N(SO_3Na)_3$, $5OH_2$.

A strong solution of sodium pyrosulphite poured upon solid sodium nitrite furnished Raschig (*Annalen*, 1887, **241**, 180 and 229) with a useful solution of the nitrilosulphate, although it contained much hydroximidosulphate, sulphite, and unchanged nitrite; sodium hydroxide he represented also to be present, but that could not have been the case (this Journal, 1900, **13**, 284). Sodium nitrilosulphate being very soluble in water, he did not isolate it in the solid state. We have found that instead of three mols. pyrosulphite, which was the quantity used by Raschig to two mols. nitrite, at least four mols. must be taken if most of the hydroximidosulphate is to be sulphonated.† By preparing

*Misled by the faulty translation in the *Annalen* (1845, **56**, 342) of Fremy's paper, Claus and Koch supposed that he had said that red fumes were evolved when the salt is heated. Fremy's statement is that the salt does not evolve red fumes, which is correct.

†Claus and Koch found it best to take still more pyrosulphite when working with the potassium salts (*Annalen*, 1869, **152**, 336), and they were right, for in the case of that insoluble nitrilosulphate, great excess of sulphite did not matter; here it does.

from the carbonate (using this in hydrated crystals, along with only half its weight of water, and saturating with sulphur dioxide) a fresh and pure solution of pyrosulphite as concentrated as possible, pouring this solution upon the nitrite dissolved in its weight of water, one mol. nitrite to every two mols. carbonate taken, and shaking the flask in cold water for a short time to moderate the rise of temperature, a solution is obtained which will in an hour or two deposit a small quantity of sparkling crystals of the nitrilosulphate, and give more when it is evaporated over sulphuric acid. The equation,

$$NaNO_2 + 2Na_2S_2O_5 = N(SO_3Na)_3 + Na_2SO_3,$$

represents the change but this would take many days to become complete, long before which time the nitrilosulphate would have all decomposed. In the mixed solution only a few hours old, some hydroximidosulphate, as well as nitrite, is still present.

The only satisfactory method of preparing sodium nitrilosulphate is a slightly modified form of that employed by us to get the imidosulphate (this Journal, 1894, 6, 65). In that process, which is after all only a development of Fremy's method of preparing the ammonium salt, the nitrilosulphate is prepared and at once allowed to hydrolyse into imidosulphate, whereas in the present case its hydrolysis is to be prevented. There a moderately concentrated solution met the end in view, whilst here the greatest possible concentration is wanted, because evaporation of the solution afterwards, though sometimes successful, is a very uncertain operation, in consequence of the short existence which can be assured to the nitrilosulphate. A solution therefore is prepared of two mols. nitrite to three mols. carbonate, so concentrated that the water is scarcely more than twice the weight of the anhydrous carbonate (for example, 10 grams nitrite, 8.5 grams hot water, and

62.2 grams carbonate crystals). Sulphur dioxide is passed rapidly into the solution in a flask continuously shaken. When, after a time, the solution grows hot the flask is immersed in cold water, and when it thickens, through temporary separation of acid carbonate, vigorous shaking of the flask is to be maintained without intermission. As the quantity of the acid carbonate suspended in the warm solution (50°-60°) lessens, the rate of current of the sulphur dioxide should be reduced and the action of the solution upon litmus paper closely watched. At the moment the solution reddens the litmus, the entrance of more sulphur dioxide must be stopped, for should acidity to lacmoid paper also be reached through the addition of much more sulphur dioxide, the nitrilosulphate will at once hydrolyse into imidosulphate. During the final slow sulphonation, the solution will generally grow cold enough to begin to deposit small crystals of the nitrilosulphate, recognisable by their brilliant lustre; these will increase largely in quantity at the temperature of the air. Without waiting too long, it is safer to add two or three drops of concentrated solution of sodium hydroxide, sufficient to render the solution faintly alkaline to litmus. It is possible to get more of the salt by evaporating the mother-liquor over sulphuric acid.

That the reaction proceeds sharply in accordance with the following equation is known from the quantity of imidosulphate which such a solution can be made to furnish:—

$$2NaNO_2+3Na_2CO_3+8SO_2=2N(SO_3Na)_3+Na_2S_2O_5+3CO_2.$$

In consequence, however, of the great solubility of the nitrilosulphate the crystals obtained amount to barely more than one-fifth of the total quantity; still, even that is 120 per cent. of the weight of nitrite taken. The crystals hold 21.8 per cent. of water, or $5OH_2$. Of this quantity it has twice been possible to

remove 15.5 per cent. by exposing the salt in a vacuum over sulphuric acid, after which the salt has hydrolysed and thereby fixed the rest of the water. The production of the unused pyrosulphite, shown in the above equation, is necessary for the safe and prompt sulphonation of all hydroximidosulphate.

Sodium nitrilosulphate crystallises in short thick prisms which melt when heated and decompose in their water of crystallisation into sulphates. The crystals cannot be long preserved under any circumstances, soon suffering decomposition and becoming opaque and acid, even in their own mother-liquor after it has been made alkaline. That is, the sodium salt is more unstable than the potassium salt. It is neutral to litmus and must be soluble in about its own weight of water, to judge from the amount of it left in the mother-liquor in its preparation, although here, no doubt, the pyrosulphite also in solution will affect its degree of solubility.

For analysis, it was drained on the tile after washing with a little strong ammonia-water, in which it is less soluble than in water. Partial water determinations have already been referred to.

	Sulphur.	Sodium.	Nitrogen.
$N(SO_3Na)_3$, $5OH_2$;	23.24	16.71	3.39
Found;	23.05	16.54	—
,,	22.96	—	3.64
,,	23.25	16.68	—
,,	23.17	—	3.45
,,	—	16.55	—

Double salts.

Potassium sodium nitrilosulphate, $N(SO_3)_3K_2Na$.—Raschig obtained this salt by adding a solution of potassium chloride

gradually to a crude solution of sodium nitrilosulphate (p. 5). We have obtained the same salt, which is like the sodium salt in appearance and like the potassium salt in being nearly insoluble. According to Raschig it is anhydrous and occurs either as a sparkling sand or in hard crystals, the size of pinheads and of adamantine lustre.

Barium salts.—By dissolving the sodium salt in a strong solution of barium chloride faintly alkaline with ammonia, a flocculent precipitate is obtained which becomes dense and crystalline on standing. It is sparingly soluble in water and very unstable. It probably contains sodium but we have not analysed it. Fremy obtained barium ammonium and barium potassium salts which he could only imperfectly analyse because of their instability. They resembled the barium sodium salt, and from the results of his analyses appear to have been two-thirds barium and one-third ammonium or potassium salt, with water of crystallisation.

Lead salts.—According to Fremy, very unstable lead salts containing potassium or ammonium are obtainable.

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Contributions to the Embryology of Amphibia:— The Mode of Blastopore Closure and the Position of the Embryonic Body.

By

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With Plates I—IV.

Although the earlier phases of the amphibian development have been studied within recent years by many able investigators such as PFLÜGER ('83), ROUX ('88*a*), O. HERTWIG ('92, '95*b*), O. SCHULZE ('88*b.c.*), MORGAN and TSUDA ('93*b*), KOPSCH ('90), ASSHETON ('94*a*) and EYCLESHYMER ('98), it is a remarkable fact that some of the most fundamental points are still far from being settled. Above all, the questions: (1) in what manner the blastopore is closed, and (2) on what portion of the egg-surface, the fundamental parts of the embryonic body are formed, have been answered by many different writers in as many different ways. The opinions held in regard to them can, however, be classified into three categories:—

(1). The blastopore is closed by the coalescence of the lateral lips (Concrescence theory), and the fundamental parts of the embryonic body are formed upon the lower hemisphere of the egg. (PFLÜGER, ROUX, HERTWIG, MORGAN, *et al.*).

(2). The blastopore is closed, mostly by the overgrowth of the ventral lip, and the fundamental parts of the embryonic body are formed upon the upper hemisphere of the egg. (O. SCHULZE, and others who accept the old views).

(3). The blastopore is closed by the overgrowth of the dorsal, ventral, and lateral lips, and the fundamental parts of the embryonic body are formed, partly upon the upper, and partly upon the lower hemisphere of the egg. (ASSHETON, KOPSCH, EYCLESHYMER, *et al.*).

As is well known, the facts on which the views of many of these writers are based, were obtained either from experimental study or from abnormally developing eggs (*spina bifida*, etc.). It has always seemed to me that one ought to exercise extreme caution in making use of this class of facts and that after all, the best way of studying normal processes of development would be to study normal eggs by some method which would not interfere with the normal course of events. For some years past I had thought that the eggs of our *Rhacophorus schlegelii* would furnish peculiarly favourable material for the investigation of the questions under discussion. As I have already stated in my former paper,[1] the eggs of this animal are absolutely without pigment, and among other favorable peculiarities, allow the invagination cavity to be seen through faintly. The "Wachsthumsrand" or the "Equatorial Zone" comparable to the "Germinal Ring" of the fish-egg, may be recognized

1) Annotationes Zoological Japonenses, Vol. I. Part III., (1897).

from the end of the blastula stage, spreading gradually upwards and downwards, until finally it covers the entire surface of the egg. And the fundamental parts of the embryonic body appear certainly to be formed within this spreading zone. My desire to subject these eggs to a thorough study by some new and suitable method was realized in the spring of 1899 and of 1900 by using, at the suggestion of Prof. K. MITSUKURI, the "Prismen-Rotator"[1] of ZEISS, obtained by him and kindly placed at my disposal. This instrument, as is probably well known, and as is fully described in in the place cited, enables one to study the upper and lower views of an egg as well as the lateral view of it from any point in 360° of its circumference, without once touching the egg and thus without the fear of calling forth abnormalities by handling. The results of this study, together with some additional matter, are recorded in the following pages; and although I am not so sanguine as to think that I have found answers to the questions propounded satisfactory to all, yet I hope that the facts set forth will be received as some contributions toward the final settlement of the problems.

For the sake of convenience, I divide the present article into four parts:—

I. General Account of External Developmental Changes in *Rhacophorus* Eggs.

II. Observations upon Eggs of *Rhacophrous*, *Rana*, and *Bufo* fixed on the Prismen Rotator of ZEISS.

III. Explanation of the Facts observed in the Second Part by Changes in the Interior of the Eggs brought out in Sections.

IV. Experiments by Puncture of Eggs.

1) Zeit. f. wiss. Mikrosk. Bd. XIV. p. 304.

Before going further, I wish to express my heartfelt thanks to Prof. K. MITSUKURI for much invaluable advice and for his kindness in working over the manuscripts of the present article.

I.

GENERAL ACCOUNT OF EXTERNAL DEVELOPMENTAL CHANGES IN *RHACOPHORUS* EGGS.

During several years past, I have frequently gone over the external changes which take place in the eggs of *Rhacophorus*. Some features which are peculiar to this species are certainly remarkable, and seem to me to deserve special notice from the point of view of comparative embryology. Some of these, as well as the general habits and the mode of the egg-deposition of this animal, I have already described briefly in my former paper (*loc. cit.*). I may, however, add a few more facts here.

As already stated, the eggs of this animal are absolutely without pigment and are placed in a frothy mass, concealed in subterranean cavities in the muddy banks of paddy-fields, ponds, and other shallow bodies of water. Deposition takes place mostly at night, during the breeding season which extends from April to May. It is an interesting fact that in the earlier part of the season, the animal deposits its eggs mostly on slopes which face east, south, or south-east, while toward the end of the season, when the atmospheric temperature has become mild, any spot favorable for deposition, even if it faces north or north-east, is indifferently chosen.

The eggs just deposited are extremely delicate and flabby,

and the frothy envelope is strongly viscous. The eggs, however, become gradually firmer in texture and are ready to begin segmentation in 4–5 hours after deposition.

During the breeding season of 1899, I kept a pair of the animals in a glass-flask on my table, in the manner already described in my former paper, and obtained a number of eggs from them. Another pair kept in a glass-cup and carried about for three days in my pocket deposited only a limited number of eggs. These eggs as well as those produced by the first-mentioned pair showed more or less abnormality in their development—due, it seems to me, to the forced arresting of oviposition and to the unnatural treatment to which the eggs had been subjected. Even the eggs deposited in a natural position are apt to show some abnormalities if they are subjected to unfavorable treatment. This tendency to abnormalities is not, however, peculiar to the eggs of this animal. So far as my experiences go, those of other Amphibians living in Japan (such as *Rana japonica* or *Bufo japonica*) show themselves similarly sensitive even to slightly altered conditions in their environment. I am now inclined to think that the process of segmentation in *Rhacophorus* eggs as described in my former paper is in some respects abnormal, as the eggs then observed were deposited by a pair of animals in captivity.

The first and second cleavage-lines make the figure of a cross, as is usual in amphibian eggs (Figs. 1–3). The third cleavage plane appears, in most eggs, symmetrically upon the second cleavage-line at nearly equal distances from the crossing point of the first two cleavage-lines (Fig. 4). Thus, the third cleavage must be considered as in a vertical, rather than in the horizontal plane, (to which I have previously referred it), and shows itself in four separate lines starting from the second cleavage-line

toward the egg periphery. In some cases, however, the third cleavage-line appears to encircle horizontally a small area around the upper pole of the egg as recorded in my former paper. There are many other individual differences with respect to the third cleavage. The fourth cleavage appears, in general horizontally encircling an area which takes in about two-thirds of the upper hemisphere (Figs. 5 and 6). It does not appear as a continuous line, but as seven or eight separate lines or pits upon the earlier (first, second, and third) cleavage-lines, and at nearly equal distances from the upper pole. The segmentation-lines which appear later than this are very irregular and are confined mostly within the circle formed by the fourth cleavage-line. Most of the later cleavage-lines including even the second and third can not be traced clearly to the lower pole of the egg. Indeed it is the first only which can be distinctly seen to reach the lower pole. In living eggs of later stages, all the cleavage-lines become superficially indistinct in the lower hemisphere while they are distinct in the upper hemisphere (Fig. 7), so that the eggs of these stages present the appearances of meroblastic forms, though when examined in sections they are seen to be truly holoblastic.

During the earlier part of the season, segmentation is finished at the end of the second day after deposition. Later, with the rise in the atmospheric temperature, the process is gone through faster, so that it is finished at the end of the first day after deposition or even in less time. Toward the end of the segmentation process, a part of the the upper hemisphere of the egg becomes gradually translucent (Fig. 8). This area which is, as a rule, circumscribed by a circular depression (Fig. 9) occupies from the first nearly two-thirds of the upper half, and corresponds approximately to the circle enclosed by the fourth

cleavage-line. As I have assured myself by a study of sections, it is the superficial expression of the segmentation-cavity within, which is seen through its very thin roof. The size of this area, therefore, indicates, in every stage, the extension of the cavity within, and naturally diminishes gradually with the progress of development, until it vanishes entirely, with the advancing closure of the blastopore (Figs. 9–14). For the sake of convenience, I shall refer to this translucent area hereafter as the "area of the segmentation cavity," and to the area encircled by the blastopore lips as the "blastoporic area."

In 24–48 hours after deposition, the dorsal lip of the blastopore makes its first appearance close below the egg-equator. Although there are many individual differences, this is seen, in most cases, 10°–20° below the equator. In some rare cases, it may approach to within 4° or 5° of the equator, while in other cases, it rarely is as far as 25° below. These cases were found mostly among eggs which had been taken out of their natural frothy envelope and reared in water. According to my experiences, however, these slight individual variations seem to make no serious differences in the development of the future animal, and indeed it seems to me that the eggs of Amphibia are endowed with a strong power of adaptation and resistance to unnatural and injurious influences.

The encircling of the egg by the blastopore lips is generally accomplished in 3–10 hours, and the complete closure of the same in 15–24 hours after the first appearance of the dorsal lip, varying according to the condition of the atmospheric temperature. The gradual diminution in the size of the translucent area of the segmentation cavity proceeds in the normally growing eggs *pari passu* with the gradual closure of the blastopore lips, so that by

the time the lips have entirely encircled the subequatorial region, the former is reduced to about two-thirds of its former dimensions (Fig. 10).

Between the translucent area above, and the blastopore lips below, a broad opaque band may be recognized encircling the equatorial zone of the egg. It gradually spreads upwards and downwards concurrently with the gradual diminution of the blastoporic area and of the area of the segmentation-cavity (Figs. 9–13). This I shall call the "Equatorial Zone." I am inclined to think that it corresponds to the embryonic zone found in the eggs of Selachii, Teleostei and other groups of fishes. It is not, however, so prominent, in normal eggs, as it is said to be by some investigators in other amphibian eggs under the name of the "Embryonic Ring," the "Medullar Ring," or the "Unwachsungsrände." It is true that in some abnormal eggs, in which the blastoporic area and the area of the segmentation cavity have been disturbed in any way, it may become as prominent as it has been figured by these writers. In such cases, the neural plate may also often become more conspicuous than usual, and the correlation which is ordinarily found between the reduction in size of the area of the segmentation cavity and of the blastoporic area may be lost. By an examination of sectioned eggs, I have assured myself that the equatorial zone is, in the first stage of gastrulation, nothing but a simple accumulation of blastoderm-cells around the egg-equator.

When the equatorial zone has come to occupy about the middle two-thirds of the egg-surface, the neural plate may be faintly detected by two shady lines within the zone, with a slightly projected anterior margin (the projecting tongue of EYCLESHYMER ['98] found in Amblystema) (Figs. 10 and 11). In some eggs,

a slight notch may be noticed at the middle point of the dorsal blastopore-lip. But such eggs can not be perfectly normal, I think, for they show sooner or later some abnormalities. In eggs which develop normally, the dorsal lip is always entire and the blastopore is a perfect circle in shape, until it is completely closed.

Up to this stage, the egg always rests on about the middle point of the blastoporic area with the translucent area of the segmentation cavity on the exact top. As the closure of the blastopore progresses, the resting-point of the egg is gradually shifted toward the ventral face of the future embryo. Thus, when we look at an egg at about the end of the gastrulation process, the now much reduced blastopore may be seen on the dorsal side of the embryo, nearer the equator of the egg than before, (Fig. 12–14). At the same time the greatly reduced translucent area of the segmentation cavity will be seen to have shifted anteriorly, in front of the future embryo.

Such a change in the position of the blastopore may be considered as due to the overgrowth of the ventral blastopore lip being greater than that of the dorsal lip, and to the eccentric closure of the blastopore thus brought about. But careful observations have convinced me that here there is a real rotation of the egg as a whole on its horizontal axis. In this, I find myself in agreement with many of my predecessors. But I am unable to follow Pflüger ('83), Roux ('88*a*), Morgan (97), *et al.*, when they state that the dorsal lip of the blastopore grows over the lower yolk hemisphere about 170° or 180° from the spot of its first appearance before the rotation of the egg takes place. Observations which I shall record in the sequel incline me strongly to the view that in normal eggs, the dorsal lip does not grow over the yolk hemisphere beyond its middle point, (the yolk pole in the

strict sense), and soon begins to move back again, to what is apparently its first starting point, *by the gradual rotation of the egg as a whole.*

At a certain stage of this rotation, the barely perceptible neural plate may be seen in a vertical position with the future head-end upward (Figs. 11 and 12), for the axis of the plate is shorter than the diameter of the egg. Even in later stages, the embryonic body does not lie in an exactly horizontal position but always at certain angles of inclination to the horizontal axis of the egg.

The neural groove is, in most eggs, to be perceived in 40–50 hours after the egg-deposition, while the neural folds appear somewhat later, becoming distinct only 10–20 hours later according to temperature. These folds are closely approximated one to the other from their first appearance.

As I have already stated in my former paper, the great peculiarity in the development of this species is that the head as well as the remaining parts of the body are up to certain late stages flattened out on the spherical mass of the yolk. Accordingly, the hyomandibular arches on both sides are strongly depressed and situated along the latero-frontal sides of the head, while the heart which usually appears below the head in anuran eggs, is here situated *in front of* the same. The body of the curved embryo is raised less than in other species and is so to speak wedged into the yolk mass, along the posterior and dorsal surface of the egg. All this reminds one strongly of what is seen in the eggs of the Ganoid fishes.

During the fifth day or the following night, the neural groove is completely closed by the coming together of the neural folds. At this stage (Fig. 15), there is around the closed blastopore a

circumjacent translucent zone which denotes the extent of the peristomal mesoblast. Now follows the gradual rising up of the embryonic body over the general surface of the spherical egg. First, the posterior portion is raised, and then the head-portion, carrying with it both the hyoid and mandibular arches which thus come to occupy the lateral and ventral portion as is usual with other forms (Fig. 16). At such stages, some of the mesoblastic somites have already been formed, these appearing first at the neck-region and gradually increasing in number in the posterior direction. The general outline of the pronephric duct may also be recognized externally, on both sides of the foremost two or three somites.

In this species, the embryo may grow to the perfect larval form within the gradually expanded vitelline membrane. The gradually growing tail may elongate and lie coiled mostly on the left side of the egg, while the head is being gradually raised. Fig. 17 represents a larva, on the eighth day after deposition, forced out of the vitelline membrane by needles. Before being taken out, it could be seen moving about in the slightly distended envelope. The yolk which is now covered by the skin is still quite spherical, and on its dorsal surface the main part of the trunk is wedged in, so that the general aspect of the larva in this stage resembles that of some teleostean embryo.

The larva, growing further, acquires gradually the usual tadpole shape. Fig. 18 represents a tadpole which, on the tenth day after deposition, had but lately hatched out of the vitelline membrane and was moving about within the partly melted frothy substance. The dendritic gills are already formed, and though small are larger than those of *Rana* or *Bufo* at the corresponding stage. Blood-circulation in the gill-filaments and the pulsation of the heart may be clearly seen from without under a dissecting

microscope. Also a small amount of blue-black pigment makes its first appearance in the pectoral region at this stage.

Fig. 19 represents a greatly advanced tadpole on the eleventh day after deposition. At this stage, the larvæ which measure 1½–2 cm. in length are already escaped into the water together with the melted frothy substance. They are now sprinkled with a large amount of pigment not only at the pectoral region but also along the dorsal surface of the head and of the thoracic region. The blood-vessels on the surface of the yolk-mass and along the mid-lateral line of the larval body are conspicuous. In the fully grown tadpole, however, the blood-vessels as well as the heart become entirely invisible, on account of the dense pigment accumulated as usual within the skin. The suctorial discs are rather rudimentary and appear late as a pair of small papilla-like spots in front of the mandibular arches. Such incomplete formation and retardation in appearance of the suctorial discs are probably due to the fact that the eggs are deposited underground and out of water. Only when the tadpoles escape into the water, is the necessity of some attaching organ felt.

Recently BUDGETT ('99) has called attention to the probable similarity in development of *Rhacophorus* as given by myself in my former paper and of *Phyllomedusa hypobranchialis* as described by him. He says: "* * * from what he (Ikeda) mentions of the appearance of the embryo which develops in a froth much as is the case with *Palidiocola*, I think the development of this form will be found quite like that of *Phyllomedusa*." Basing my judgment on the description and figures of *Phyllomedusa* given by BUDGETT, I may perhaps point out the points of similarity and difference between that genus and *Rhacophorus*, some of which are by no means insignificant.

In *Phyllomedusa*, segmentation seems to approach the typical holoblastic mode much more closely than in other anurans, the blastomeres of the upper and lower hemispheres showing less difference in size. In *Rhacophorus*, quite the contrary is the case, segmentation approximating more to the meroblastic type as already stated. Again, the head of the *Rhacophorus* embryo when raised over the general surface of the egg is not proportionally as large as that of *Phyllomedusa* as figured by BUDGETT. Also, the gills are not so enormously large in the Japanese genus as in the South American. The embryo of *Rhacophorus* as a whole is rather small in comparison with the yolk-mass which remains almost always spherical in shape. Moreover, the main part of the embryonic body when seen in sections is wedged into the yolk along its dorsal surface. On the other hand, the mode of the tail formation and the shape and the formation of the mesoblastic somites, as well as the rudimentary condition of the suckers, are strictly alike in the two genera.

II.

OBSERVATIONS UPON EGGS OF *RHACOPHORUS, RANA,* AND *BUFO* FIXED ON THE PRISMEN-ROTATOR OF ZEISS.

The Prismen-Rotator of ZEISS, as I have before stated, enables one to study the upper and lower views of an egg as well as the lateral view of it from any point of its circumference, without once touching the egg and thus without the risk of calling forth abnormalities by handling. A full description of it

is given in the place already cited, and therefore it suffices here to say that it is a system of mirrors placed in the center of a small dish which can be rotated around its vertical axis at will, and has a graduated scale around its rim to enable one to locate any point exactly on its circumference. The upper view of an egg is obtained by looking at it from above, as in any ordinary case of examination by microscope. The view of its lower hemisphere is reflected on a mirror, and is observed by slightly sliding the dish and thus bringing the objective of the microscope above this mirror. In a similar way, another mirror gives the reflection of the lateral view of the egg, and by rotating the dish, and with it the egg, a view from any point of its circumference can be obtained.[1]

Method :—My first care was of course to find a suitable method for fixing an egg on the mirror-surface. I proceeded at first in the manner of PFLÜGER. That is, an egg was taken out of the frothy substance, with the thin layer of the innermost egg-envelope, placed on the dry surface of the mirror and exposed to the air for two or three minutes. When the egg seemed safely fixed, water was poured into the revolving-dish by means of a pipette, until the egg was submerged. This method was not entirely satisfactory, for an egg fixed in this way was apt to detach itself from the mirror surface, thus vitiating the experiment.

I next tried collodion which, I believe, has already been used by some investigators. This was far from satisfactory, for although it is a good fixative, it becomes opaque on the application of water, and often kills the egg, probably by the injurious effect

1) While this instrument has been designed for special use in investigations such as I have undertaken, it has not, so far as I know, been used in the study of frog's eggs.

of the ether used in dissolving it. I now tried gelatine. An egg was taken out of the forthy substance with forceps, and its orientation was noted. Gelatine which had previously been warmed on a sand-bath was dropped from the point of a needle on the mirror-surface and on its cooling, the egg was placed in the desired position on the gelatine drop which was then further cooled by pouring water into the mirror-dish. This gave some very satisfactory results (see Egg C). But if the drop of gelatine be too large or too thick in its consistence, the egg will be strongly compressed and may become deformed by the swelling up of the gelatine caused by absorbing water. Moreover, in the later part of the breeding season when the weather gets warm, the gelatine seems to become too soft to keep the egg in its place and it may thus become detached.

The method I have found most satisfactory so far, is the following:—instead of fixing the egg directly on the mirror, a small square piece of cover-glass, about the size of the mirror is cut, and this being gently heated for about 20–25 seconds, on the warm edge of a sand-bath which should never be hotter than one's finger can bear, the egg is quickly placed on it. The coagulation of the egg-envelope attaches the egg firmly on the glass which is then to be fixed on the mirror by gelatine in the manner described above. The least possible quantity of gelatine, of very weak consistence is enough to fix a small piece of glass on the mirror, the two surfaces coming into close contact with each other and not detaching to the end of the experiment. A very necessary precaution is that the water should be poured into the mirror-dish with the greatest possible gentleness. Eggs prepared in this way can be observed continuously for five or more days without the least inconvenience.

Owing partly to the fact that I spent the first three weeks or more of the breeding season (in 1899) in making tentative experiments in methods, and partly to the fact that I was determined to observe only absolutely normal eggs, removing from the mirror-surface eggs that showed the least sign of abnormality, the number of cases I have observed is not at all proportionate to the large number of eggs which I have examined. Only thirteen eggs (Eggs A–M), have been subjected to continuous observation, and of these only three (Eggs C, H, and I) gave entirely satisfactory results, while the remaining ten showed more or less imperfection during the observation.

During the spring of 1900, I subjected the eggs of two other anurans found in Tokyo, (*Rana japonica* and *Bufo japonica*) to the same observations. The number of the eggs employed by me three were of the former, and seven of the latter species. Of these, those that gave sufficient results were respectively one of the first, and two, of the second species. This small number was owing partly to the brevity of the breeding season in these species, as compared with *Rhacophorus*, and partly to the clouding of the mirror, the amalgam appearing unable to stand prolonged submergence in water.

I may here add a few words of explanation in regard to Figs. 20–50 which have been selected out of innumerable drawings to illustrate this section. The heavy horizontal line under every lateral, and some posterior views of the egg is the level of the mirror surface and is to be referred to as the standard line. The second thick horizontal line through the centre of the egg is drawn parallel to the standard line in order to denote approximately the equator of the egg. The third line, vertical to the standard line, passing through the egg-centre gives the vertical

axis of the egg. In a similar manner, the position and size of the blastopore in every stage is indicated by the vertical and horizontal dotted lines, respectively vertical or parallel to the standard line. I may also explain here that when a "side-view" of an egg is mentioned, that view of the egg is meant which has the middle point of the dorsal lip of the blastopore *just on the edge of the egg-outline*. When this edge is on the right hand of the figure, the view is the "left" view; and if on the left hand, it is the "right" view. The "posterior view" of an egg is the one which has the middle point of the dorsal lip of the blastopore on the middle line of the microscopic field. When once the positions of these views are accurately determined on the graduated scale on the rim of the rotating dish, they are very useful in orienting the egg and in finding these same views again in the further course of development. I generally made it my practice to prepare two sets of drawings at every stage. The first set was drawn from what I judged by my eye to be the side and posterior views of the egg without reference to the graduated scales of the rotating dish. The second set was drawn by turning the dish to points on the graduated scale which ought to give these views as determined in the earliest observed stage of the egg. When discrepancies appeared between these two sets, I generally determined what ought to be considered as the true side and posterior views, by a comparison of the drawings and a careful study of the actual object.

I had hoped to give the exact measurements of the changes in the position and size of the gradually closing blastopore at every stage. I found, however, that basing such measurements on sketches or drawings which necessarily have to be made rapidly in order to mark the conditions at any given moment, would lead

to errors. One or more accessory apparatuses specially designed for the purpose may be necessary to accomplish this object.

I should like to add here that my observations thus far have determined the fact that all the eggs within the vitelline membrane of *Rhacophorus* as well as of other Amphibians studied by me are in their earliest stages incapable of executing any horizontal movements around the vertical axis, *if they are carefully placed in their natural position:*

Having premised these remarks, I may now proceed to describe the eggs observed. I begin with *Rhacophorus.*

An egg-mass of that animal was obtained at 10 a.m., April 27th. It had probably been deposited during the preceding night. From it an egg (Egg C, Figs. 20–38) was taken out and fixed on the mirror by the gelatine method at 2:25 p.m., on the same day. It had already gone through the earlier phases of segmentation: cell-outlines were visible on its upper hemisphere, while obscure on its lower half (Fig. 20). At 2 p.m., on the next day (April 28th), the first trace of the dorsal blastopore lip had appeared at about 6° below the equator (Fig. 21). The posterior view of the egg (Fig. 21) was seen at 217° of the rotating mirror-dish. At 130° and 310° (217−90=127; 217+90=307), were respectively the right, and left side views. Fig. 23 gives the left side view sketched at 3:20 p.m., on the same day: the middle point of the dorsal lip has moved slightly downward and its limbs have grown longer. Fig. 22 gives a view of the upper hemisphere of the egg seen from above at 2:30 p.m. The large translucent area of the segmentation-cavity is found occupying the centre of the egg-outline. On that day, the blastopore lips did not quite encircle the lower hemisphere but on the next morning (April

29th), they had already united in a circle. The area enclosed by them is circular in shape as may be seen in Fig. 24 which gives a view of the lower half of the egg seen from below at 7 a.m. The egg is resting upon the exact middle point of this area (Figs. 26 and 27). The upper view of the egg is given in Fig. 25 which shows that the somewhat diminished but still large area of the segmentation cavity is in the center and stands in exact opposition to the blastoporic area of the lower view (Fig. 24). Figs. 26 and 27 represent respectively the left, and right side views of the egg at 8:10 a.m., on the same day: the dorsal and ventral lips of the blastopore are exactly equidistant from the standard line (the mirror-surface), showing that the rims of the blastopore are growing over the yolk mass at the same rate of progress all around its circumference.

When seen at 9:43 a.m., on the same day, the egg was seen to have rotated about 20° backward (Fig. 28, left side view) so that instead of resting on the middle point of the blastoporic area as heretofore, it was now resting nearly on the ventral lip of the blastopore, and the dorsal lip had again moved slightly upward. The area of the segmentation cavity at the upper pole had also rotated slightly forward and kept its position exactly opposite the blastoporic area of the lower pole. Both these areas had now dwindled to about two-thirds of their original size. Note on the right edge of the egg-outline the faintly marked embryonic body stretching between these two areas. Figs. 29 and 30 give respectively the lower, and upper views of the egg sketched fifteen minutes later, and in them the eccentric position of the blastoporic area, and of the area of the segmentation cavity is well brought out.

At 11:27 a.m., on the same day, the dorsal lip of the blastopore (Fig. 31, left side view) had moved upward greatly, and

the closure of the blastopore had further progressed and the ventral lip had been brought further backward so that the entire blastoporic area could now be seen in the posterior view of the egg. The area of the segmentation cavity still kept its position exactly opposite the blastoporic area.

Further progress in these changes may be seen in Figs. 32–35 which give the left side view of the same egg at successive stages of development sketched respectively at 12:50, 2:04, 3:30 and 4:50 p.m., on the same day. These figures show that while the closure of the blastopore is going on, both the dorsal and ventral lips of it are gradually taken upward toward the equator by rotation. There is, however, an apparent difference between the behavior of the dorsal, and of the ventral lips beyond a certain point. When the dorsal lip in its upward return has reached about the point where it made its first appearance, it apparently ceases to rise any further, while the ventral lip continues to do so (Figs. 33, 34 and 35). This is undoubtedly due to the fact that the dorsal lip, partaking of the general growth of the whole periphery of the blastoporic area toward its center, grows downward toward the center of the area, and the rate of this downward growth must be exactly the same as that of its upward progress by the rotation of the whole egg, so that it remains apparently stationary. On the ventral lip, these two motions are combined, and there is naturally a steady upward progress. In earlier stages, the rate of the egg-rotation must be faster than that of the growth of the blastopore lip, so that there is an upward movement of the whole blastoporic area toward the equator. Conditions like those just described might lead one to suppose that the blastopore in certain later stages is closed only by the rapid overgrowth of the ventral lip.

At 10:30 a.m., next morning (April 30th., the fourth day after deposition), the blastopore was nearly closed, leaving only a small yolk-plug, and the general outline of the neural plate could be well recognized. Fig. 36 gives the left side view, and Fig. 37 the posterior view of the egg at this stage, sketched respectively at 10:37 and 10:50 a.m. These figures show that the dorsal lip of the blastopore has again grown a little downward while the position of the blastopore remains nearly unaltered. This is probably due to the fact that the rotation of the egg had ceased before the closure of the blastopore was finished, and thus bereft of the counterbalancing movement the downward growth of the dorsal lip toward the center of the blastopore is able once more to make itself felt. It seems to me that this is a strong argument in favour of the view that the blastopore is closed by the overgrowth of its lip all around its circumference.

Fig. 38 shows the left side view of the egg on the morning of May 1st., (the fifth day). The neural groove is already closed, and the first traces of the mandibular arches, the optic vesicles etc. are visible.

The facts brought out by the study of this egg (Egg C) are as follows :—

1). The entire lip of the blastopore, the dorsal, ventral and lateral parts, grow equally from all around toward the center of the blastoporic area, from the first until the blastopore is completely closed, although at certain late stages, this is obscured by the rotation of the egg.

2). The rotation of the egg as a whole by which the dorsal blastoporic lip is again brought back to its first starting point begins when the dorsal lip has grown downwards over the yolk mass about 70°, proving that the dorsal lip never travels beyond the yolk-pole.

3). The arc passed by the egg in its rotation as a whole around its horizontal axis is about 80°, because the final position of the closed blastopore or the former yolk-pole never reaches the equator and is about 10° below it.

4). The rate of the egg-rotation in earlier stages is obviously faster than the rate of the downward growth of the dorsal blastoporic lip. Gradually slowing, it becomes equal to the latter, at about the time when the lip has returned to its first starting point. Finally it becomes slower, before it entirely stops.

5). As to the location of the embryonic body in the egg, the main parts of it appear between the upper and lower poles of the egg, along the meridian of the middle point in the dorsal lip of the blastopore (the blastoporic meridian). The lower yolk-pole corresponds to the tail end, while the foremost point of the head end is about 30°–35° from the upper pole.

6). The initial vertical axis of the egg is at the end brought nearly to concide with the antero-posterior axis of the embryonic body, while the lateral axis of the egg remains strictly unaltered.

The egg-mass from which Egg H was taken, was deposited early on the morning of May 11th. As the eggs were in the first cleavage stage, I tried to fix an egg in haste in order to determine, if possible, the relations of the plane of the first cleavage to the future axis of the embryo. But the three eggs I mounted one after another all showed abnormalities and in the mean time the first cleavage processes were passed. I therefore on the next morning fixed a new egg (Egg H) on the mirror surface and made the following observations.

The first appearance of the dorsal blastopore lip was noticed

at 2:15 p.m., on the same (2nd) day, (May 12th). The right, and left side views were respectively at 97° and 277° (=97+180), and the posterior view at 187° (=97+90) of the rotating dish. In this case, the spot where the dorsal lip of the blastopore first appeared was far below the egg-equator, being about 20° below that line. By 5:25 p.m., on that day, the lip had travelled about 15° further downward and the edges of the blastopore hardly extended to the lateral meridian of the egg (*i.e.* the meridian 90° from that of the dorsal lip at its first appearance). During the night which was quite warm, a great progress was, however, made, and when observed next morning (the 3rd day or May 13th) the blastopore was completely encircled and the rotation of the egg had begun. At 6:50 a.m., the egg was resting, just on the ventral lip of the blastopore, which was very much smaller in diameter than (only about $\frac{1}{3}$) that of Egg C at the corresponding stage.

At 8:40 a.m., the resting point of the egg was found at a certain distance ventrad of the ventral lip of the blastopore so that the whole of it could be seen in the posterior view of the egg. The position of the dorsal lip at the time was about 30° below the equator.

During this day, I was able to look at the egg every hour up to 5:30 p.m., and had thus the opportunity of observing the actual closure of the blastopore under microscope, which event took place at 4 p.m. The shape of the gradually diminishing blastopore was always circular, while the yolk-plug ready to be withdrawn into the interior was elongated a little dorso-ventrally. Also, no doubt owing to the small size of the blastopore to start with, its closure in this egg was finished sooner than the rotation of the egg, which was continued after that event over an arc of

about 10°. When seen at 7:30 next morning (the 4th day), the general outline of the embryonic body with the well defined neural groove and folds was already established. The posterior end of the neural groove at the time was about 25° below the equator showing that the spot which was the dorsal blastoporic lip must have returned, as in the first egg (Egg C), nearly to its starting point, after the blastopore was completely closed.

Egg H was therefore like Egg C in the mode of the blastopore closure, and in the location of the embryo. But the spot at which the dorsal blastoporic lip first appeared was much lower down than in the egg first observed, and was closed much earlier, while the arc travelled by the whole egg in its rotation was less, being about 60°.

Egg J was taken out from a mass obtained on the morning of May 20th. The eggs were all in the first cleavage, but I again failed in my attempt to observe the relation of that plane to the future embryo, as an egg of that stage on being fixed on the mirror showed signs of abnormalities. So, Egg J was fixed on the mirror the next morning. At 9:50 a.m., of the same day (May 21th.), the dorsal blastopore lip made its first appearance at a spot about 12° below the equator. The left, and right side views of the egg were to be seen respectively at 160° and 340° (=160+180) of the rotating dish, while the posterior view was at 250° (=160+90). When seen about ten minutes later (10 a.m.), the dorsal lip had grown about 6° downwards, and at 2 p.m., about 9° further down. Thirty minutes later (2:30 p.m.), in the left side view the dorsal lip had grown down another 9°, so that the lip had moved downwards over the yolk altogether about 24° in 4 hours and 40 minutes. The rate of growth was not con-

stant in this case, being rapid at the first and last observations and quite slow in the interval. The acceleration in the first instance was without doubt to be accounted for by the artificial application of heat as the means of fixing the egg, while the speed in the last period mentioned was perhaps due to the rise of the atmospheric temperature. Such irregularities are, however, of common occurrence and may be due to both artificial and natural causes.

By 3 p.m., of the same day, the dorsal lip had moved to about 60° below the equator, and now the blastopore lip was closed in a complete circle. At this time, the ventral lip was found at a level 5° or 6° higher than the dorsal lip. In the posterior view, also, there could be noticed some differences (2° or 3°) in the level of the right and left lateral lips. The rim of the blastopore was, therefore, in this egg somewhat wavy. The highest part was at a point about 25°–30° to the right of the middle line of the dorsal lip (*i.e.* of the blastoporic meridian), showing that some trouble in the overgrowth of the lip had unfortunately occurred in this region. When, however, I looked at the egg at 6:30 p.m., all such unevennesses of the blastopore lip had disappeared and it had now grown downward evenly all around to about 66° below the equator.

When seen at 7:30 on the next morning (the third day), the blastopore had completely closed and the yolk-mass had been withdrawn into the interior. The closure had taken place during the night which was unusually warm. The point at which the blastopore was closed was to be found about 30° to the right of the former middle line of the posterior view, and moreover was far below the equator (about 20°). Such an irregularity was no doubt due to the same cause as that which produced the uneven

downward growth of the blastopore rim, although there is a possibility that I may have mistaken the exact position of the posterior view at the start. The dorsal lip of the blastopore in this egg did not return to its first starting point, though the egg rotated 5° or 6° further after the complete closure of the blastopore. The embryonic body formed of this egg was entirely normal.

Although observations on the later features of the blastopore closure and the beginning of the egg-rotation are wanting, the earlier phases observed in this egg clearly show that the blastopore was closed, as in the former cases, by the equal downward growth of the lips all around over the yolk-mass, until the dorsal lip arrives at about 66° below the equator. The position of the embryo also coincides in the main with that in the former eggs, although somewhat swung out of the blastoporic meridian in its posterior portion. The only difference is that the dorsal blastoporic lip did not return to its starting point, which is to be accounted for by the smaller amount of the egg-rotation.

I shall add a few more details obtained from the study of Egg G which unfortunately became unstable in the later stages through the insufficient fixation of the cover-glass upon the mirror-surface. In this egg, the dorsal lip of the blastopore appeared first at 5 p.m., on the second day at about 10° or 12° below the equator, and had moved down to about 50° by 9:50 a.m., on the third day by which time also the blastoporic-circle was completed. Afterwards, the whole edge of the blastopore moved further down, until at 3:10 p.m., on the third day the circle arrived at about 66° below the equator. When seen at 5:30 p.m., from the left side, the egg had rotated about 35°. At this time, the dorsal lip of the blastopore was found at 32° or 33° below the equator, and

the ventral lip at about 80°, so that the diameter of the blastopore occupied about 50° of the egg circumference, whereas it had occupied 70° or more when it first assumed the circular shape.

At 6 p.m., the blastopore has diminished to about 35° in diameter, but the egg had become somewhat unstable, rotating also when the mirror-dish was rotated, and by the next morning had become utterly useless from the same reason.

In the spring of 1900, I studied the subject under consideration in the eggs of two other anurans (*Rana japonica* and *Bufo japonica*). The spawning season of the former species about Tokyo is from February to March, and of the latter species from the end of March to the middle of April.

On the morning of February 2nd, several masses of the *Rana* eggs were obtained. From among them I fixed on the Prismen Rotator an egg in the early gastrulation stage in which the dorsal lip of the blastopore had already appeared and encircled about one-third of the circumference. This year, I carried on my researches in a basement room in order to have a perfectly steady floor, but, although this end was attained, the temperature of the room was comparatively low, and the development of the egg progressed very slowly. This proved very unfortunate, as owing probably to prolonged submergence the mirror became clouded, until on the sixth day nothing more could be done with it.

As the gastrulation process had been going on for sometime, I could not ascertain on this egg the actual starting point of the dorsal blastopore lip. Fig. 39 represents the view of the egg from the lower pole at 4:30 p.m., on Feb. 2nd (the first day). The right and left side-views were obtained respectively at 20°

and 200°, and the posterior view at 290°, of the rotating dish. The complete encircling of the blastopore-lips was observed at 2:17 p.m., on the next day, when the lips had grown down to about 64° or 65° below the equator. At this time and later, up to the morning of the fourth day, the gradually diminishing blastoporic area was always found at the exact center of the lower hemisphere (Fig. 40). At 4:05 p.m., on the fourth day, the blastopore lips had grown down to 72° or 73° below the equator, and the egg was just beginning its rotation as a whole. On the next morning (the fifth day), the egg was found to have rotated about 13° or 14° and the closure of the blastopore had also advanced somewhat. At 3:32 p.m., on the same day, the right side view of the egg was as given in Fig. 41: the egg had rotated so that it was now resting, nearly though not yet quite, on the ventral lip of the blastopore which was now reduced in diameter to $\frac{4}{5}$ of the size in Fig. 40. Further progress is seen in Fig. 42 sketched at 3 p.m., on the sixth day: the egg is now resting exactly on the ventral lip of the blastopore which is still further reduced in size. I could unfortunately make no observation beyond this point, owing as before mentioned to the clouding of the mirror, but the facts brought out prove that in the main, the mode of the blastopore-closure and of the egg rotation in *Rana* are exactly like the same processes in *Rhacophorus*.

In the case of *Bufo*, the eggs whose development I was able to follow during the breeding season of last year were only three in number. Of these the third did not produce satisfactory results, owing to the clouding of the mirror again. Even in the case of the first two eggs which gave tolerably good results, the lower view was rather imperfect from the same cause. I tried therefore to supplement my observations by following three or

four eggs during the spawning season of the present year, but again all were failures. I ought to state also that I tried at first to study the *Bufo* eggs in the basement-room before referred to, but I found the constant low temperature of the room acted injuriously on the *Bufo* eggs and I was therefore obliged to return to the up-stairs laboratory which is kept warmed by hot-water pipes. I will now proceed to describe the two eggs whose development I followed, and in which I was able to make out the relations between the first two cleavage-planes and the future embryonic axis.

The first egg was taken from a mass obtained from the pond in our University grounds on the morning of March 16th. The egg had not yet begun segmentation, when it was fixed on the mirror at 10 a.m. The first cleavage line appeared at 2:50 p.m. on the same day, and had nearly reached the lower pole by 5:30 p.m. When the first cleavage line was placed approximately in the middle vertical line of a side view of the egg the rotating dish was at 175° (Fig. 44) or at 355°. At 6 p.m., the second cleavage line appeared making right angles with the first (Fig. 43). When this line was placed in the middle vertical line of the side view, the dish stood at 85° (=175°−90) or at 265° (=175+90=355 −90). Having made out the positions of these two cleavage lines on the scale, I was now able to identify the same positions by simply turning the rotating dish to these several readings.

At 8 a.m, on the next morning, the egg had advanced to a stage with 64 or more cells. The first dorsal blastoporic lip appeared at 8:15 a.m., on the third day, about 30°–34° below the equator. When the middle point of the dorsal lip was brought on the middle vertical line of a side view, the rotating dish stood at 85°, corresponding to the similar position of the second

cleavage line. In the same way, in the left and right side views of the egg, when the dorsal lip came to be in the profile, the rotating dish stood at 175° (Fig. 45) and 355° respectively. This showed that the meridian of the blastopore coincided with the plane of the second cleavage and stood at 90° to the plane of the first cleavage.

The blastopore did not close in a circle during this day, but when seen next morning, (the 4th day), the encircling had been completed and the blastopore rim had grown downward all around to about 70° below the equator. Fig. 46 represents the left side view at 8:20 a.m., and corresponds to Figs. 26 and 27 of the *Rhacophorus* egg, and to Fig. 40 of the *Rana* egg. As in all these figures, the dorsal, ventral, and lateral lips of the blastopore are all at the same level and the egg rests on the approximate middle point of the blastoporic area.

At 1:30 p.m., on the same day, the egg (Fig. 47) had rotated about 32° or 33° and the blastopore had diminished to about $\frac{2}{13}$ in its diameter as compared with that in Fig. 46. One hour later at 2:30 p.m., the dorsal lip had returned to its first starting point and the ventral lip was about 20° dorsad of the resting point of the egg, for in addition to the egg rotation the blastopore was now still more diminished in diameter. Toward the evening of that day, the closure of the blastopore was far advanced but the general outline of the embryonic body could not yet be recognized (Fig. 48). On the morning of the fifth day, the blastopore was nearly closed, leaving only a small yolk-plug exposed, and the first trace of the neural groove and folds could be detected in front of the small blastopore. Fig. 49 gives the posterior view of the egg at 8:30 on that morning (the fifth day). Although the embryonic body is not sketched in, it will

not be difficult, I think, to orientate the egg. The final point of closure coincided nearly with the starting point of the dorsal lip, showing that the egg did not rotate more than 55°–60°. After this, the egg unfortunately became somewhat unstable by the swelling up of the egg envelope. The egg seemed also to show at times certain independent movements which I attributed at the time to some accidental causes.

The second *Bufo* egg that was studied was taken from a mass that was obtained from the same pond on the morning of March 21st. All the eggs had not yet begun segmentation. The particular egg to be studied was soon fixed on the mirror at 10 a.m. The first cleavage line appeared about one hour later (11 a.m.) and reached the lower pole at 12 m. when the second cleavage line began to appear nearly at right angles to the first. In this egg, the frontal view of the first cleavage plane was seen at 115° and 295° of the rotating-dish, and the same view of the second cleavage-plane at 20° (=115−95) and 200° (=295−95). The dorsal blastoporic lip appeared first at 8 a.m., on the third day, at about 28° or 29° below the equator. In this egg also, the frontal view of the blastopore (the posterior view of the egg) coincided with the frontal view of the second cleavage plane, while the profile or side view of the dorsal lip was the same as the frontal view of the first cleavage plane.

The blastopore rim completed its circle when it had grown downward to about 60° below the equator (2:30 p.m.). At this stage, all the circumference of the rim was at the same level. The same state of things still continued at 3:10 p.m., when the lip had grown down to about 70° below the equator. At 3:30 p.m., the egg had just begun its rotation. At that time, I noticed that the egg moved in an unexpected direction. Thus

at 4:45 p.m. it had rotated about 20° in the opposite direction *i.e.* obliquely ventrad. By careful examination, I was convinced that such unexpected movements of the egg were due to accidental causes, such as the too rapid rotation of the mirror dish, since the vitelline membrane and the outer envelope had become very loose by absorbing a large amount of water. By allowing the egg to stand quietly without any disturbance for ten or fifteen minutes, it gradually returned to its normal position and was rotating in the usual way. Thus, at 5:10 p.m., the dorsal lip had returned to about 50° below the equator, and the ventral lip was at the resting point of the egg, the blastopore being thus 40° in its diameter.

At 8:30 on the next morning (the fourth day), the ventral lip of the blastopore was 6° or 7° posteriorly from the resting point of the egg, while the dorsal lip had again grown *downwards* 4° or 5°, showing that very slight, if any, egg rotation had occurred during the night, although the blastopore had lessened 3° or 4° in its diameter. This was probably due to the fact that the temperature was low during the night. During the day, I was not able to observe the egg up to 2 p.m., when the blastopore had already nearly closed and the yolk-plug had mostly withdrawn into the interior. The neural groove and fold were also recognizable. The arc of rotation in this egg was evidently less than in the first, and the final position of the closed blastopore did not coincide with the first starting point of the dorsal lip, being at about 55° below the equator. The difference between the former and the latter was thus about 25°, and the arc passed over by the egg rotation was about 35°. I thought it quite probable that in this case the rotation would be continued after the complete closure of the blastopore, as in one of the *Rhacophorus* eggs, but

unfortunately I could not observe this, for the egg began at this stage, to show some independent movements within the vitelline membrane, which seemed to increase gradually. As the first *Bufo* egg had showed similar movements at the corresponding stage, I gave close attention to the matter. After examining the conditions carefully, I came to the conclusion that such movements were not natural but, were caused in some way by the strong sun-light which was falling at the time directly on the mirror and the egg —a conclusion which was soon proved to be false, as will be seen further on.

The third egg of *Bufo* was taken from a mass deposited in the same pond early on the morning of March 27th. The eggs had not yet begun segmentation, and one of them was fixed on the mirror at 8:30. The first cleavage appeared at 9:45 a.m., and the second cleavage at 11 a.m. I watched this egg at intervals of time up to the fourth day, but unfortunately owing to the clouding of the mirror, and to the unusual amount of pigment in the egg, I am unable to give details about the exact spot at which the dorsal blastopore lip appeared, or about the mode of the blastopore closure. While, however, looking at the egg at 12 m., on the fourth day when the blastopore had just closed and the neural groove and folds had become clearly recognizable, I noticed a curious behavior of the egg. As in the last two cases, the egg began to show movements within the vitelline membrane. The outer envelope was not swollen out as in the first egg nor was the sun-light at the time falling on it, as in the second egg. The movements were at first very slow and seemed somewhat irregular so that they seemed somewhat accidental, and due to some external stimulus. After a while, however, the motion became more rapid and seemed to show

a considerable regularity. By continuous observation for one hour or more, I finally made out that the egg was rotating regularly with the axis of the embryo for its rotation-axis. Fig. 50 is the anterior view of the egg. The direction of rotation as indicated by the arrows, was from right to left *i.e.* contrary to the motion of the clock-hands. The thick lines on the figure show the different positions of the neural groove during rotation. The time occupied by one turn of the egg was not quite constant, but the differences were small, as the following measurements show : 2′.17″, 2′.21″, 2′.20″, 2′.30″, 2′.20″, 2′.15″ *etc.* Thus the mean time required by one turn was 2′.20″. The position of the anterior and posterior ends of the neural groove also varied slightly at every turn. Such regular rotating motion of course could not have been accidental, and the somewhat irregular movements which I had noticed in the first two eggs must have been the beginning of this regular motion. I can not understand the purpose of such motion. Nor have I been able to ascertain the means by which it is performed. If minute cilia are present, I could not bring a sufficiently high power to bear on the egg to make them out. So far as I know, such motion has not been noticed by any previous observer.

Although my observations on the eggs of both *Rana* and *Bufo* are somewhat meagre, the results as regards the most important points, such as the mode of the blastopore closure and the location of the embryo body, are exactly similar to what have been obtained from the study of the *Rhacophorus* eggs. The principal points in these results in the three genera may be summed up as follows :—

1). The dorsal lip of the blastopore always makes its first

appearance some degrees below the equator, although there are variations in this distance from the equator according to different species and to different individuals of the same species. In *Rhacophorus*, it is generally at 10°–20° below the equator, although in rare cases it may go up to 5° or down to 25°. In *Bufo*, it is at about 28°–30°. The same fact may be made out from the papers of MORGAN and TSUDA ('95*b*), ASSHETON ('94*a*), KOPSCH ('00), EYCLESHYMER ('95*a*, '98), and other authors.

2). The completion of the blastopore lip in a circle, or in other words the first appearance of the ventral lip may also vary: it takes place generally at about the time when the dorsal lip has grown down to about 50° below the equator in *Rhacophorus*, and to about 60° in *Rana* and *Bufo*.

3). The blastopore remains circular throughout its existence, since the dorsal and the ventral, as well as the lateral lips close in towards the center of the blastopore area equally from all around. Owing to the rotation of the egg as a whole, it looks, however, in later stages as if there were a difference of growth in different parts. That the entire rim of the blastopore takes part in its closure has already been recognized directly or indirectly by SCHULZE ('88*b.c.*), ASSHETON ('94*a*), EYCLESHYMER ('95*a*, '98), and KOPSCH ('00), in opposition to the views of PFLÜGER ('83), ROUX ('88*a*), MORGAN ('93, '97) *et al.* The former authors, however, seem to me to be of the opinion that there is a certain difference between the growing ratios of the dorsal and the ventral lips of a blastopore. And moreover, the fact that the actual final closing point of the blastopore is the former yolk pole (the center of the blastopore area), of the egg, has not yet been maintained by any previous writer. This is probably due to the circumstance that the methods hitherto employed are not

calculated to bring out clearly the equal growth of the whole periphery of the blastopore lips towards the yolk pole.

4). The point at which the blastopore finally closes is in reality always what is the lowest point of the yolk hemisphere (or the yolk pole *in s. str.*) of the unsegmented egg. The distance traversed by the dorsal lip from its first appearance to the time of closure is therefore about 70°–80° in *Rhacophorus*, and 68°–70° or less in *Bufo* and *Rana;* that by the ventral lip is about 20°–40° in all the species; and that by the lateral lip ranges between these two extremes. This estimation agrees well with those given by ASSHETON (60°–70°), and KOPSCH (*circa* 75°–80°).

5). When the dorsal, ventral, and lateral lips of the blastopore have grown down to a level which is 60°–75° below the equator, the whole egg begins to rotate slowly on its transverse horizontal axis, the resting point of the egg gradually shifting toward what becomes the ventral face of the future embryo, and the dorsal lip apparently returning toward its starting point. This greatly obscures the actual mode of the blastopore closure (or the equal growth of the lips over the yolk hemisphere).

6). This rotation of the egg within the vitelline membrane is very slow and may cease, sometimes before, and sometimes after the process of the blastopore closure is completed.

7). The dorsal lip of the blastopore in normally growing eggs, therefore, never goes ventrad beyond the middle point of the blastoporic area (the yolk-pole).

8). In *Rhacophorus*, the correlation in size between the upper translucent area of the segmentation cavity and the lower blastoporic area becomes specially obvious in eggs fixed on the mirror. They not only diminish in size together, but also maintain their position opposite each other, even after the rotation

of the egg has begun. If pigment were absent in the eggs of *Rana* and *Bufo*, similar relations could no doubt be made out.

9). As to the location of the embryonic body, the results of the present investigation are in entire accord with the views of ASSHETON ('94*a*), and EYCLESHYMER ('98). The anterior half of the embryonic body is formed on the upper hemisphere of the egg, and the posterior half on the lower hemisphere. I can not, however, in any way make out that the two halves of the embryonic body are in reality formed from the two separate centers of the formative area, as was supposed by these two authors. And as to the orientation of the embryonic axis, I agree in the main with KOPSCH's statements :—"Und dass die von Pol zu Pol gezogene Axe der Furchungsstadien nicht die dorsoventrale Axe des Embryos ist, sondern dass sie beim jungen Embryo schräg von caudal oben nach cranial unten verläuft," (p. 21, '00). I have only to point out that according to the present observations the head portion of the young embryo is turned always *upwards* (not "unten") and at the same time the caudal portion is turned *downwards* (not "oben").

I have described above how I found in two *Bufo* eggs that the axis of the embryonic body coincided with the plane of the second cleavage line. According to many previous investigators, the coincidence ought to be with the first cleavage plane rather than with the second, while some recent writers, as JORDAN and EYCLESHYMER ('94*b*), entertain the view expressed in the following quotation :—"It seems to us a more reasonable supposition that the direction of the early cleavage planes and the embryonic axes have not vital connection, and

that the coincidence, where it exists, is in itself of no fundamental significance" (p. 413).

In order to test this point further, I made the following observations on *Rhacophorus* eggs. I took eggs either before, or just after, the first appearance of the first cleavage line and fixed them, each on a cover-glass, in the manner already described at the beginning of the article, by heating it slightly on the edge of a sand-bath for about 30 seconds. The eggs were put as quickly as possible in a vessel full of water, and reared in the usual way. As soon as the first cleavage appeared, its direction was plainly marked on the cover-glass by two points. In this way, I prepared four groups of eggs.

In the first group, only one egg out of five was found to have been well fixed on the cover-glass: the rest unfortunately fell off. In the single egg, the axis of the embryo, when it appeared, was exactly at right angles with the plane of the first cleavage.

In the second group, three eggs out of five were well fixed. In the first of these the embryonic axis (the head end) was inclined 50° to the right of the first cleavage plane. In the second egg, the embryonic axis was at right angles to the first cleavage plane. In the third, the anterior half of the embryo was at right angles to the first cleavage plane, while the posterior half was somewhat oblique and deflected about 30° to the left from the embryonic plane. The main axis of the embryo must, however, be considered to be at right angles to the first cleavage plane.

In the third group, only three out of ten eggs were found to have been well attached to the cover-glasses. In one of the three, the inclination of the embryonic axis to the first cleavage plane was about 50°, and in another 20° to the right. In the

third egg, the embryonic axis was exactly at right angles to the same plane.

In the fourth group, only three eggs out of five were good. In one of these, the embryonic axis nearly coincided with the first cleavage; in the second, it was inclined about 60°, and in the third, 40° to the first cleavage plane.

When tabulated, the results may be expressed as follows:—

	The no. of eggs fixed well on the cover glass.	Eggs with the inclined embryonic axis —.	Eggs in which the embryonic axis was at right angles to the first cleavage plane.	Eggs in which the embryonic axis coincided with the first cleavage plane.
Group I.	1 (among 5)		1	
Group II.	3 (among 5)	1 (50°)	2	
Group III.	3 (among 10)	2 (1 – 50°, 1 – 20°)	1	
Group IV.	3 (among 5)	2 (1 – 60°, 1 – 40°)		1
Total	10.	= 5 +	4 +	1

When the two *Bufo* eggs in which the embryonic axis was at right angles to the first cleavage plane are added to the list, the total of such eggs becomes 6 out of 12. I admit that the number is altogether too small to allow us to draw any general conclusion, but the fact remains that the percentage of amphibian eggs in which there is a definite relation between the embryonic axis and the earlier cleavage planes is quite large, and that in such cases, the first named axis coincides with the second

cleavage plane and is at right angles to the first. I am inclined to think that the first segmentation cleavage divides the substance of the egg into two parts corresponding to the dorsal, and ventral halves of the future embryonic body, supposing that there is on the whole no interchange of substance during the course of development.

III.

EXPLANATION OF THE FACTS OBSERVED IN THE SECOND PART BY CHANGES IN THE INTERIOR OF THE EGG BROUGHT OUT IN SECTIONS.

Method :—The *Rhacophorus* egg presents several peculiar difficulties for sectioning: the closely applied chorion and the innermost envelope of the frothy substance become exceedingly tough, when hardened, so that it is very difficult for paraffin to penetrate into the egg. Moreover, the egg being absolutely without pigment, becomes so translucent, when clarified in turpentine or xylol, that orientation becomes impossible. Again, owing to the extreme tenuity of the roof of the segmentation cavity, it is apt to cave in when the egg is transferred from one liquid into another of a different specific gravity.

After some experiments, the mode of procedure I adopted was as follows :—The orientation of the egg was secured by first fixing it in any desired position on a thin triangular sheet of frog's liver by a drop of albumen. This was then passed through the ascending grades of alcohol, being kept 13 or 14 hours in absolute alcohol at the end. If a shortening of the time was desired, absolute alcohol was changed two or three times in one

hour. This was then imbedded in the usual celloidin-paraffin method, care being taken that the egg should be thoroughly permeated by celloidin by being kept two or three days in a weak solution of it in a mixture of absolute alcohol and ether in equal parts.

After cutting and mounting, sections were stained on slides. Most satisfactory results were obtained by subjecting them to double-staining with the water solution of the acid fuchsin G (*ca* 2%) and methyl-blue (*ca* 3%). This is a slight modification of Auerbach's method for the double-staining of certain sexual elements. A slide is placed in a solution of the first dye for 15–20 minutes, washed, and then put in a solution of the second dye for 20–30 minutes. After a second washing, it is put into absolute alcohol and the excess of blue colour is washed out by stirring it with forceps. At a certain stage of discoloration, it becomes very beautiful. The sections stained in this way show the nucleus in blue, while the cytoplasm as well as the yolk-spherules are red or reddish purple in colour.

Fig. 51 is a cross-section passed through the approximate center of a *Rhacophorus* egg which is at about the stage represented in Fig. 8 and is intermediate between Figs. 20 and 21 of Egg C. The segmentation cavity has not yet become enlarged to its full extent, and its roof is still thick being composed of three or four layers of cells.

The next two figures are the median sagittal (Fig. 52), and the middle transverse (Fig. 53), section of eggs corresponding to the stage represented in Fig. 23 of Egg C. The segmentation cavity has now become enormously enlarged, and its roof very thin, being composed of only two layers of cells (the epi-

dermal, and the neural layers of the epiblast). The shaded portions on both sides of the segmentation cavity are the sections of the equatorial zone. Fig. 62 gives a magnified view of the roof drawn from another section of the same series : the cells of the outer layer are somewhat flattened and slightly smaller than those of the inner layer. As before mentioned, this extreme tenuity of the roof accounts for the translucency of the area of the segmentation cavity in an external view.

Fig. 54 is the median sagittal section of an egg corresponding to Figs. 10 and 11 or to Figs. 26 and 27 of Egg C. The blastopore lip has already closed in a circle and is at the same level all around.

The next two figures (Figs. 55 and 56) are respectively a median sagittal, and a middle transverse section of the eggs corresponding to that represented in Fig. 12 or in Figs. 28–30 of Egg C in which the rotation of the egg has commenced. The archenteron has acquired a distinct lumen along the posterior and dorsal surface of the yolk-mass, while the segmentation cavity has become pushed forward and diminished in size in an inverse ratio to the enlargement of the first named cavity.

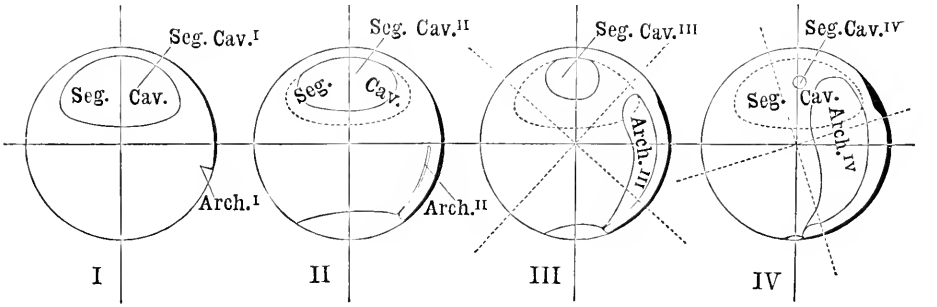
Fig. 57 is a median sagittal section of an egg corresponding to Fig. 13 or to Figs. 32 and 33, in which the blastopore has become moderately closed and the rotation has progressed considerably. The segmentation cavity has now vanished, leaving only small irregular cavities at the anterior end of the large archenteric cavity. This accounts for the fact that the translucent area can no longer be recognized in a superficial view.

Figs. 58 and 59 give respectively a median sagittal, and a middle transverse section of the stage represented in Fig. 14 or in Figs. 36 and 37. Here the blastopore is on the point of being

closed, the yolk-plug having already been withdrawn into the interior. The first trace of the neural groove may be recognized along the median line of the neural plate which is now raised somewhat over the general surface of the egg.

Figs. 60 and 61 give the corresponding sections of the stage represented in Fig. 15 or in Fig. 38. The blastopore is completely closed, and the neural groove is nearly so, so that the general outline of the embryo-body can now be distinctly made out.

In the above series of figures, the horizontal line drawn under each gives the surface on which the egg was resting at a given stage, and the lines drawn parallel and vertical to this show the horizontal and vertical axes of the egg as it then stood. The dotted line in Figs. 55, 57, 58, and 60 shows, on the contrary, the ideal vertical axis or the line which passed through the upper and lower poles of the egg at the blastula stage, and which shifts its position through the gradual rotation of the egg around its transverse horizontal axis. If the egg were supposed to be held to its initial vertical axis without any rotation, the series would become as in Woodcuts I–IV. The two dotted lines at right



The thick line in front of the blastopore indicates the extension of the neural plate. Seg. cav.I, Seg. cav.II, Seg. cav.III, Seg. cav.IV, the gradually diminishing seg. cavity. Arch.I, Arch.II, Arch.III, Arch.IV, the gradually enlarging archenteron.

angles to each other in Woodcuts III., and IV., indicate the *actual* vertical and horizontal axes drawn in full lines in the plate figures. The relative position and size of the segmentation cavity and the archenteron are easily understood from this series.

If now these two series (Figs. 51–61 and Woodcuts I–IV.) are carefully studied, it will become clear that the position of an egg at a given stage is entirely due to the position of its center of gravity. Up to the stage of Fig. 54 or of Woodcut II., the mass and the segmentation cavity are uniformly disposed with regard to the initial vertical axis of the egg, hence the center of gravity lies in this axis, and on its lower pole the egg rests. With the formation and gradual enlargement of the archenteric cavity which goes on hand in hand with the reduction in size of the segmentation cavity, (Figs. 66–61 and Woodcuts III–IV.), the dorsal and posterior part of the egg gradually diminishes in weight while, on the contrary, the anterior and ventral part becomes heavier by degrees. The center of gravity is, naturally, shifted forward, but as the archenteron keeps its symmetry in regard to the sagittal plane, this shifting takes place along that same plane. Hence such rotation of the egg as we have noticed in the study of the external features. In fact, it would be impossible for an egg constituted as given in Woodcut IV., to keep the unnatural position given in that figure: if left to itself, it must fall forward and assume the position given in Fig. 60.

That the rotation is sometimes continued after the complete closure of the blastopore, is due without doubt to the fact that at the time of the latter event, the archenteric cavity has not yet reached its maximum size. The archenteron, however, at its highest development never extends below the original vertical axis and is confined within the dorsal half.

As above stated the egg-rotation is a fact which has already been noticed by all investigators in the growing eggs of Amphibia. But the degrees passed by the rotation as well as the period at which the rotation takes place, are different according to different writers. Thus according to PFLÜGER ('83), ROUX ('88*a*), MORGAN ('93*b*, '97) *et al.* the egg has to rotate, about 170° or more, after the complete closure of blastopore. O. SCHULZE ('88*b*) maintains that the egg rotation begins from the beginning of the gastrulation process, so that it counterbalances, from the first, the downward growth of the dorsal blastopore lip over the yolk hemisphere.

Meanwhile the fact that the egg-rotation takes place by the change in position of the center of gravity of the egg during the gastrulation, has already been noticed by all my predecessors, and is now generally accepted.

I will now go over categorically some of the other points brought out in the study of the sections :—

1). The growth in size of the segmentation cavity from the condition seen in Fig. 51 to that of Fig. 53 is no doubt due to the thinning out of the roof which, as mentioned before, is three or four cells thick at first but becomes later strictly two-cell layered. This thinning appears to me to be brought about by the downward shifting or migration toward the equator of the cells composing the roof which no doubt also increase by division.

2). It seems probable to me that the equatorial zone is produced by the accummulation of these downward migrating cells rather than by the multiplication of the cells which were here from the first. This view is strengthened, it seems to me, by what is seen in Figs. 63 and 64 which are respectively the magnified sagittal sections of the zonal mass at the blastoporic,

and the anti-blastoporic regions of an egg in the stage of Fig. 52. In each of these figures, although the lower part of the zonal mass passes over gradually to the yolk mass, it is in its upper part sharply marked off by a line from the yolk cells, as if the cells had moved down from the roof of the segmentation cavity with which they form one continuous sheet, and accummulated in a mass here.

In Fig. 63, the first trace of the blastoporic slit interrupts the region of transition from the equatorial zone to the yolk-mass. In other words the cells directly above the first blastoporic slit are not the true zonal cells, but forms transitional between them and the yolk cells. Such a state of things leads me to accept the statement of MORGAN and TSUDA that "the blastopore makes its first appearance on the less pigmented and further developed side of the egg, and, moreover, at a short distance only from the group of large cells around the lower pole," (p. 381), although there is no pigment in my materials. These transitional cells are however doubtless soon transformed into the zonal cells which are in their turn differentiated into the permanent tissue cells roofing over the Archenteron. This process of transformation no doubt goes on all around the lower edge of the equatorial zone, while the cells themselves multiply also by division *in situ*. As the result of these formative changes, the equatorial zone spreads downward toward the yolk pole. I am therefore able to agree with ASSHETON ('99), when he says: "In this way there is a gradual apparent creeping of small (black) cells over the surface of the egg—though in reality it is conversion of large cells into smaller *in situ* as, I believe, is now generally accepted," (p. 227).

3). The equatorial zone is, however, only a temporary structure. The blastopore lip extends gradually on each side from the

point of its first appearance in the dorsal median line, along the lower edge of the equatorial zone, until the two limbs of it meet each other and complete the circle in the ventral median line. Before the lip becomes established, the component zonal cells are no doubt differentiated into the permanent epiblast cells, and by this means the epiblast grows down toward the lower pole. As the downward growth of the epiblast is thus taking place before the lip is definitely established, it seems to me that the individual or specific variations in the exact spot at which the dorsal blastoporic lip first appears, are not of so much importance as some writers believe.

The establishment of the lip itself, we must regard as due to invagination. While this goes in deep at the dorsal portion, it becomes at all other portions of its circumference evaginated again to add to its downward growth, as explained below.

The growth downward of the blastopore lip, after once it is established, is, I believe, nothing but the continuation of the cell mutiplication and the differentiation of these cells *in situ*. Although I have no direct proof of it, yet I have no doubt that the most active seat of cell-multiplication is at the edge or just inside the edge of the lip. Cells multiplied accummulate inside the lip as a sort of invagination and become soon evaginated to add to the downward growth of the lip. This takes place all around the circumference of the lip except at the dorsal median line where the underlying archenteric cavity has to be deepened and enlarged. The formation of the blastopore lips and their growth are therefore not produced by either invagination or cell-multiplication alone, but conjointly by these two processes.

4). As to the manner in which *the segmentation-cavity diminishes in size*, most previous writers have stated that this

is caused by the gradual growth of the archenteron which pushes the yolk-mass ventrad and thus causes the segmentation cavity to become obliterated in the *ventral half* of the egg. But according to my own observations, the segmentation cavity becomes smaller not only by the gradual growth of the archenteric cavity but also by the rising upward of the yolk-mass which takes place along the inner face of the epiblast, roofing over the segmentation-cavity as the blastopore lips grow downward. In other words, the cavity becomes reduced centripetally, *in situ*. Thus as shown in Figs. 57, 58, and Woodcut IV., the segmentation cavity and the blastoporic area both of which have now become very small are found, as before, at, or close to the former vertical axis of the egg, without changing their original positions relative to each other. So far as my experience goes, I have never yet met with a case in which the final point of the disappearance of the segmentation cavity was *below* the original vertical axis of the egg (the dotted line in Figs. 57, 58, and 60), although I am not in a position to assert that such cases may not occur as individual variations. Also, according to my views, the atrophy of the segmentation cavity has not so much to do with the rotation of the egg as the growth of the archenteric cavity, since in the former process the yolk cells rise *en masse* so to speak, and do not greatly change the position of the centre of gravity.

As a case of individual variation, there is that of Fig. 57 in which the segmentation cavity communicates freely with the archenteric cavity, the barrier separating the two cavities having broken down. Such a case is often met with. In fact, seeing that the yolk mass in the living egg is not solid but must be very mobile, being composed of loosely associated yolk cells, it would be strange if such and other slight differences were not found in various individuals.

5). As to the *first formation and growth of the archenteric cavity*, I agree mostly with ASSHETON ('94*a* p. 225–226, p. 227, p. 229). As represented in Fig. 63, the first trace of the blastopore appears as a horizontal linear slit below the cell mass of the equatorial zone, and is bounded by a kind of cells which is not the true zonal but transitional between the yolk-cells and the zonal. With ASSHETON we may consider this as formed by a splitting between cells, or as a linear accummulation of the intercellular space; but we ought to remember that it is produced in direct continuation of the *intra-vitelline* space, and must be regarded morphologically as a modified form of invagination, the only difference from the typical form being that cells situated on the outer surface of the egg do not actually migrate inward.

The further growth of the archenteron seems to take place very much as ASSHETON describes:—posteriorly the cavity elongates by the downward growth of the dorsal blastopore lip, in the manner already described, while anteriorly it grows upward by a splitting between cells or by an accummulation of intercellular space accompanied by the multiplication and differentiation of cells *in situ*. This splitting process for the formation of the archenteron cavity and the growth of the blastopore lips seems to me also to be the direct continuation of the segmentation process. For my part, however, I do not quite see the necessity for distinguishing primary and secondary centres of growth, as ASSHETON has done.

The enlarging of the archenteric cavity as well as the reduction of the segmentation-cavity may be regarded from another point of view, simply as a process of rearrangement of the yolk cells in the egg interior. This has already been expressed by KOPSCH ('00) when he says "Da unsere Urteil hinsichtlich der

Verwertung des durch die Furchung geschaffenen Zellenmaterials beim Aufbau des Embryos in erster Linie abhängt von der Zellenumlagerungen, welche bei der Gastrulation von sich gehen, so müssen wir die Vorgänge bei der Gastrulation zum Mittelpunkte der Betrachtung machen," (p. 19).

6). In *Rhacophorus* eggs, the gradual *growth of the neural plate* is indicated by a very conspicuous structural change. It is that the lower or neural layer of the epiblast becomes two-cell layered. This has been already noticed by ASSHETON ('94*a*) who says: "In the frog the nervous layer soon becomes thickened" (the number of cell-layers not specified) "along the future dorsal surface of the embryo, and over the rest of the embryo the nervous layer becomes reduced to a layer of one cell only in thickness, like the epidermic layer" (p. 169). This process takes place not only on the dorsal blastopore lip (Fig. 65 *a*) but also all around the rim of the blastopore (Fig. 65 *b*). However, no further progress is made except at the dorsal median portion where it spreads gradually forward and laterally over the dorsal surface of the future embryo (Fig. 66[1]), until it reaches a point 30°–40° distant from the original upper pole when it stops. The region over which this change takes place I have indicated in Figs. 54–60 by crossed lines and in Woodcuts I–IV., by heavy black lines. By a careful study, I have ascertained that the upper limit of the two-cell layered portion of the mucous layer coincides approximately with the forward extension of the archenteric cavity at every stage, and therefore at the final stage marks the head end of the future embryo. This again shows clearly that the location of the embryo in the egg is along the meridian of the blastopore with its head end situated 30°–40° from the original upper pole of the egg.

1) Dorsal portion of the egg in Fig. 57 under a higher power.

7). The growth in length of the embryo in *Rhacophorus* and other anuran eggs takes place after the complete closure of the blastopore or the finishing of the gastrulation process.

IV.

EXPERIMENTS BY PUNCTURE OF EGGS.

After making out the normal course of development in the anuran eggs, it occurred to me that experiments by puncturing eggs would be interesting, if for no other reason than to determine what effects these injuries would have on the normal course. Accordingly during the breeding season of 1900, some experiments were made on the *Rhacophorus* eggs.

The puncturing of the eggs was done with the sharpest possible needles from the outside through the vitelline membrane and the thin innermost compact layer of the frothy substance. The injuries which I inflicted on the eggs proved on the whole rather severe, but this was an advantage than otherwise, for slight punctures heal rapidly and often leave no trace as has been observed by many previous investigators. Moreover as remarked already by Assheton, punctures however slight have the effect of causing eggs to deviate from the normal course, and if such is the case, it is preferable to have the deviations stand out unmistakably by making the injuries somewhat severe.

I will anticipate the main result of these experiments by the statement that the *normal course of development can not be made out from the results obtained by the experiments.*

The number of eggs experimented upon was in all forty,

divided into four groups, each of which consisted of ten eggs from the same egg-mass. As I was most successful with the fourth group, I shall begin with that:

Fourth Group. (Figs. 67–84).

This series of eggs was obtained from a mass probably deposited on the morning of May 4th. When taken on the morning of May 5th, the eggs were near the end of the segmentation process. At 2:20 p.m., of the same day, the first trace of the dorsal blastopore lip appeared. Puncturing operations were commenced at 2:30 p.m., (*i.e.* 10 minutes after the first appearance of the dorsal lip), and finished at 4:30 p.m. I will give my remarks on each egg observed in the form of notes[1]:—

Egg No. 1. (Figs. 67 *a–e*, Fig. 76).

May 5, 2:30 p.m.:—(Fig. *a*). Punctured just a little to the right of the yolk-pole (as seen in the posterior view).

4:04 p.m.:—(Fig. *b*). Left side of the blastopore lip more inclined downwards than the right, and the ex-ovate moved also slightly to the right. Otherwise normal. (Fig. *b*).

May 6, 7:55 a.m.:—(Fig. *c*). Ex-ovate attached to the right lip of the now circular blastopore. Otherwise normal.

4:11 p.m.:—(Fig. *d*). Medullary folds and groove faintly recognizable. Ex-ovate attached to the right margin of the now very much smaller circular blastopore.

May 7, 4:00 p.m.:—(Fig. *e*). Blastopore already closed. Medullary groove ready to be closed by the closely approximated folds. Embryo normal, except that the ex-ovate is at the right margin of the posteriormost part of the medullary groove.

This egg shows that an injury near or at the yolk pole causes perhaps the least amount of deviation from the normal course.

1) The time given in the notes is in many cases only approximate within few minutes

In fact the development of the embryo in this egg is entirely natural and proves again the correctness of the observations before recorded. Fig. 76 shows diagrammatically the location of the embryo and the closing point of the blastopore.

Egg No. 2. (Figs. 68 *a–f*, Fig. 77).

May 5, 12:37 p.m.:—(Fig. *a*). Punctured a little to the left of the median line, closely under the left limb of the dorsal blastopore lip.

4:06 p.m.:—(Fig. *b*). Previously distinctly visible dorsal lip utterly unrecognizable. Ex-ovate visible. Area of the segmentation-cavity diminishing in the normal manner.

May 6, 8:02 a.m.:—(Fig. *c*). Blastopore with distinct lips, again visible, closed in circle, but larger as compared with the same stage of Egg No. 1 (Fig. 67 *c*). Ex-ovate attached inside the blastopore near its left margin. In front of, and parallel to the dorsal lip, another *accessory* lip present, formed perhaps by the reappearance of the vanished original lip. Area of the segmentation-cavity irregular in shape and found eccentrically a little to the left of the median line, showing that the rearranging process of the yolk-cells inside was disturbed.

4:14 p.m.:—(Fig. *d*). Area of the segmentation-cavity entirely vanished. Shape of the greatly diminished blastoporic area triangular, being a little elongated dorso-ventrally. Ex-ovate still attached within the blastopore near the left margin. Accessory dorsal lip still visible and in its front the neural plate and the shallow medullary groove faintly recognized.

May 7, 9:40 a.m.:—(Fig. *e*). Blastopore completely closed; ex-ovate still attached to the part where the blastopore closed. *Accessory lip has now grown around and enclosed a new circular patch in front of the actual closed blastopore.* Embryonic body raised a little over the egg-surface in front of the accessory lip; comparatively short and somewhat incomplete.

4:03 p.m.:—(Fig. *f*). Embryonic body, medullary groove and folds distinctly recognizable. Distal end of the medullary groove has not yet reached the final closing point of the actual blastopore, there being interposed the circular patch formed by

the accessory dorsal lip. Ex-ovate attached to the closing point of the actual blastopore. Embryo short, although otherwise not greatly abnormal, its axis turned about 14°–15° to the right.

It seems to me that in this egg, the blastoporic area has somehow become divided into two entirely separate parts: (1) the upper smaller part which was represented by the accessory lip and which completed itself in a circle separate and smaller; and (2) the lower, larger, main part which closed itself finally at the point of puncture, the overgrowth of the yolk having been effected mostly by the ventral and lateral lips. Moreover the embryonic body must all have been formed in front of the injured spot, *i.e.* mostly in the original upper hemisphere of the egg. Fig. 77 gives the diagram of this egg.

Egg No. 3. (Figs. 69 *a–f*, Fig. 78).

May 5, 12:40 p.m.:—(Fig. *a*). Punctured at the periphery of the translucent area of the segmentation cavity, in front of, and in the line of the first appearing dorsal blastopore lip.

4:40 p.m.:—(Fig. *b*). Dorsal blastopore lip has grown somewhat downward and in length, and shows a more than usually crescentic curve at the highest point of which the ex-ovate is now attached.

May 6, 8:12 a.m.:—(Fig. *c* = posterior, Fig. *d* = left side view). Very peculiar aspect. Blastopore circle finished, but unusual in appearance. Ventral part round and smooth, but a gradually narrowing arm sent dorsad, and the ex-ovate attached at the tip. Segmentation cavity likewise peculiar, and elongated antero-posteriorly.

4:19 p.m.:—(Fig. *e*). Area of the segmentation cavity entirely vanished. Blastopore grown much smaller and almost circular, but still connected by a narrow slit with the ex-ovate. General outline of the embryonic shield faintly recognized.

May 7, 9:10 a.m.:—(Fig. *f*). Medullary groove and folds well re-

cognizable. Blastopore already closed leaving a small yolk-plug. Ex-ovate detached and adhering to the inner face of the vitelline membrane, but its scar still visible as a plug-like protuberance of the yolk substance in front of the normal yolk-plug. Embryonic body shorter than normal.

The first effect of puncture in this egg seems to have been a sending upward of the dorsal blastopore lip toward the point of injury. This was probably due to the exudation of more or less yolk substance in the intervening space. The dorsal lip does not seem to have grown downward at all after this, thus causing a peculiar pointed bay in the blastoporic area. The growth over the yolk seems to have been undertaken entirely by the ventral and lateral lips. The whole of the embryo was without doubt formed entirely on the upper hemisphere, as in No. 2. Fig. 78 represents diagrammatically the location of the embryo body. The oblique dotted line indicates the part over which the dorsal blastopore lip was pulled upward.

Egg No. 4. (Figs. 70 *a–f*, Fig. 79).

May 5, 2:43 p.m. :—(Fig. *a*). Punctured on both sides of the dorsal blastopore lip slightly below its level, and at about equal distances from it (the right puncture just a little nearer and heavier, hence its ex-ovate larger).

4:14 p.m. :—(Fig. *b*). No noteworthy peculiarity. Translucent area grown smaller. Dorsal lip grown slightly downwards.

May 6, 8:19 a.m. :—(Fig. *c*). Blastopore circle completed but very peculiar in appearance. Two horns sent outward from its upper lateral corners to each ex-ovate. Dorsal lip curved contrary to the usual way and has a hanging festoon-like outline, showing that the middle point is growing fastest downward. Segmentation cavity of an unusual shape, being elongated antero-posteriorly and somewhat out of the median line on the left side.

4:22 p.m. :—(Fig. *d*). Segmentation-cavity entirely vanished.

Blastopore circle grown much smaller and become oval in shape; detached from the smaller left ex-ovate, although the basal part of the horn by which it reached the latter still remains; approximated much closer to the larger right ex-ovate; dorsal lip again in the normal crescentic curve.

May 7, 9:14 a.m.:—(Fig. *e*). Blastopore just closed leaving a comparatively large yolk-plug to which the right ex-ovate is attached. Left ex-ovate found near the left margin of the neural plate, though its distance from the right ex-ovate is nearly the same. Neural plate clearly recognizable.

4:15 p.m.:—(Fig. *f*). Yolk-plug withdrawn into the interior. Right ex-ovate attached to the posterior end of the right medullary fold. Left ex-ovate in the same position as before. Embryo itself normal, but its position greatly revolved leftward.

In this egg, the blastopore was closed at the heavier right puncture, and the growth over the yolk was undertaken mostly by the ventral, and left lateral lips. As the axis of the embryo-body has veered greatly leftward with the right ex-ovate as the center, the main part of the body has been formed within the early equatorial zone, as shown in Fig. 79.

Egg No. 5. (Figs. 71 *a–f*, Fig. 80).

May 5, 3:57 p.m.:—(Fig. *a*). Punctured at the approximate middle point of the region antipodal to the first appearing dorsal blastopore lip, at about the level of the equator.

4:14 p.m.:—(Fig. *b*). Dorsal lip grown somewhat downward seems to be moving leftward.

May 6, 8:25 a.m.:—(Fig. *c*). Blastopore circle completed, and grown moderately small; ex-ovate attached within it near the ventral lip. Area of the segmentation cavity diminished in size, and has shifted its position opposite as usual to the blastopore circle.

4:26 p.m.:—(Fig. *d*). Broad neural plate faintly recognized. Blastopore greatly reduced in size; ex-ovate attached to its left margin. Area of the segmentation-cavity entirely vanished.

May 7, 9:16 a.m.:—(Fig. *e*). Blastopore completely closed; yolk plug no longer visible. Ex-ovate attached to the left side of the closing point of the blastopore. Head and neural folds faintly recognizable although the posterior part of the neural groove is not yet distinct.

4:18 p.m.:—(Fig. *f*). Embryo formed and quite normal. Ex-ovate attached to the left side of the tail end.

Here we must conclude that the blastopore was closed at the punctured point (*i.e.* at the point opposite the first dorsal lip) by an excessive growth of the dorsal and lateral lips: as the embryo was situated in front of this, it must have been formed entirely on the lower hemisphere as given diagrammatically in Fig. 80.

Egg No. 6. (Figs. 72 *a–g*, Fig. 81).

May 5, 1:35 p.m.:—(Fig. *a*). Punctured closely below the dorsal blastopore lip, slightly to the right of the median line.

4:17 p.m.:—(Fig. *b*). Nothing noteworthy.

May 6, 8:32 a.m.:—(Fig. *c*=posterior, Fig. *d*=left side view). Segmentation-cavity and blastoporic area similarly acuminated toward the punctured point.

4:30 p.m.:—(Fig. *e*). Blastopore circle much smaller and now circular in shape. Ex-ovate attached to its upper margin. Area of the segmentation cavity entirely vanished.

May 7, 9:20 a.m.:—(Fig. *f*). Embryonic area defined. Ex-ovate attached to its posterior end. Blastopore already closed.

4:21 p.m.:—(Fig. *g*). Embryonic body well formed, normal in every way except that it is short. Ex-ovate attached to its posterior end.

Fig. 81 gives a diagrammatic representation of this egg. The embryo except for its shortness is entirely normal as is also its location. This is probably due to the circumstance that the puncture was slight, compared with Egg No. 2. Although the point of injury was different, the results in this egg are very much like those seen in Egg No. 3.

Egg No. 7. (Figs. 73 *a–f*, Fig. 82).

(*Cf.* Egg No. 3).

May 5, 12:50 p.m. :—(Fig. *a*). Punctured closely above the approximate middle point of the dorsal lip.

4:19 p.m. :—(Fig. *b*). Dorsal lip slightly grown downward ; its middle point bent and drawn upward toward the ex-ovate with which it is in direct contact. (*Cf.* Fig. 69 *b*).

May 6, 8:35 a.m. :—(Fig. *c*). Area of the segmentation-cavity unusually small. Blastopore circle still large and acuminated toward the ex-ovate. (*Cf.* Figs. 69 *c* and *d*).

4:32 p.m. :—(Fig. *d*). Segmentation-cavity no longer visible. Blastopore circle small, and somewhat triangular in shape, with the apex touching the ex-ovate. Neural plate faintly recognizable.

May 7, 9:24 a.m. :—(Fig. *e*). Blastopore closed ; ex-ovate attached to the point of its closure. Yolk-plug withdrawn. Neural plate distinct.

4:25 p.m. :—(Fig. *f*). Medullary groove ready to be closed. Ex-ovate attached to the posterior end of the right medullary fold.

The results as represented in Fig. 82 are very much as in Egg No. 3, as well as in Egg No. 6.

Egg No. 8.

Failure.

Egg No. 9. (Figs. 74 *a–f*, Fig. 83).

May 5, 1:15 p.m. :—(Fig. *a*). Punctured at the approximate animal pole, somewhat heavily, as the fluid contents of the segmentation cavity were forcibly ejected at the time.

4:23 p.m. :—(Fig. *b*). Segmentation cavity collapsed ; even a small quantity of yolk substance had been protruded. Dorsal blastopore lip growing downward normally.

May 6, 8:44 a.m. :—(Fig. *c*). Shrinkage of the upper hemisphere recovered ; area of the segmentation-cavity disproportionately

small compared with the blastoporic area. First ex-ovate had been lost without leaving any trace; in its place a large globular mass protruded anew. Blastopore lip grown considerably downward.

4:41 p.m. :—(Fig. *d*). Segmentation cavity no longer visible. Blastopore considerably reduced in size. Large ex-ovate in front, and to the right of the now faintly recognizable medullary plate.

May 7, 9:30 a.m. :—(Fig. *e*). Medullary groove and folds evident. Ex-ovate in front.

4:27 p.m. :—(Fig. *f*). Embryonic body distinct, shorter than normal. Medullary groove ready to be closed. Ex-ovate a short distance in front of the head end.

The shortness of the embryonic body is probably due to the exudation of the second large ex-ovate. The development is otherwise normal. Compare Diagram Fig. 83.

Egg No. 10. (Figs. 75 *a–f*, Fig. 84).

(*Cf.* Egg No. 5).

May 5, 2:43 p.m. :—(Fig. *a*). Punctured on the ventral median line, approximately 45° below the equator.

4:26 p.m. :—(Fig. *b*). Normal.

May 6, 8:46 a.m. :—(Fig. *c*). Blastopore circle complete. Ex-ovate at its ventral lip.

4:45 p.m. :—(Fig. *d*). Area of the segmentation cavity vanished. Blastopore much smaller and the ex-ovate attached to its left dorsal margin.

May 7, 9:30 a.m. :—(Fig. *e*). Blastopore nearly closed, leaving a small yolk-plug. Ex-ovate out of the blastopore, but still closely attached to the dorsal lip. Neural plate faintly visible.

4:30 p.m. :—(Fig. *f*). General outline of the embryo formed. Ex-ovate attached to the posterior end of the left neural fold.

We must conclude that the blastopore was finally closed at the point of injury, the ventral lip ceasing to grow when it reached the ex-ovate about 45° below the equator, the task of overgrowth

being thereafter undertaken by the dorsal and lateral lips. The location of the embryo must therefore be, as in Fig. 84, on the lower hemisphere of the egg. The results are exactly as in Egg No. 5, the only difference being that as the puncture was further down in this egg, the position of the embryo was different in the same degree.

First Group. (Figs. 85–86).

The eggs of this group were picked out from a mass obtained on the morning of April 17th., (deposited in all probability on the morning of the 16th.) and were at the time at the end of the segmentation process. The first trace of the dorsal blastopore lip appeared at 2 p.m., of the same day. Puncturing was begun at 2:30 and finished at 4:40 p.m.

Egg No. 1.

April 17, 2:30 p.m. :—Punctured at the approximate center of the lower yolk hemisphere as in Group IV., Egg No. 1. (*Cf.* Figs. 67 *a–e*). Watched until April 19, 2:12 p.m. when the embryo had been completely formed in the normal way, with only the ex-ovate attached to the distal end of the embryonic body. Results are exactly as in the egg referred to : hence details may be omitted.

Egg No. 2.

April 17, 2:30 + p.m. :—Punctured below the dorsal lip slightly to the left of the median line, at a point which was two-thirds of the distance from the yolk polo to that lip (as in Group IV., Egg No. 2, Figs. 68 *a–f*). Puncture unfortunately rather severe and an excessive amount of egg-contents exuded.

April 18, 2:00 p.m. :—Upper hemisphere strongly depressed. Area of the segmentation cavity vanished. Still more matter exuded

and covered the posterior portion of the egg so that the blastopore could not be seen.

April 19, 2:20 p.m.:—Head portion of the embryo seen beneath, and in front of, the exuded matter.

Although exact observation could not be made, there is no reason to suppose that the course of development was, in the main, otherwise than was seen in Group IV., Egg No. 2, or Nos. 6 and 7.

Egg No. 3.

April 17, 2:30 + p.m.:—Punctured as in Group IV., Egg No. 3, (Figs. 69 *a–f*), but somewhat higher. Liquid-contnets forced out of the segmentation cavity whose roof became shrunken. Exudation, however, soon ceased, and the roof recovered its usual appearance. Then the distance between the punctured point and the dorsal lip became reduced to about $\frac{1}{2}$. This is in all probability due to the upward movement of the blastopore toward the punctured point as in Group IV., Egg No. 3.

April 18, 2:15 p.m.:—Area of the segmentation cavity and blastoporic area reduced in size in equal proportions. But the distance of the ex-ovate from the dorsal lip remains the same.

April 19, 2:30 p.m.:—General outline of the embryonic body evident. Blastopore completely closed, and at the same distance from it as before, the ex-ovate.

How the ex-ovate keeps its distance from the dorsal lip unaltered, after the first rather sudden approximation of the two may probably be explained in this way. After the first pulling upward of the dorsal lip toward the injured point, it ceases to grow any further downward, due somehow to the injury inflicted above it and the consequent exudation of the contents within, and the overgrowth of the blastopore lips over the yolk is performed mostly by the ventral and lateral lips. The final point of the blastopore closure is therefore near the point of the

first appearance of the dorsal lip, and the embryo is formed almost entirely on the upper hemisphere of the egg (Fig. 78). The results are therefore identical with those of the egg of the corresponding number in Group IV.

Egg No. 4. (Figs. 85 *a–c*, Fig. 85′).

April 17, 2:30 + p.m. :—(Fig. *a*). Punctured on both sides of the dorsal blastopore lip, slightly below its level and at equal distances from it (as in Group IV., Egg No. 4, Fig. 70 *a*). Right puncture heavier than left in this egg also, but the two punctures further from the dorsal lip and from each other than in that egg. Ex-ovate produced from the punctures in profile in the posterior view and about 20° below the equator. Another unexpected small and transverse ex-ovate formed directly beneath the dorsal lip which, however, disappeared next day.

April 18, 2:30 p.m. :—(Fig. *b*). Right and left ex-ovates somewhat nearer each other. Blastopore oval and closer to the right (larger) ex-ovate. Main axis of the future embryo-body somewhat curved toward the right side. This state of things can probably be explained by unequal growth of blastopore lips. Punctures and ex-ovates must check the growth of the blastopore lips, when they are reached in the downward growth. In this case, the growth has not entirely ceased but been only retarded, longer on the right side than on the left, because the injury was severer on the former side. Dorsal lip was also somehow checked in its further downward growth, when it reached the level of the ex-ovates, and overgrowth must have been done mostly by the ventral lip. Hence the position of the blastopore at the level of the ex-ovates; its shape is transversely oval, because retarded at the ex-ovates; it is nearer the right ex-ovate because the left lip was, so to speak, released sooner at the left ex-ovate and had grown more than the right.

April 19, 2:30 p.m. :—(Fig. *c*). Blastopore nearly closed, leaving only a small yolk-plug. Right ex-ovate detached from the punctured

point and carried to the right of the middle of the embryo. Left ex-ovate in the former position. Embryo entirely recovered from its lateral curvature and quite normal.

As in Group IV., Egg No. 4, the blastopore closed in this egg near the right point of puncture, the growing of its rim over the yolk being performed mostly by the ventral and left lateral lips. But unlike that egg (Fig. 79), the position of the embryo was more normal, although the main portion of it was formed on the upper hemisphere (Fig. 85′).

Egg No. 5. (Figs. 86 *a–c*, Fig. 86′).

April 17, 2:30 + p.m.:—(Fig. *a*). Punctured at the approximate middle point of the region opposite the first appearing dorsal lip, as in Group IV., Egg No. 5, Fig. 71 *a*. The point of injury was a little below the level of the dorsal lip, and the exudation of the contents somewhat serious so that the upper hemisphere shrunk near the puncture. It soon recovered, however.

April 18, 2:40 p.m.:—(Fig. *b* = view from the original lower pole). Area of the segmentation cavity disappeared. Blastopore small, and about 30° dorsad from the ex-ovate.

April 19, 3:30 p.m.:—(Fig. *c*). Blastopore closed, about 35° dorsad of the ex-ovate, the increased distance (5°) being equal to about half the diameter of the blastoporic circle in Fig. *b*. Embryonic body distinct.

Unlike the egg of the corresponding number in Group IV., the blastopore did not close in this egg at the punctured point, but 35° below it (Fig. 86′). We must suppose that the ventral lip was able to overcome the obstacle presented by the puncture and to grow further downward. The final closing point of the blastopore becomes as usual the tail end of the embryo which is not therefore formed entirely on the lower hemisphere as in Fig. 80.

Egg No. 6.

April 17, 2:30 + p.m.:—Punctured in the middle line, close below the dorsal blastopore lip (*Cf.* Group IV., Egg No. 6, Figs. 72 *a–g*).

Blastopore finally closed at the point of puncture, and the embryo stretching forward from this point was distinctly shorter than normal, though not as short as in Group IV., Egg No. 6.

The results are therefore exactly like those in the egg of the corresponding number in Group IV.

Egg No. 7.

Not punctured, as there were 1 large, and 3 or 4 small natural ex-ovates in the anti-blastoporic region. Twenty four hours later, they had, however, all become detached from the egg and attached to the inner surface of the vitelline membrane. Hence quite useless for the present investigation.

Egg No. 8.

Punctured closely above the first dorsal blastopore lip as in Group IV., Egg No. 7 (Figs. 73 *a–f*, Fig. 82). In this egg the injury was slightly to the right of the middle line and lighter. Twenty-four hours later, blastopore circular and already small; ex-ovate attached to its right margin. Hence stage like Fig. 73 *c* missed, if present. Another twenty-four hours, ex-ovate attached to the right margin of the closed blastopore. Embryo short but otherwise normal.

The results are therefore absolutely the same as those in Group IV., Egg No. 7.

Egg No. 9.

Chiefly instructive in regard to the formation of ex-ovates. Punctured at the approximate lowest point of the yolk

hemisphere as in Egg No. 1 of this and Group IV. Exudation rather less than usual.

Twenty-four hours later, ex-ovate enormously elongated, within the vitelline membrane, into a long streak the base of which was attached to the left margin of the moderately closed blastopore. The point in the vitelline membrane through which puncturing was effected shifted considerably upward from the dorsal lip. Careful examination showed that as after puncture I had placed the egg with the yolk hemisphere to one side, the egg rotated within the vitelline membrane to its natural position, and in so doing the ex-ovate was gradually elongated into a long streak by additional exudation. I tried to restore the egg to the proper position, but the exudation outside the outer envelope had become firmly attached to cotton-fibres in a bed of which substance the egg had been placed. So the egg was left as it was.

Another twenty-four hours, some more exudation which seemed, however, completely detached from the egg-surface. Embryo seems normal in its formation beneath the large, but detached ex-ovate.

The final closing point of the blastopore and the location of the embryonic body were somewhat obscured by the exudations, but as the ex-ovate was seen distinctly attached to the dorsal lip up to the evening of the second day after puncture, we may suppose that the final point of the blastopore closure was, as usual, at the punctured point (in this case, the yolk-pole), and that the embryo must have been formed as in other cases of this kind.

Egg No. 10.

Punctured on the ventral median line about 68° below the equator, *i.e.* somewhat lower than in Group IV., Egg No. 10 (Figs. 75 *a–f*).

Twenty-four hours later, blastopore diminished to a very small circular spot to the ventral margin of which the ex-ovate was attached.

After another twenty-four hours, the ex-ovate had un-

fortunately become detached and was found a short distance forward from the tail end of the embryo. Embryo quite normal.

Although the detaching of the ex-ovate obscured the final stage, there can be no reasonable doubt that the closing point of the blastopore was at, or near, the punctured point, and so the location of the embryonic body nearly the same as Egg No. 5 of this group or Egg No. 10 of Group IV.

Second Group. (Figs. 87–92).

The eggs of this group were obtained from a mass deposited late on the morning of April 21. The dorsal blastopore lip appeared at 8:10 a.m., April 23. Punctures were carried out between 8:20 and 9:30 of the same morning.

Egg No. 1. (Figs. 87 *a-d*, Fig. 88).

April 23, 8:20 a.m. :—(Fig. *a*). Punctured at the approximate middle point of the yolk-pole (*Cf.* Group I., Eggs Nos. 1 and 9, and Group IV., Egg No. 1). Needle-point somewhat blunt and did not penetrate well. Only a small ex-ovate.

April 24, 7:20 a.m. :—(Fig. *b*). Blastopore much reduced in size, and somewhat oval in shape. Ex-ovate near the dorsal lip showing that this lip has grown faster than the ventral. Another unexpected ex-ovate discovered near the ventral lip, accounting for the slow growth of that lip, and the shape of the blastopore.

3:30 p.m. :—(Fig. *c*). Blastopore much smaller. Dorsal ex-ovate now excluded out of it and found to the left of the median line of the neural plate consisting of a larger anterior, and a smaller posterior globule arranged close together longitudinally. Former no doubt the original ex-ovate, and the smaller formed at the time of exclusion. Ventral ex-ovate at the ventral blastopore lip.

April 25, 5:40 p.m. :—(Fig. *d*). General outline of the embryonic body distinct. Embryo, however, somewhat incomplete and its axis

more or less curved to right and left in a wavy manner. Dorsal ex-ovate as before. Ventral ex-ovate unfortunately detached and lost without trace. But a mass of yolk has exuded anew from the closing point of the blastopore, probably at the time the ventral ex-ovate was detached.

In the normal course of development, the dorsal lip ought to have stopped when it reached the first ex-ovate formed by puncturing. But in this egg there was formed unexpectedly a second ex-ovate which stood in the way of the ventral lip growing over the yolk. Accordingly the dorsal lip went on growing toward the ventral lip, until the blastopore finally closed at or near the second ex-ovate. The embryo was thus formed as in Fig. 88. Seeing that all these disturbances took place, it is no wonder that the embryo body is somewhat abnormal.

Egg No. 2.

Punctured at the point intermediate between the dorsal lip and the yolk-pole, on the blastopore meridian. (Therefore lower and more in the middle line than in the eggs of the corresponding number in Groups I., and IV.). Although exudation at the moment was slight, the pressure of the somewhat blunt needle must have been rather strong. At any rate a number of larger and smaller unexpected ex-ovates appeared below the dorsal lip and near the puncture-point. Afterwards there were further exudations at this point as well as from the left limb of the dorsal blastopore lip when it had grown down some distance, showing that the arrangement of the internal contents must have been considerably disturbed. The results of all these were that the dorsal, ventral and lateral, lips were all hindered in their growth and the embryo was abnormal, being shorter, and the neural groove and folds being incomplete, especially near the head-end. The closure of the blastopore was also incomplete, a large yolk-plug being present at the end of the third day. But in the main,

the closing point of the blastopore was at or near the punctured point, and the anterior $\frac{2}{3}$ or $\frac{3}{4}$ of the embryonic body were formed in front of the point at which the dorsal lip first appeared.

Egg No. 3.

April 23, 4:15 p.m. :—Taken from another egg-mass and punctured at the upper pole, as in Group IV., No. 9. Fluid contents of the segmentation cavity forcibly ejected, and the upper hemisphere shrunken. After a few minutes ejection stopped.

April 24, 7:25 a.m. :—Shrinkage of the upper hemisphere recovered, although the area of the segmentation cavity unusually small. Blastopore circle completed and grown down to about 50° below the equator. Neural plate faintly visible.

2:20 p.m. :—Small ex-ovate found in front of the neural plate and to the right of the median line.

April 25, 5:00 p.m. :—Blastopore completely closed. General outline of the embryonic body evident, with well-defined neural groove and folds. Ex-ovate found 20°–50° forward from the head-end of the embryo, about opposite the final closing point of the blastopore. Embryo quite normal.

This egg was therefore like Group IV., Egg No. 9 with this difference, that in the latter the embryo was shorter, owing to the exudation of the large secondary ex-ovate after the first was lost.

Egg No. 4. (Figs. 89 *a–e*, Fig. 90).

April 23, 8:20 + a.m. :—(Fig. *a*). Punctured on both sides of the dorsal blastopore lip, some distance below its level (as in the eggs of the corresponding number in Groups I., and IV.). In this egg, left puncture at the periphery of the egg when seen in the posterior view ; right puncture higher and nearer the dorsal lip.

2:40 p.m. :—(Fig. *b*). Dorsal lip grown downwards and elongated, left limb reaching the left ex-ovate ; right limb slightly above the right ex-ovate.

April 24, 7:00 a.m. :—(Fig. *c*). Blastopore lips grown further down-

ward, and the circle complete; ovate in shape, the pointed extremity extending toward and reaching the right ex-ovate. Place of the small left ex-ovate already grown over and now outside the blastopore.

2:15 p.m.:—(Fig. *d*). Blastopore circle grown much smaller. But quite unexpectedly a new short transverse ex-ovate was found attached close to the ventral lip. Right ex-ovate now also outside the blastopore circle, but a string-like prominence with rough surface connecting it with the new transverse ex-ovate (its nature not clear). We may suppose that a large new obstacle appearing to hinder the progress of the ventral lip, the right lip had time allowed it to overcome the right ex-ovate and to grow down toward the ventral lip producing somehow the string-like prominence in its track. If this new obstacle had not appeared, the blastopore might have closed much nearer the right ex-ovate. Neural plate not yet recognizable, although usually seen at this stage.

April 25, 5:07 p.m.:—(Fig. *e*). General outline of the embryonic body with partly closed neural groove and closely approximated folds distinct. Embryo short and curved to the right. Right ex-ovate attached to the right side of the embryonic head; left ex-ovate in the former position. Transverse ex-ovate at the posterior end of the embryo.

The actual point of the blastopore closure and the location of the embryonic body were somewhat obscured in this egg. But the former, we may suppose, was a little to the left of the yolk-pole, and the embryonic body was directed from this point obliquely toward the point which corresponded to the right edge of the dorsal blastopore at its first appearance, as shown in Fig. 90. The inclination of the embryonic axis to the former blastopore meridian is about 45°.

Egg No. 5.

April 23, 3:40 p.m.:—Taken out of another egg-mass. It was intended to puncture the egg at the anti-blastoporic region as in the

eggs of the corresponding number in Groups I., and IV. But when punctured, the point was found to be above the level of the dorsal lip, and to the right of the anti-blastoporic meridian (*i.e.* to the left when seen from the blastoporic meridian). Then another puncture was made: This time, it was at the level of the dorsal lip but slightly to the left of the anti-blastoporic meridian (*i.e.* to the right, when seen from the blastoporic meridian). From the first puncture, a small quantity of the egg-contents exuded through the egg envelope; from the second, a small protuberance was produced as an ex-ovate inside the envelope.

April 23, 7:15 a.m.:—Blastopore circle already finished. Ventral lip has overcome the first ex-ovate and passed 10° beyond it, but not yet detached from the second ex-ovate which is found close to this lip. Dorsal and lateral lips grown much further, 55°–60° below the equator.

3:07 p.m.:—Blastopore reduced to a small circular spot; second ex-ovate close to the right lateral lip; first ex-ovate about double the same distance from the ventral lip.

April 24, 5:15 p.m.:—Blastopore closed. Embryonic body formed normally. First ex-ovate at the distal end of the embryonic body; second ex-ovate, on the right side of the right neural fold; distance between the two unaltered. Growth therefore must be by the dorsal and lateral lips.

As the blastopore was closed about 10° below the first ex-ovate, and the embryo extended dorsad from this, nearly the whole of the embryonic body must have been formed on the yolk hemisphere, below the first dorsal blastopore lip. (*Cf.* Fig. 80).

Egg No. 6.

Punctured close below the dorsal blastopore lip in the median line, (*Cf.* the eggs of the corresponding number in Groups I., and IV.). Twenty-four hours later, the blastopore circle completed and all going well. Eight hours later, a large new tubercle-like ex-ovate was discovered close below the ventral lip. This was probably due in some way to the

pressure of the left hand needle during the operation which was done with the right. After this, the course of development was checked, and the egg was set aside.

Egg No. 7.

April 23, 8:20 + a.m. :—Punctured at the yolk-pole (as in Group I., Eggs Nos. 1 and 9, and Group IV., Egg No. 1). The point of puncture was however not at the actual pole, but slightly to the left when seen in the posterior view. Exudation somewhat forcible.

April 24, 7:22 a.m. :—Egg entirely recovered. Blastoporic area and area of the segmentation cavity diminishing in the normal way. Ex-ovate near the middle point of the blastoporic area.

2:50 p.m. :—Blastopore now reduced to a small spot. General outline of the neural plate faintly recognizable. Ex-ovate close to the left blastopore lip.

April 25, 5:20 p.m. :—Blastopore completely closed. General outline of the embryonic body well established, entirely normal. Ex-ovate attached to the left margin of the closed blastopore.

Except that the axis of the embryo did not coincide with the blastopore meridian, but was bent slightly to the left, because the punctured point which became the tail end was slightly to the left, the results agree entirely with those in the above mentioned eggs in Groups I., and IV.

Egg No. 8.

April 23, 9:00 a.m. :—Punctured near the right limb of the dorsal blastopore lip. Injury not severe, and a small quantity of the egg-contents exuded outside the egg-envelope.

2:50 p.m. :—Dorsal blastopore lip increased in length and grown a little downward, so that the right limb nearly reached the ex-ovate.

April 24, 7:30 a.m. :—Moderately reduced blastoporic area peculiar in shape : ovate with the pointed end turned toward the ex-ovate with which it was directly connected. Lateral and ventral lips not distinct.

2:50 p.m. :—Blastopore greatly reduced in size, but a very large mass of the egg-contents had been exuded from the ventral margin of the small blastopore, concealing from view that region. First ex-ovate still attached to the right lateral lip. Neural groove faintly recognized in front of the dorsal lip.

April 25, 5:25 p.m. :—Large secondary ex-ovate larger in size by additional exudation covering the posterior part of the egg but apparently entirely detached from the exuding part. Blastopore barely closed in front of the large ex-ovate. Original ex-ovate attached to the right margin of the tail fold, showing that the blastopore must have closed near the region when the dorsal lip first appeared. Embryo very imperfect: head could not be detected in the external view; tail well formed.

Although the formation of the embryonic body in this egg was very incomplete, the results in regard to the final point of the blastopore closure and the location of the embryonic body agree in the main with those obtained from Group IV., Nos. 2, 6, and 7; Group I., Nos. 6 and 8, and No. 6 of this group.

Egg No. 9. (Figs. 91 *a-d*, Fig. 92).

April 23, 9:00 + a.m. :—(Fig. *a*). Puncturing was intended to be at the exact yolk-pole, but was in fact slightly to the right in the posterior view.

April 24, 7:55 a.m. :—(Fig. *b*). Blastoporic area greatly reduced, circular in shape but connected with the ex-ovate by an arm on the right lip.

2:45 p.m. :—(Fig. *c*). Blastopore now reduced to a very small circle. Ex-ovate now outside of it to the right. Neural plate faintly visible.

April 25, 5:30 p.m. :—(Fig. *d*). Blastopore completely closed. General outline of the embryonic body well made out: *ex-ovate is at the exact axial end of the embryo, and the final closing point of the blastopore a little to the left of it did not become the tail end.*

The diagram of this egg is given in Fig. 92. Although there

is the last-noted peculiarity, the main results are the same as in other eggs which received similar injury: the blastopore closed at the punctured point and the embryo was situated as in the normal course of development.

Egg No. 10.

Intended to be punctured as in the eggs of the corresponding number in Groups I., and IV., but owing to failures in manipulation the results were meagre and not worth recording; there was nothing contrary to what had already been made out in other eggs.

Third Group. (Figs. 93–96).

The eggs of this group were taken from the mass deposited probably on the early morning of April 27. The dorsal blastopore lip appeared at 12:30 p.m., April 28. Puncturing operations were performed on them between 12:40 and 1:35 p.m. of the same day.

Egg No. 1.

Punctured at the approximate yolk-pole, as in the eggs of the same number in the three other groups. Ex-ovate not large. All went well during the first two days, excepting that at one time the surface of the yolk-hemisphere near the ventral lip had a rough appearance. But when examined 8:55 a.m., third day (April 30) a large and oval mass of yolk substance had been protruded outward as a yolk-plug along the dorsal blastopore margin. Original ex-ovate a little outside of the much reduced blastopore area near its ventral lip. At 5:06 p.m., (the same day) the peculiar yolk-plug had been largely withdrawn within the egg-interior. Blastopore become a small circular spot. Ex-ovate further removed from the

blastopore on the ventral side. Neural groove, though not neural folds, recognizable. At 7:00 a.m., fourth day (May 1), embryo formation not advanced. Blastopore completely closed at about the place of the exuded yolk-plug.

This egg must somehow have been damaged by the puncturing operation more than at first appeared. The final closing point of the blastopore was not at the point of puncture, which was the yolk-pole in this case, but at a point about 20° dorsad of that pole on the blastopore meridian, and the incomplete embryo stretched forward from that point. This is undoubtedly to be accounted for in this way: the ex-ovate stood for a time in the way of the growth of the ventral lip, but a greater obstacle in the shape of a peculiar yolk-plug effectually stopped the downward progress of the dorsal lip. Thus the ventral lip had time to overcome its obstacle and to grow up toward the dorsal lip, where the blastopore closed.

Egg No. 2.

Punctured about 20° below the dorsal blastopore lip in the middle line (as in the corresponding number of the other groups). Exudation little and injury seemed not very great. Four hours later, dorsal lip grown down close to the ex-ovate. Blastoporic region somewhat bulged outward. At 8:23 a.m., next day (April 29), a large new tubercle-like ex-ovate had been produced hanging down from the dorsal region, in front of the dorsal blastopore lip. Original ex-ovate now attached to the middle basal part of the new tubercle, anteriorly from the dorsal lip. At 5:35 p.m., blastoporic circle completed but acuminate toward the tubercle, as also the area of the segmentation-cavity from above. At 9 a.m., third day (April 30), area of the segmentation cavity disappeared. Blastopore area still large. At 5 p.m., blastopore greatly reduced in size. Tubercle-like ex-ovate smaller and grown in length, whereby the original ex-ovate was pushed further

forward from the dorsal lip. At 7 a.m., fourth day (May 1), blastopore closed just behind the tubercle. Embryonic body not recognizable.

Although the definite location of the embryonic body was not made out, it is evident that the blastopore was closed at the place of the greatest obstacle to the growth of the blastopore lips.

Egg No. 3. (Figs. 93 *a–h*, Fig. 94).

April 28, 12:50 p.m.:—(Fig. *a*). Punctured about midway from the upper pole to the dorsal blastopore lip on the blastopore meridian. Not only liquid contents but also some solid yolk-substance exuded.

4:34 p.m.:—(Fig. *b*). Dorsal lip grown downward and increased in length; its right limb and ex-ovate nearer each other, as has been often noticed before in similar cases.

April 29, 8:30 a.m.:—(Fig. *c* = left side view, Fig. *d* = posterior view). Blastopore circle completed and grown small; shape ovate, acuminate toward the ex-ovate, which is found in the same relative position to the dorsal lip.

5:38 p.m.:—(Fig. *e*). Blastopore greatly reduced in size. Area of the segmentation cavity completely disappeared. Ex-ovate in the same relative position.

April 30, 9:04 a.m.:—(Fig. *f*). Blastopore nearly closed, leaving a small oval yolk-plug exposed. Ex-ovate about 30° above it in the same relative position. Medullary groove and folds not distinct.

5:12 p.m.:—(Fig. *g*). Blastopore completely closed. General outline of the embryonic body well made out: at its tail end is attached the ex-ovate.

May 1, 7.04 a.m.:—(Fig. *h*). Embryo formation more advanced. Ex-ovate in the same position as before.

The blastopore was probably closed in this egg at about the point where the dorsal lip first appeared, if we may judge from the position of the ex-ovate. But curiously enough, the tail end of the embryo did not coincide with the closing point of the blastopore,

but with the punctured point, as happened in Group II., Egg No. 9. The main parts of the embryonic body were, therefore, formed entirely upon the upper hemisphere (Fig. 94).

Egg No. 4. (Figs. 95 *a-g*, Fig. 96).

April 28, 1:35 p.m. :—(Fig. *a*). Punctured on both sides below the level of the dorsal lip as in the eggs of the same number in other groups. Punctures just seen in profile in the posterior view ; about 20° below the level of the dorsal lip which was 8° or 9° below the equator. Right puncture heavier and exudation outside the envelope. Left puncture much lighter, slightly protruded.

4:38 p.m. :—(Fig. *b*). Dorsal lip grown downward and increased in length : its left limb longer and nearly reaching the left ex-ovate which has grown a little larger ; its right limb still some distance from the right ex-ovate.

April 29, 8:45 a.m. :—(Fig. *c*=left side view, Fig. *d*=ventral view). Blastopore circle completed and ovate in shape, touching with the acuminate end the right ex-ovate. Left ex-ovate grown a little larger but firmly attached to the punctured point is far outside the blastopore circle (about 70° from the left lip), and curiously near the area of the segmentation cavity.

5:42 p.m. :—(Fig. *e*). Area of the segmentation cavity nearly disappeared. Left ex-ovate in the same relative position. Right ex-ovate still attached to the right lip of the further reduced blastopore.

April 30, 9:07 a.m. :—(Fig. *f*). Blastopore become still smaller and circular in shape, with the right ex-ovate yet closely attached to the right lip. Left ex-ovate more remote from the left lip. In front of the blastopore neural plate recognizable.

5:14 p.m. :—(Fig. *g*). Left ex-ovate approximately in the neck region of the future embryo. Right ex-ovate still attached to the right lip of the now greatly reduced blastopore. The egg unfortunately died after this.

As the blastopore closed at the right point of puncture where the injury was greater, the growth of the blastopore rim must

have been performed mostly by the dorsal, ventral, and left lips. And to judge from the relative position of the two ex-ovates, in regard to each other and to the embryo, the location of its body must have been as in Fig. 96. This is similar to Group IV., Egg No. 4 (Fig. 79), but the inclination of the embryo body to the blastopore is greater in this case than in that egg.

Egg No. 5.

Punctured at a point approximately antipodal to the place of the first appearance of the dorsal lip, as in the eggs of the corresponding number in the other groups. Development proceeded as in those eggs. Blastopore gradually closing presented an ovate shape, with the ex-ovate attached to its acuminate ventral end. When it was nearly closed, there was found a small yolk-plug on the top of which the ex-ovate somewhat reduced in size was attached. It finally closed here. Embryonic body extended from here forward.

This egg, therefore, substantiates the results obtained in the eggs of the same number in Groups II., and IV.

Egg No. 6.

Punctured at the approximate middle point close under the dorsal lip (see the corresponding number in other groups). $3\frac{1}{2}$ hours afterward, appeared somewhat peculiar: two dorsal lips, one above the other, as in Group IV., Egg No. 2 (Fig. 68 *d*). The higher was probably the original one. A large, rectangular ex-ovate exuded afresh behind the lower and actual dorsal lip. Original ex-ovate attached to the middle point of this. At 9.05 a.m., next day (April 29), the area of the segmentation-cavity strongly acuminated toward the ex-ovates (as in Group IV., Nos. 3 and 6 and No. 2, of this group), and much more than the blastopore.

Blastopore grew smaller and in the end would probably have closed somewhat behind the large secondary ex-ovate

which was futher enlarged by fresh exudation. Embryonic body stretched forward from this and was short compared with the normal. Original ex-ovate on the middle point of the secondary ex-ovate as before.

Although the results are imperfect, the manner of the blastopore closure and the location of the embryonic body are as in Egg No. 6 of Groups I., and IV.

Egg No. 7.

Punctured close above the middle point of the dorsal blastopore lip. Injury very slight. Ex-ovate very small. Dorsal lip seems to have been able to overcome the injury easily, for at 9:09 a.m., next day (April 29), it had already cleared from the ex-ovate and grown downward to about 35° below the equator. Segmentation cavity grown moderately small as also the blastopore circle which was not entirely circular but somewhat oval, the dorsal lip being drawn up a little toward the ex-ovate. Soon after this, ex-ovate became detached, and therefore development proceeded entirely normally and the embryo formed was normal.

This incidentally proves that, as remarked by ASSHETON ('94*a*), an egg is able to recover sooner or later from a slight injury, and is able to develop in an entirely normal manner.

Egg No. 8.

April 28, 1:24 p.m.:—Punctured at both the upper and lower poles. Lower injury much more severe, so much so that one hour after the lower hemisphere was somewhat diminished in size and there was a distinct space between the egg and the vitelline membrane.

April 29, 9:20 a.m.:—Blastopore area and area of the segmentation cavity equally diminished in a moderate degree. Unfortunately a new ex-ovate produced in front *i.e.* dorsad of the lower puncture. Dorsal lip had, however, already grown over the new obstacle and was nearer the original ex ovate than the ventral lip.

5:55 p.m.:—Blastopore area and area of the segmentation cavity much diminished in size in equal ratio. Dorsal lip, however, not grown any further, so that the reduction of the blastopore area at this stage is due to the growth of the ventral and lateral lips. Secondary ex-ovate beneath the dorsal lip still more increased in size.

April 30, 9:23 a.m.:—Blastopore area diminishing faster than the area of the segmentation cavity, though both are now much reduced. Two ex-ovates still at their antipodal positions.

5:25 p.m.:—Area of the segmentation cavity disappeared. Blastopore closed. Rudiment of medullary groove (primitive groove?) seen in front of the lower ex-ovate. Other embryonic parts not made out.

May 1, 7:14 a.m.:—No further progress.

The final point of the blastopore closure in this egg was no doubt about the lower punctured point (yolk-pole), and the location of the imperfect embryo was as usual between the two poles.

Egg No. 9.

Punctured at the approximate upper pole (see Group IV., Egg No. 9; Group II., Egg No. 3). Puncture light. Development proceeded normally and the embryo formed normal. At the end the ex-ovate found about 20° forward from the embryonic head-end, and about 25° to the right of the produced embryonic axis.

Egg No. 10.

April 28, 1:30 p.m.:—Punctured at a point approximately midway between the yolk-pole and the anti-blastoporic point, as in the eggs of the corresponding number in other groups. Piercing rather forcible, but not as much as in Group II., Egg No. 10. Soon afterwards a second small ex-ovate was produced unexpectedly close under the dorsal lip.

5:10 p.m.:—Dorsal lip grown somewhat downwards and increased in length, has changed its position very peculiarly to the left side of the egg between the first and second ex-ovate.

April 29, 9:36 a.m. :—Blastopore circle already completed, and somewhat reduced in size; not circular in shape, but elongated antero-posteriorly between the two ex-ovates, showing that growth downward has been accomplished mostly by the lateral lips. Secondary ex-ovate nearly excluded out of the area, although still connected with the dorsal lip by a narrow channel. Original ex-ovate still attached to the ventral lip. Left lip entire but right lip somewhat zigzag, showing that growth was more or less disturbed.

6:02 p.m. :—Area of the segmentation cavity already disappeared. Blastopore a very small but elongated spot in front of the original ex-ovate which is attached to the ventral lip. Secondary ex-ovate in the same position as before. Neural plate not recognizable.

April 30, 9:30 a.m. :—Blastopore closed. Location of the embryonic body still doubtful.

5:30 p.m. :—Embryonic body with the neural plate and the primitive groove faintly recognizable in front of the original ex-ovate. Secondary ex-ovate entirely disappeared.

May 1, 7:24 a.m. :—Embryonic body distinct. Original ex-ovate attached to the tail end. Embryo has its axis somewhat curved to the left; otherwise normal.

The results in this egg are in the end strictly like the eggs of the corresponding number in the other groups.

For the sake of convenience I append here a table showing where the punctures were made on each egg :—

	Where punctured.	At yolk pole.	At upper pole.	Close below dorsal blast. lip.	Close above dorsal blast. lip.	At anti-blastopore region.	On both sides of dors. blast. lip.	At a point midway from yolk pole to dors. lip.	At a point midway from yolk pole to anti-blast. region.	At a point midway from dors. lip to upper pole.	Figures.
Group I.	1	×	—	—	—	—	—	—	—	—	
	2	—	—	—	—	—	—	×	—	—	
	3	—	—	—	—	—	—	—	—	×	
	4	—	—	—	—	—	×	—	—	—	Figs. 85 *a–c*, Fig. 85′.
	5	—	—	—	—	×	—	—	—	—	Figs. 86 *a–c*, Fig. 86′.
	6	—	—	×	—	—	—	—	—	—	
	7	—	—	—	—	—	—	—	—	—	not punctured.
	8	—	—	—	×	—	—	—	—	—	
	9	×	—	—	—	—	—	—	—	—	
	10	—	—	—	—	—	—	—	×	—	
Group II.	1	×	—	—	—	—	—	—	—	—	Figs. 87 *a–d*, Fig. 88.
	2	—	—	—	—	—	—	×	—	—	
	3	—	×	—	—	—	—	—	—	—	
	4	—	—	—	—	—	×	—	—	—	Figs. 89 *a–e*, Fig. 90.
	5	—	—	—	—	×	—	—	—	—	
	6	—	—	×	—	—	—	—	—	—	
	7	×	—	—	—	—	—	—	—	—	
	8	—	—	—	×	—	—	—	—	—	
	9	×	—	—	—	—	—	—	—	—	Figs. 91 *a–d*, Fig. 92.
	10	—	—	—	—	—	—	—	×	—	
Group III.	1	×	—	—	—	—	—	—	—	—	
	2	—	—	—	—	—	—	×	—	—	
	3	—	—	—	—	—	—	—	—	×	Figs. 93 *a–h*, Fig. 94.
	4	—	—	—	—	—	×	—	—	—	Figs. 95 *a–g*, Fig. 96.
	5	—	—	—	—	×	—	—	—	—	
	6	—	—	×	—	—	—	—	—	—	
	7	—	—	—	×	—	—	—	—	—	
	8	×	×	—	—	—	—	—	—	—	
	9	—	×	—	—	—	—	—	—	—	
	10	—	—	—	—	—	—	—	×	—	
Group IV.	1	×	—	—	—	—	—	—	—	—	Figs. 67 *a–e*, Fig. 76.
	2	—	—	×	—	—	—	—	—	—	Figs. 68 *a–f*, Fig. 77.
	3	—	—	—	—	—	—	—	—	×	Figs. 69 *a–f*, Fig. 78.
	4	—	—	—	—	—	×	—	—	—	Figs. 70 *a–f*, Fig. 79.
	5	—	—	—	—	×	—	—	—	—	Figs. 71 *a–f*, Fig. 80.
	6	—	—	×	—	—	—	—	—	—	Figs. 72 *a–g*, Fig. 81.
	7	—	—	—	×	—	—	—	—	—	Figs. 73 *a–f*, Fig. 82.
	8	—	—	—	—	—	—	—	—	—	Failure.
	9	—	×	—	—	—	—	—	—	—	Figs. 74 *a–f*, Fig. 83.
	10	—	—	—	—	—	—	—	×	—	Figs. 75 *a–f*, Fig. 84.

The results of the above experiments by the puncture of eggs may be summed up as follows:—

1). When the puncture is single and is at or below the level of the dorsal blastopore lip at its first appearance, the blastopore always closes at the point of puncture. Group I., No. 5, Group II., No. 1, Group III., Nos. 1, 2, and 3 appear to be exceptions to this general statement, but in these cases there were disturbing conditions such as the detachment of the ex-ovate formed, or the formation of unexpected accidental ex-ovates on other parts of the egg-surface.

2). When two or more punctures are made at the same time on different parts of an egg, the blastopore closes at the one which causes the greatest injury. In Eggs No. 4 of Groups I., III., and IV., the right puncture was always the severer of the two: hence the blastopore closed at that point. The egg of the same number in Group II., is only an apparent exception, for an accidental ex-ovate had been produced near the ventral lip which hindered its further growth.

3). The extent to which various portions of the blastopore lip grows is not constant in punctured eggs but depends entirely on the position of the pierced point. When the blastopore arrives in its downward growth at any injury, the portion of its lip which touches the injury is hindered in its further course, and while this portion is, so to speak, trying to get over the obstacle, the other parts of the lip have time to grow over a larger extent than is normal. If the obstacle is not serious (Group I., No. 5), or is in some way detached (Group I., Nos. 4, 9, and 10, Group III., No. 7), the hindered portion may get over it and grow beyond, showing only more or less retardation. If the obstacle si serious, the downward growth may be entirely stopped and

other portions of the blastopore lip have to compensate by covering a larger extent.

4). When the puncture is made on any part of the upper hemisphere it has no direct effect on the closure of the blastopore but may affect more or less the process of the reduction of the segmentation cavity or in other words the internal re-arrangement of the egg materials, and may thus indirectly cause disturbance in the growth of the blastopore lip as well as in the formation of the embryonic body.

5). In most cases, the final closing point of the blastopore coincides with the tail end of the future embryo. But occasionally there are exceptions to this general rule. In Group II., No. 9, and Group III., No. 3, the ex-ovates were at the tail end of the embryo.

6). In accordance with the statement under the last heading, the location of the embryonic body in punctured eggs is very variable. Thus, in Group I., Nos. 3, 6, and 8; Group II., No. 8; Group III., Nos. 3 and 6, and Group IV., Nos. 2, 3, 6, and 7, the embryonic body was formed almost entirely upon the upper hemisphere. But in No. 5 of all the groups, it was on the lower yolk hemisphere. And several intermediate stages between these two extremes may be seen in other eggs. The most peculiar cases are the eggs No. 4 of Groups II., III., and IV., in which the embryos appeared obliquely along the former equatorial zone.

7). There is, however, a general tendency in every egg for the blastopore to close at the yolk-pole (*s. str.*), and for the embryonic body to have its axis coinciding with the plane of the blastoporic meridian. The former fact is evident in No. 5 of Groups I., and II., and Group III., Nos. 7, and 9, while the latter appears in Group I., No. 4 in which the curved axis of

the embryo has entirely recovered its normal condition by the detachment of the right ex-ovate.

Such a tendency we must regard as the result or sum of the long course of selection, and is the reason of the great resisting power which amphibian eggs offer to various disturbing circumstances. And there must be many such disturbances, sometimes no doubt very slight, to which the frog's egg-masses must be subjected in their natural environment in spite of their gelatinous envelope. This probably accounts for the fact that individual variations in the frog's egg in regard to the segmentation process and the blastopore closure are so innumerable, that one is at a loss to find out exactly which is the normal course.

8). The greatest injury is done to the process of the blastopore closure and to the formation of the embryonic body when an egg is punctured at the region of the first appearance of the dorsal blastopore lip, and the resulting embryo shows more or less imperfections. This has already been remarked by ASSHETON and others.

9). The least injury is done, when an egg is punctured at either pole or on the future ventral side along the anti-blastoporic meridian.

10). Not only a puncture, but various other causes such as misplacement of eggs, insufficient supply of fresh water, unconscious pressure of a blunt needle during an experiment *etc.*, may disturb the internal arrangement of the egg-contents and cause abnormalities in development, often greater than those produced by a puncture.

11). From what has been detailed, it seems evident to me that the results obtained by puncturing eggs or by other similar methods, can never be depended on to reveal the normal course

of development in the naturally growing eggs. Thus, in the above experiments the facts observed in Group I., Nos. 5 and 10; Group II., Nos. 1 and 10; and Groups III., and IV., No. 10 appear to speak strongly for MORGAN'S views, while the eggs, No. 5 of Groups II., III., and IV., would become data favorable to the opinions of PFLÜGER, ROUX and others. Again a third group (Group I., Nos. 3, 6, and 8; Group II., Nos. 6 and 8; Group III., Nos. 3 and 6; Group IV., Nos. 2, 3, 6, and 7) would go to support SCHULZE and others who maintain the older views. The truth is: none of these shows the normal cousre, and we must look deeper for a general law that would include and explain all these cases.

12). For my own part, I am inclined to accept the *isotropism* of the frog's egg. According to this view there is no fixed law that the embryonic body in Amphibia must be formed in one particular region and in no other part of the egg-surface. Whenever there is sufficient reason, the embryo can be formed at any part of the egg-surface. It is true that in the ordinary course of the normal development the embryonic body, as already mentioned, appears along the meridian of the blastopore within the equatorial zone. This general rule has been produced by a long course of inheritance and is no doubt most beneficial to the growth of the egg. But whenever there occurs any great obstacle so that the egg can not grow in the usual way, it does not stop its growth at all, but seems always trying to overcome the obstacle in one way or another, and to continue its development. In this manner there result various kinds of abnormalities, natural or artificial. If it were true that the embryonic body could be formed only at one particular region and in one particular manner, there ought not to be normal embryos produced in

abnormal ways. That such are actually produced, as has been brought out by the foregoing investigation, is solely due to the isotropic nature of the Amphibian eggs so far as regards the formation of the embryonic body.

Consequently, I feel justfied in saying that the embryonic body in Amphibia may be formed indifferently at any part of the egg-surface according to the nature of the disturbing circumstances.

It is needless for me to state that the isotropic nature of Amphibian eggs has already been insisted on by many eminent writers beginning with PFLÜGER, and including BORN, O. SCHULZE, etc. But it seems to me that most of these writers have been inclined to place too much weight on the influence of gravity, and have overlooked or underestimated a factor much more efficient in the production of abnormalities, namely, the varying ratio in which different parts of the blastoporic lip accomplish their task in enclosing the yolk-mass—the ratio which determines, in reality, the final closing point of the blastopore, and consequently the position of the embryo.

Postscript.

While I was engaged in writing the present paper, an interesting article by H. V. WILSON on the "Formation of the Blastopore in the Frog Egg" appeared in Anat. Anzeiger Bd. XVIII., No. 9–10, 1900, pp. 209–239. It appears that WILSON and I have been studying independently the same subject at the same time. Moreover the main results of his observations and experiments by pricking the eggs, are largely in agreement with mine, though there are some differences in details.

The most striking points of agreement in our observations are as follows:—1. The blastopore is closed by the equal overgrowth of the blastopore lip all around. 2. The first appearance and later growth of the archenteric cavity is brought about, by the gradual accumulation of the intercellular spaces. 3. The vertical rotation of the Amphibian egg is caused by the change in the specific gravity of the different parts of the egg during the gastrulation. 4. The change in the specific gravity of the different parts is brought about by the enlargement of the archenteric cavity, with the concomitant suppression of the segmentation cavity, etc.

The points of more or less difference between the results of WILSON and of myself are as follows:—1. WILSON observes that the "Ectoderm cells close to the dorsal lip, and close to the ventral lip, gradually disappear round their respective lips. Such cells, it would seem, must become a part of the archenteric lining." This I can not corroborate from my materials. 2. WILSON states that the ex-ovate produced by pricking the eggs cannot be regarded as necessarily a fixed point, for the ex-ovate

in some instances shifts its position. As already mentioned, the ex-ovates produced from heavy punctures in my materials, may undoubtedly be regarded as fixed point of reckoning.

This view of WILSON on the second point is, probably, based upon a notion which I believe to be untenable, *viz.* that every part of the blastopore lip in pricked eggs always grows over the yolk in equal proportion just as in the normally growing egg. For this reason, WILSON's explanation of the result of his experiments 9 and 10 seems rather hard to understand.

There is no difficulty in reality, I think, in explaining these complex phenomena when we once admit, that inequality in growth in various parts of the blastopore lip may, and does usually, occur in eggs punctured or otherwise experimented upon.

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PLATE I.

Explanation of Letters in the Figures.

A. C. = Archenteron cavity.
E. = Equatorial line.
S. C. = Segmentation cavity.
V. = Vertical axis of the rotating eggs.
V′ = Original vertical axis of eggs.

Plate I.

Figs. 1–19. Developmental stages of the *Rhacophorus* egg, magnified about 11 or 12 times.

Figs. 1–6.—Segmentation up to the fourth cleavage stage.

In Fig. 2, the second cleavage-plane should have been marked II instead of IV, which is an error.

Fig. 7.—Side view of an egg, 7–10 hours after deposition.

Fig. 8.—Side view of an egg, 10–15 hours after deposition, when the first dorsal lip of the blastopore has not yet appeared.

Fig. 9.—Posterior view of an egg, 20–25 hours after deposition, with the dorsal lip just appeared, and with the fully grown upper area of the segmentation cavity which had already begun to appear in the last stage.

Fig. 10.—Posterior view of an egg, 35–45 hours after deposition, with the broadly spread equatorial zone interposed between the blastopore lip, which has just completed its encircling, and the tolerably reduced area of the segmentation cavity.

Fig. 11.—Posterior view of another egg, 40–50 hours after deposition, still standing vertically upon the middle point of the blastopore area.

Fig. 12.—Posterior view of another egg, 45–55 hours after deposition, with the faintly visible outline of the neural plate.

Fig. 13.—Left side view of a much advanced egg, 50–60 hours after deposition, when the egg has rotated considerably and the blastopore and the area of the segmentation cavity placed opposite each other have been greatly reduced in nearly equal ratio.

Fig. 14.—Left side view of an egg, 55–65 hours after deposition, when the blastopore was completely closed and the axial region of the embryonic body may be well recognised by the presence of the deep neural groove as well as the slightly raised neural folds.

Fig. 15.—Left side view of another egg, 60–70 hours after deposition, when the neural groove has just closed. The embryo body is flattened out over the surface of the yolk mass.

Fig. 16.—Left side view of a much advanced egg, 70–90 hours after

deposition, when the body of the embryo with several mesoblastic somites and the rudiments of the Wolffian duct etc. are hardly raised over the general surface of the egg.

Fig. 17.—Fish-like stage of an embryo on the 8th day after deposition. Sketched after being taken out of the vitelline membrane.

Fig. 18.—(*a*) Tadpole on the 10th day after deposition when it has just hatched out but is still within the frothy substance. Number of pigment spots beginning to appear at the thoracic region of the tadpole over the upper surface of the spherical yolk mass. (*b*) Blood-vessels in the external gills.

Fig. 19.—Tadpole on the 11th day after deposition.

Figs. 20–38. Successive stages of the *Rhacophorus* Egg C, fixed on Zeiss's "Prismen Rotator" and observed with Zeiss's Oc. I × Obj. a^2. The horizontal basal lines under many of the figures in this Plate indicate the surface of the mirror on which the egg is resting. The lines drawn vertical to these show the vertical axis of the rotating egg in successive stages. The horizontal lines parallel to the basal lines through the central point of these figures denote the equatorial plane of the rotating egg in successive stages. The dotted and other lines in several figures all denote the approximate position of the blastopore in the successive stages of the rotating egg.

Fig. 20.—Side view of Egg C sketched at 2:30 p.m. of the first day after deposition. Stage intermediate between Figs. 7 and 8.

Fig. 21.—Posterior view of Egg C sketched at 2:25 p.m. of the second day after deposition and at the scale 217° of the mirror dish.

Fig. 22.—Upper view of Egg C showing the centrally situated large area of the segmentation cavity, sketched at 2:30 p.m. of the second day.

Fig. 23.—Left side view of Egg C, sketched at 3:20 p.m. of the second day.

Fig. 24.—Lower view of Egg C, sketched at 7:00 a.m. of the third day after deposition. The blastopore is diminishing by equal growth of every part of its lip.

Fig. 25.—Upper view of Egg C, sketched about 5 minutes later than Fig. 24. It shows that the diminution of the area of the segmentation cavity is also taking place centripetally.

Figs. 26, 27.—Left, and right, side views of Egg C just before its vertical rotation begins. Sketched at 8:10 and 8:15 a.m. of the third day.

Fig. 28.—Left side view sketched at 9:43 a.m. of the third day when the egg has just begun to rotate, resting now on the ventral lip of the gradually reducing blastopore.

Figs. 29, 30.—Lower, and upper, views of Egg C, sketched respectively at 9:57 and 10:00 a.m. of the third day. Both the areas of the

segmentation cavity and the blastopore are equally diminished in size and similarly acquiring an eccentrical position.

Fig. 31.—Left side view of Egg C, sketched at 11:27 a.m. of the third day.

Fig. 32.—Left side view of Egg C, sketched at 0:50 p.m. of the third day.

Fig. 33.—Left side view of Egg C, sketched at 5:14 p.m. of the third day.

Fig. 34.—Left side view of Egg C, sketched at 3:30 p.m. of the third day.

Fig. 35.—Left side view of Egg C, sketched at 4:50 p.m. of the third day.

Fig. 36.—Left side view of Egg C, sketched at 10:37 a.m. of the fourth day, when the blastopore has nearly closed, and the general outline as well as the location of the embryonic body become equally well recognisable.

Fig. 37.—Posterior view of Egg C, sketched at 10:50 a.m. of the fourth day.

Fig. 38.—Left side view of Egg C, sketched at 8:30 a.m. of the fifth day.

Figs. 39–42. Selected to show successive stages of a *Rana* egg (*R. japonica*) observed in the same way as Egg C, Zeiss Oc. 1 × Obj. a^2.

Fig. 39.—Lower view of the *Rana* egg sketched at 4:30 p.m. of the first day, after being fixed on the "Prismen-Rotator."

Fig. 40.—Lower view of the same egg sketched at 7:40 a.m. of the fourth day, after being fixed on the "Prismen-Rotator."

Fig. 41.—Right side view of the same egg sketched at 3:32 p.m. of the fifth day, after being fixed on the "Prismen-Rotator."

Fig. 42.—Right side view sketched at 3:00 p.m. of the sixth day.

Figs. 43–49. selected to represent successive stages of a *Bufo* egg (*B. japonica*) No. 1. Observed in the same way as Egg C.

Fig. 43.—Upper view of the egg at the first appearance of the second cleavage line.

Fig. 44.—Frontal view of the first cleavage line, corresponding to the later left side view of the egg.

Fig. 45.—Left side view of the egg sketched at 8:30 a.m. of the third day after deposition.

Fig. 46.—Left side view of the egg sketched at 8:20 a.m. of the fourth day.

Fig. 47.—Left side view sketched at 1:30 p.m. of the fourth day, when the egg has rotated around its transverse horizontal axis about 30°–35°.

Fig. 48.—Posterior view of the egg, sketched at 4:00 p.m. of the fourth day.

Fig. 49.—Posterior view of the egg, sketched at 8:30 a.m. of the fifth day.

Fig. 50.—Anterior view of a *Bufo* egg No. 3 in its peculiar rotatory motion. The arrows show the direction of the rotation, and the meridional lines represent successive positions of the neural groove in the moving egg. The dots made between the thick lines indicate different positions which the anterior extremity of the neural groove assumes by oscillation in every turn of the egg.

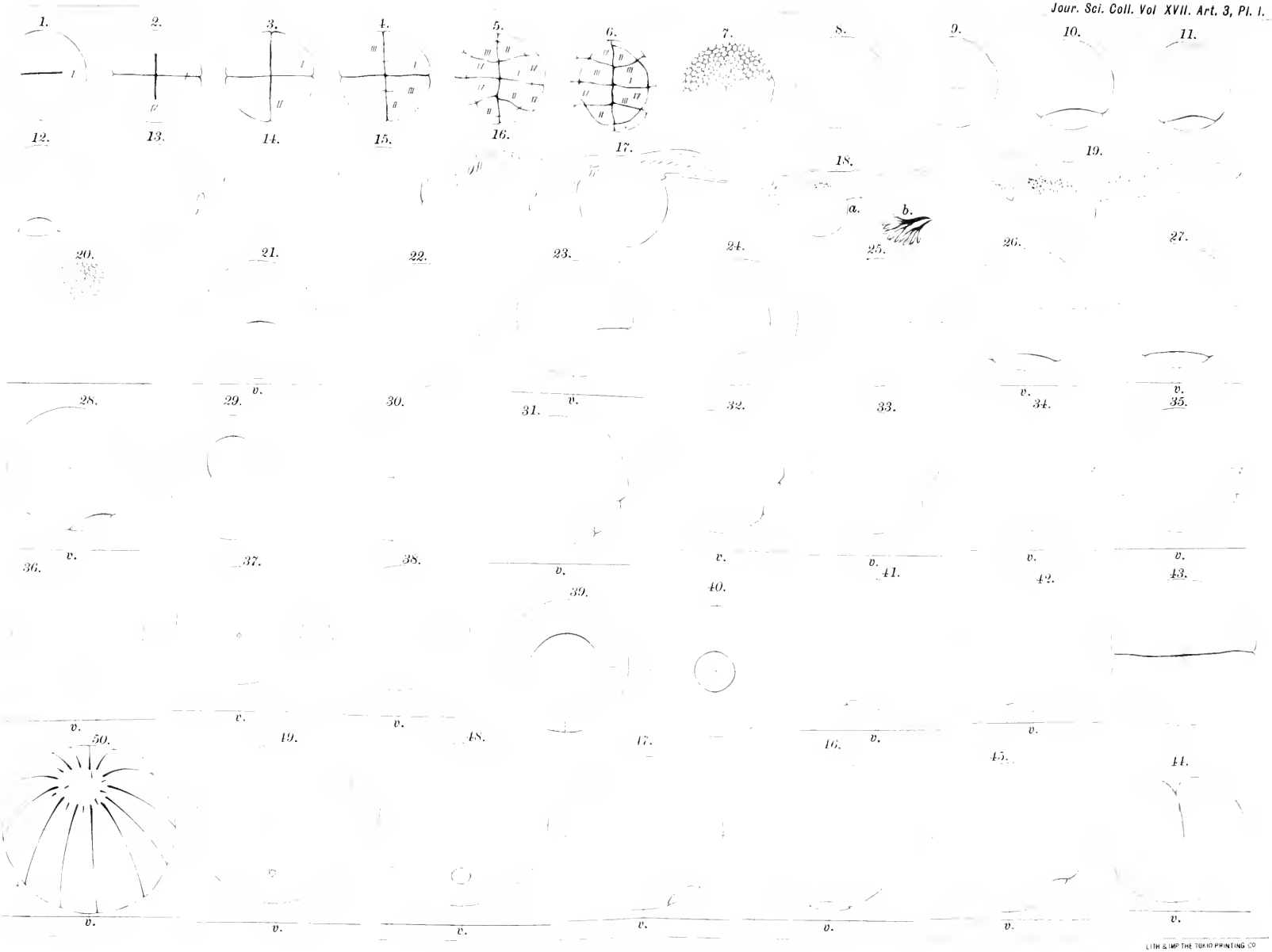

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PLATE II.

Plate II.

In the figures contained in this plate, the sparingly dotted spaces indicate the yolk-mass. The closely dotted parts in Figs. 52 and 53 are sections of the equatorial zone overlaid by the recently differentiated epiblast, while in all other figures they represent both the hypoblast and epiblast without discrimination. In Figs. 54–61, the parts of the epiblast, which are marked with a double row of lines, show the thickening of that layer around the blastopore and in the neural plate.

All the figures drawn from the middle sagittal or the middle transverse sections of the *Rhacophorus* eggs in their successive stages by the drawing prism. WINKEL Oc. 1 × Obj. 1.

Fig. 51.—Cross-section through the approximate center of an egg which corresponds to about the stage represented in Fig. 20.

Fig. 52.—Middle sagittal section of an egg which corresponds to the stage represented in Figs. 9 and 23.

Fig. 53.—Cross-section of another egg in the same developmental stage as the above, through the approximate center but in a somewhat oblique direction. The parts densely dotted in Figs. 52 and 53, are the sections of the equatorial zone overlaid by the recently differentiated epiblast.

Fig. 54.—Middle sagittal section of an egg which corresponds to the stage represented in Figs. 10 and 24–27, when the egg has not yet begun its rotation.

Figs. 55, 56.—Median sagittal and middle transverse sections of two different eggs in a stage corresponding to that represented in Fig. 12 and Figs. 28–30.

Fig. 57, *a*, *b*.—Two median sagittal sections of two different eggs which correspond to the stage represented in Figs. 13, 32 and 33.

Fig. 58.—Sagittal section of another egg corresponding to the stage represented in Figs. 14, 36 and 37.

Fig. 59.—Middle transverse section of another egg in the same stage.

Figs. 60, 61.—Middle sagittal, and middle transverse, sections of the eggs in a stage which is represented in Figs. 15 and 38.

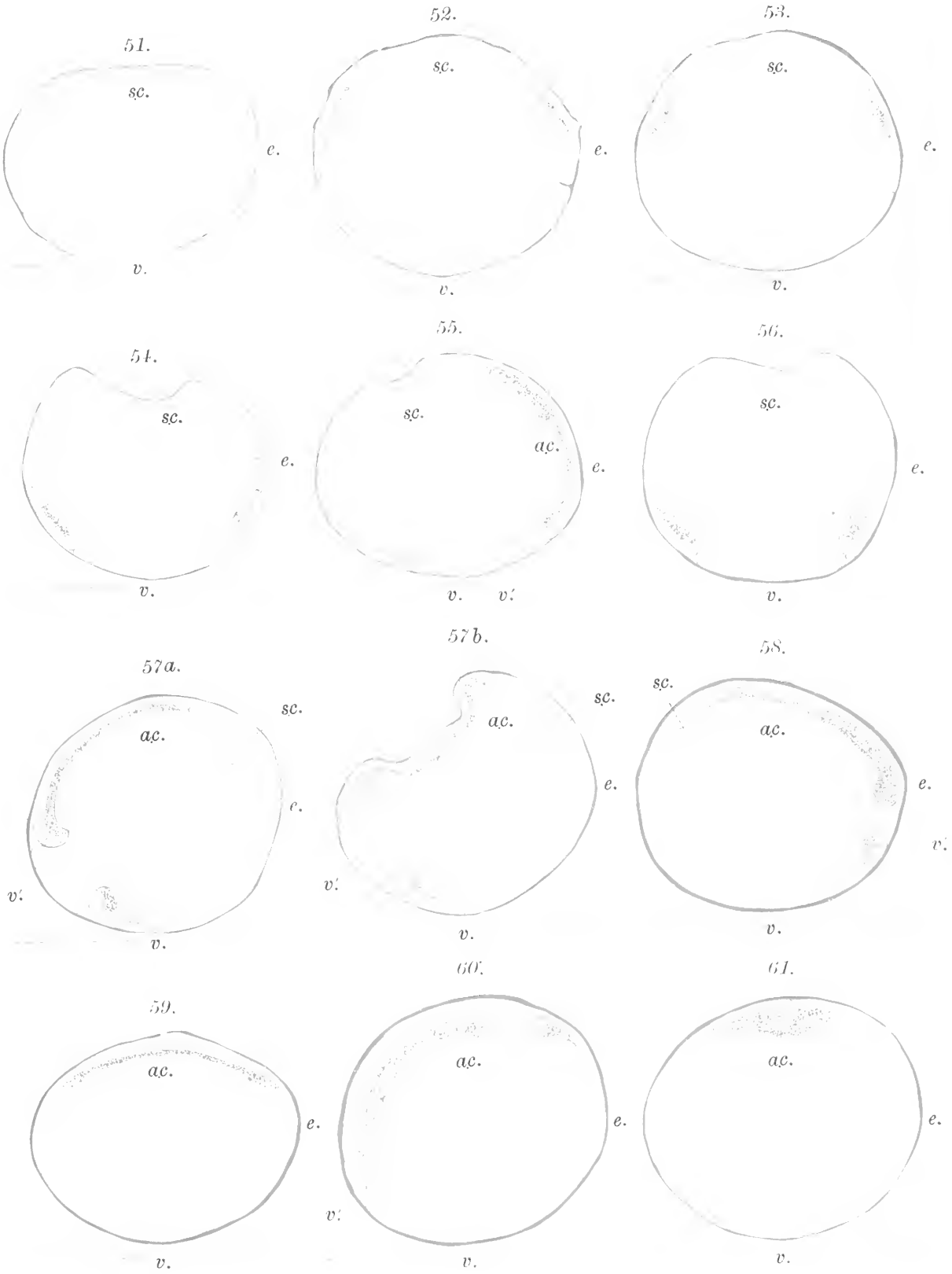

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PLATE III.

Plate III.

Fig. 62.—Roof of the segmentation cavity, under strong magnification, drawn from a section of the same series as that from which Fig. 53 was drawn. WINKEL, Oc. 3 × Obj. 5.

Fig. 63.—Blastoporic region of the same section as Fig. 52, under a higher magnification. WINKEL, Oc. 3 × Obj. 5.

Fig. 64.—Antiblastoporic region of the same section as the last. WINKEL, Oc. 3 × Obj. 5.

Fig. 65.—Blastoporic region drawn from the same sagittal section as Fig. 54, under a higher magnification. WINKEL, Oc. 3 × Obj. 5.

Fig. 65′.—Antiblastoporic region of the same section as above. WINKEL, Oc. 3 × Obj. 5.

Fig. 66.—Roof of the archenteron cavity in the section represented in 58. WINKEL, Oc. 3 × Obj. 5.

Fig. 67 *a–e*.—Successive stages of Egg No. 1, Group IV., which was punctured at the approximate yolk pole.

Fig. 68 *a–f*.—Successive stages of Egg No. 2. Group IV., which was punctured closely under the left side rim of the first dorsal lip of the blastopore.

Fig. 69 *a–f*.—Successive stages of Egg No. 3, Group IV., which was punctured directly above the first dorsal lip of the blastopore on the median line.

Fig. 70. *a–f*.—Successive stages of Egg No. 4, Group IV., which was punctured on both sides below the first dorsal lip of the blastopore.

Fig. 71 *a–f*.—Successive stages of Egg No. 5, Group IV., which was punctured at the approximate middle point of the antiblastoporic region.

Fig. 72 *a–g*.—Successive stages of Egg No. 6, Group IV., which was punctured closely under the first dorsal lip of the blastopore.

Fig. 73 *a–f*.—Successive stages of Egg No. 7, Group IV., which was punctured directly above the middle point of the first dorsal lip of the blastopore.

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PLATE IV.

Plate IV.

Fig. 74 *a–f.*—Successive stages of Egg No. 9, Group IV., which was punctured at the upper pole.

Fig. 75 *a–f.*—Successive stages of Egg No. 10, Group IV., which was punctured at the sub-antiblastoporic region.

Figs. 76–84. Diagrammatic representations of the results obtained from Eggs Nos. 1, 2, 3, 4, 5, 6, 7, 9, 10, Group IV., in regard to the location of the embryonic body and the punctured region of every egg. The curved line above the egg-equator, in every figure, is the lower limit of the early area of the segmentation cavity, while the dotted line denotes the future lip of the blastopore.

Fig. 76.—Diagram of Egg No. 1, Group IV., Figs. 67 *a–e.*

Fig. 77.—Diagram of Egg No. 2, Group IV., Figs. 68 *a–f.*

Fig. 78.—Diagram of Egg No. 3, Group IV., Figs. 69 *a–f.*

Fig. 79.—Diagram of Egg No. 4, Group IV., Figs. 70 *a–f.*

Fig. 80.—Diagram of Egg No. 5, Group IV., Figs. 71 *a–f.*

Fig. 81.—Diagram of Egg No. 6, Group IV., Figs. 72 *a–g.*

Fig. 82.—Diagram of Egg No. 7, Group IV., Figs. 73 *a–f.*

Fig. 83.—Diagram of Egg No. 9, Group IV., Figs. 74 *a–f.*

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Fig. 85 *a–c.*—Successive stages of Egg No. 4, Group I.

Fig. 85′.—Diagrammatic representation of the results obtained from the above egg.

Fig. 86 *a–c.*—Successive stages of Egg No. 5, Group I.

Fig. 86′.—Diagrammatic representation of the results obtained from the above egg.

Fig. 87 *a–d.*—Successive stages of Egg No. 1, Group II.

Fig. 88.—Diagrammatic representation of the results obtained from the above egg.

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Fig. 90.—Diagrammatic representation of the results obtained from the above egg.

Fig. 91 *a–d.*—Successive stages of Egg No. 9, Group II.

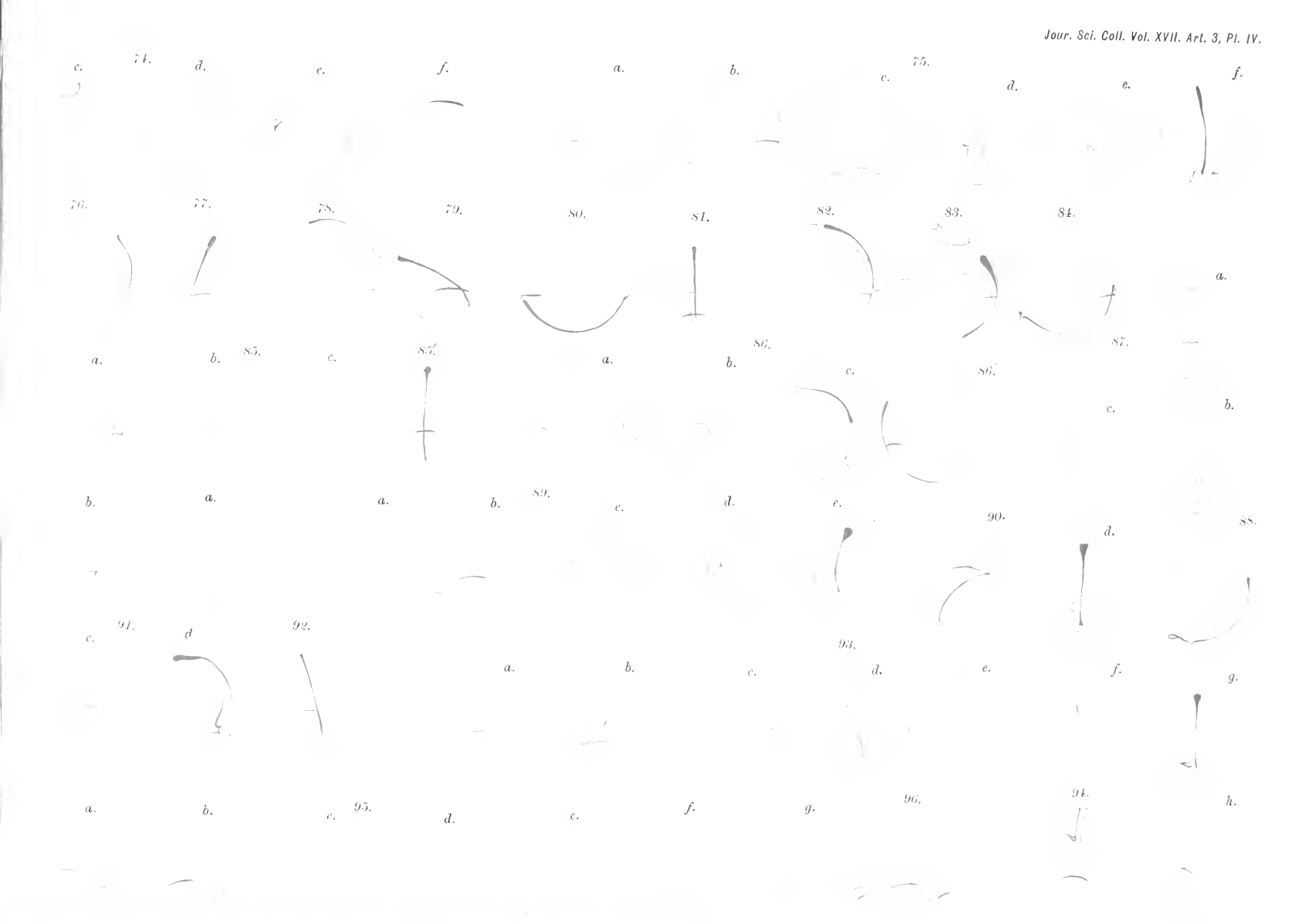
Fig. 92.—Diagrammatic representation of the results obtained from the above egg.

Fig. 93 *a–h.*—Successive stages of Egg No. 3, Group III.

Fig. 94.—Diagrammatic representation of the results obtained from the above egg.

Fig. 95 *a–g.*—Successive stages of Egg No. 4, Group III.

Fig. 96.—Diagrammatic representation of the results obtained from the above egg.



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S. Ikeda. *Contributions to the Embryology of Amphibia.*

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On the Development of *Lingula anatina*.[1]

By

Naohidé Yatsu, *Rigakushi*.

With Plates I—VIII.

CONTENTS.

1. The substance of this paper was presented as a dissertation on my graduation from Science College in June 1900; since that time some new facts have been added by further investigation.

I. INTRODUCTION.

Although the adult anatomy of *Lingula* has been thoroughly studied by many investigators, its embryology has not received the amount of attention which it deserves, owing no doubt to the difficulty of obtaining materials. The observations[1] of McCrady ('60), of Semper ('61), and of Simroth ('97), are but fragmentary. Brooks ('78) is the only author who has dealt with it with anything like adequacy, but even in his case, older larvæ alone were treated. The early history of development has thus remained entirely unknown to this day.

Lingula anatina Bruguière occurs in great abundance in the neighborhood of the Marine Biological Station at Misaki. The efforts to make the Brachiopod breed in captivity had, however, repeatedly failed there as elsewhere. It was, therefore, by a specially good fortune that during the summers of 1899 and 1901 I was enabled to observe the spawning habits and to collect a moderately complete series of the early developmental stages of *Lingula*. On this the present investigation has been carried out. The work was undertaken at the suggestion of Professor K. Mitsukuri, to whose generous aid I am greatly indebted. My sincere thanks are also due to Professor I. Ijima and Professor S. Watasé for much valuable advice. I wish also to express my obligations to Professor B. Dean of Columbia University, U. S., who during his stay in Japan kindly read the manuscript in one of the stages of its preparation, and suggested many improvements in the language employed.

1. R. Owen ('35, '53) must have mistaken the blood corpuscles of *Lingula* for the embryos.

II. DISCHARGE OF THE SEXUAL ELEMENTS.

The mode in which the sexual elements are discharged is described at great length in *Terebratulina septentrionalis* Couth, by Morse ('73). I have found another short account of ovulation by Wilson in Whitman's ('85) work on microscopical technique (p. 136). Other writers on the Brachiopod are silent on its breeding habits, though Kowalevsky, Joubin, *et al.* must have been familiar with them. My notes from personal observations on the emission of eggs and sperm in *Lingula* may, therefore, not be wholly superfluous.

In the summer of 1899 when I was staying at the station, I collected a number of *Lingula* on July 24th and kept them in two large bowls. I had then little hope of securing eggs and embryos, since similar efforts had been made in vain by many of my predecessors. About ten of the specimens were of the maximum size for that locality, and many others were the medium ones, the remaining few being very young. Natural conditions were as far as possible imitated. The mud in the bowls was brought from the same mud flat where the individuals were collected. The water was almost all drawn off by means of a siphon once or twice a day, and the bowls were gradually filled up with freshly drawn sea water containing ample food. There was thus caused an artificial ebb and flood tide. The specimens thus kept appeared in an excellent condition protruding from their burrows the anterior edge of the mantle which appeared drawn out into the form of three funnels arranged side by side. Several days passed without any change.

Early in the morning of the fifth day *i.e.* at about 6 a.m.

on July 28th (21st of the lunar calendar), Mr. MORIWAKI came and told me, that he saw in the bowl yellow dust which was perhaps eggs. At this information I hastened into the room and to my great joy, I found that the yellow dust was nothing other than the sexual elements. This was probably the first instance in which the spawning of the Ecardines has been observed.

Four individuals of the maximum size, two males and two females, were discharging their sexual elements. The eggs were of an yellow color and so small that we could hardly distinguish them individually with the naked eye. They were forcibly discharged from the median funnel of setae like a jet of water. As the clouds of eggs came out constantly during the spawning, they assumed the form of an inverted cone, ascended nearly perpendicularly to the surface of water and then gradually sank to the bottom. The process appeared like the eruption of a miniature volcano. After a few minutes the spawning ceased. The fallen eggs covered in a tolerably thick layer an area of some thirty square centimeters. As some mucus was secreted from the mantle at spawning, the egg-layer was found to be like a scum, when I tried to dip the eggs up with a pipette. Sperm was emitted in the same manner as the eggs, but it was milky white in color. In nature these elements discharged simultaneously are no doubt mixed up by the current and fertilization is thus secured.

As I did not witness the beginning of the process of spawning I am unable to say which sexual element was the first to be discharged. But the following hypothesis would not be incredible. Until the sperm is discharged the eggs even when well ripened seem to be retained within the body. When the sperm is emitted some chemical substances discharged in the spermatic fluid may diffuse in the water and irritate the female, thus awakening the

mechanism of ovulation; for the females, even when the sexual product is actually ripe, will, when kept separate from the males, fail to deposit eggs. JOUBIN ('86) also observed that on keeping *Crania* the eggs atrophy, and turn into yellow bands (p. 255). In order to test the above hypothesis an experiment was tried last summer: several full grown males[1] were cut open and sperm was taken into a glass vessel. It was then scattered by means of a pipette around the females or injected in to the mantle cavity of the latter. But unfortunately this did not produce any result, owing perhaps to the fact that the female element was not ripe enough.

During the summer just past (1901) *Lingula* spawned twice in captivity; the first spawning took place early in the morning of August 7th (23rd of the lunar calendar). The water had been changed on the previous day after standing several days. Unfortunately I failed to observe the process of spawning and only found fertilized eggs on the mud in which the specimens were kept. In the second case the eggs discharged did not develop at all owing perhaps to the fact that the water had already become stagnant. From the above three cases I cannot find out exactly what it is that induces spawning. Anyhow it is almost certain that the spawning seems to take place under unfavorable, rather than favorable, circumstances.

In *Lingula* the breeding season is, in Misaki at least, very short and certainly restricted to one month and a half of the summer, *i.e.* from the middle of July to the end of August, as

1. It has become not a very difficult matter to me to distinguish the sexes of *Lingula* from without. As the shell is translucent, the female appears dark brown while the male is of lighter brown colour or rather whitish. The distinction of colours between sexes is most conspicuous in individuals of a medium size; for in older animals the shell increases in thickness, so that light hardly passes through it.

I have never met with larvæ at any other time during the year. The spawning season is about one month and a half later than that of *Terebratulina*, which, according to MORSE, spawns at the end of May and in the early part of June at Eastport. As to how many times *Lingula* spawns in a season I have not yet any positive proof, as the young free-swimming larvæ have not been caught in the past three years in sufficient number to enable me to decide the question. I can, however, safely declare that the youngest larvæ appear at least twice in a season. Professor MITSUKURI informs me that in his experience the youngest larvæ appear after every spring tide. And this being the case, the spawning must occur twice a month *i.e.* three or four times during a season.

III. MATERIALS AND METHODS.

Early stages of the embryo were secured from the two spawnings described above. The eggs mixed with sperm were kept in several chemically clean glass vessels with covers, which were placed within another larger vessel filled with cold fresh-water in order to keep the inner vessel as far as possible at a low temperature, as we usually do at the station in rearing various larvæ during the hot season. In both cases in spite of the utmost care it became most difficult after three days to keep the embryos living, and on the fourth morning the last of the materials was killed.

Artificial fertilization was afterward tried several times, but always in vain, owing to difficulties of obtaining mature sexual elements simultaneously and of separating the eggs from the

blood corpuscles, whose decomposition soon takes place making the fluid unfit for the development of eggs.

Free-swimming larvæ provided with 3–10 pairs of cirri were captured by means of a tow-net drawn at, or a few feet below, the surface.

The stalked young of *Lingula* were secured by keeping larvæ with 8–9 pairs of cirri in a vessel in which the water was changed every two or three days. I afterward searched for the stages a little more advanced than those I was able to rear from the free-swimming larvæ, by examining in various ways the mud from the flats where *Lingula* flourishes, but all in vain.[1]

From the beginning of fertilization up to the end of the blastula stage all material was fixed with FLEMMING'S fluid (weaker formula), and VOM RATH'S picro-platin-acetic mixture; these yielded fairly satisfactory results. For the later stages several fluids were used; among them saturated solution of sublimate in distilled water with the addition of a little acetic acid, and VOM RATH'S picro-sublimate-acetic mixture proved best.

All the embryos and larvæ were cut in 5 μ, and stained on slides, except some of the older larvæ, which were stained *in toto*.

Of the stains used, HEIDENHAIN'S iron-hæmatoxylin with orange G or Bordeaux red proved best for the eggs. As for embryos, borax carmine or carmalum followed by picric acid or orange G gave the most satisfactory results. Haemalum with counter-stain of erythrosin was also extensively used, especially when it was desired to differentiate muscles from other tissues.

1. BROOKS, it may be recalled, was successful in securing the young of *Glottidia* at this stage by an examination of the mud of their habitat, but he has unfortunately omitted to describe his mode of procedure.

In imbedding eggs in parafin the following method was used. The eggs were first thoroughly dehydrated and put in a short test tube (30 mm. in length and 20 mm. in diameter) into which benzine was introduced at the bottom by the aid of a fine pipette and the mouth of the test tube was tightly closed with a cork stopper. After about half an hour the liquid was thoroughly dipped out and pure benzine was poured in. After the benzine had been changed once more, finely cut paraffin was melted in the tube. The latter was now allowed to remain uncorked; paraffin was put piece by piece into the mixture and a slight degree of heat was applied. On cooling the tube or by slightly heating the sides of the tube the entire mass could now be readily removed. From this block the smallest possible portion containing objects may next be cut, and placed in a little paper case filled with, and placed in, melted hard paraffin. A few minutes after the objects had sunk to the bottom the case was taken out of the melted paraffin, and was rapidly cooled. The paper was now removed, and the block was ready for cutting. In case the block was not hard enough a small portion of it containing the objects was again cut out, and melted with pure paraffin in another paper case as just described. In some other cases I used pieces of *Ulva* to stick eggs upon, as HÄCKER describes (Praxis u. Theorie d. Zellen u. Befruchtungslehre p. 111).

The embryos and larvæ were imbedded by a modification of the above test-tube method. When orientation was necessary, as in the case of advanced larvæ, the block prepared as above described was examined with a low power and the desired section-plane was then marked on the block. Since the larvæ are disc-shaped, orientation in two directions is sufficient. Sometimes, after APATHY, a small piece of gelatine plate cut into a convenient

shape was used to attach larvæ according to PATTEN's method (Zeit. wiss. Mikr. Bd. XI., 1894. p. 13–15). The plate proved better than a piece of granulated paper on account of its transparency and its being easily detached from the paraffin block. I prefer gelatine to the glass slip proposed by HOFFMANN (Zeit. wiss. Mikr. Bd. XV., 1898. p. 312–316.) for the same purpose, as gelatine is easily marked with a knife.

My present studies on the embryology of *Lingula* have naturally been divided into two parts. The studies from the egg up to the oldest embryo I obtained by rearing the egg, constitute the first part; those on the free-swimming larvæ and young sedentary *Lingula* obtained by keeping the latter, the second part.

A. EMBRYONAL STAGES.

IV. MATURATION AND FERTILIZATION.

In describing the phenomena of maturation I shall begin with the ovum which has just been deposited, and is at a stage slightly prior to the casting out of the polar bodies. The ovum (Pl. II., Fig. 14.) is spherical, its diameter measuring 90 μ–100 μ, just the same size as the largest yellow yolk-granules of the hen's egg. It is enclosed by a thin vitelline membrane, a covering apparently secreted from the surface of the ovum, while floating either inside or outside the body, since no trace of it could be found in eggs still enclosed in the follicle. Needless to say, it is formed before fertilization. Under a high power the membrane shows radial striations. In the ovum one may distinguish the

nucleus occupying a central position : a single and well marked vesicular principal nucleolus (plasmosome) is present, the accessory nucleolus which had been found in the ovarian ovum having disappeared in the course of growth.[1] The principal one too degenerates *in situ* shortly after the accessory one has disappeared, and at the time when no changes as yet have taken place in the nucleus. The nucleus is vesicular and stains in any basic dyes far less intensely than the cytoplasm, a small amount of chromatic substance being scattered on the nuclear reticulum—showing that the ovum is very poor in nucleïnic acid at this stage. Surrounding the nucleus is a thick layer of cytoplasmic net-work, in whose meshes fine yolk-granules are packed, which, of course, take readily erythrosin or any other plasm stains. On examining iron-hæmatoxylin preparations we can distinguish two kinds of ova according to the appearances presented by the yolk : the one with yolk-granules of an almost uniform size (Pl. II., Fig. 14.), and the other with those of different sizes (Pl. II., Fig. 15.). This distinction may be due not to natural differences, but to the state of precipitation brought about by the action of fixing reagents, and to the degree of extraction in the iron-hæmatoxylin method. Exterior to this yolk layer is a layer of vacuoles, 2–4 vacuoles in thickness. They are probably formed by the dissolving away of yolk-granules by the action of certain enzymes ; for the vacuoles increase in number with the age of the egg. In some cases external to the vacuolar layer and just beneath the vitelline membrane is found a layer of yolk-granules varying somewhat in thickness, while in others no such layer is found. There are some cases in which no vacuolar layer is present at all. All these variations

1. *Cf.* N. YATSU :—Notes on the Histology of *Lingula anatina* BRUG. This Volume Art. 5.

cannot be looked upon as pathological, since they occur in eggs, in which the various processes of maturation or of cleavage seem to have gone on normally.

In studying the living material I could observe in the ovum (Pl. I., Fig. 1.) a translucent central portion of a light yellow color corresponding to the yolk part, and a transparent peripheral portion of a still lighter yellow color with a slightly greenish tint representing the vacuolar layer. The outermost layer of vacuoles is found to be a little thicker than the inner ones, all the vacuoles being arranged or each vacuole being disposed with great regularity. In one ovum it was actually observed that the first polar body pushed up the vitelline membrane into a hillock, and then under the first polar body was raised up, a still smaller portion of cytoplasm which must, of course, represent the second polar body (Pl. I., Fig. 1.). In section, only in three cases out of several hundreds of eggs were polar bodies seen (Pl. II., Fig. 20.). From this fact it seems most probable that the polar bodies soon after they are cast off, disorganize and are absorbed by the ovum. The exact time when the first polar body is given off I could not positively determine, but as in the ova just laid, the second polar mitosis has in most cases proceeded to the meta- or ana-phases, it may be concluded with reasonable accuracy that the first polar body is produced in a majority of cases either while the egg remains in the body cavity or at the moment after its deposition.

As the metaphase of the second polar mitosis is the earliest stage I have showing anything concerning the phenomena of the formation of the polar bodies, I cannot at present give any account of the changes antecedent to it.

The second polar mitotic figure lies in a somewhat clear space

(Pl. II., Figs. 15–18.) free from yolk-granules. At both ends of the spindle are centrosomes, around each of which a "heller Hof" is present. The zone gradually passes over into a darker zone (cortical layer) and this again into the clear outer zone of the centrosphere : throughout all these layers astral rays are distinctly seen. It may be noted that the distal region of the mitotic figure is slightly elevated above the surface contour of the egg, the vacuolar layer at this point having been thrust aside (Pl. II., Fig. 15.). The axis of the spindle seems to stand in no definite relation to the egg surface ; sometimes the axis is almost paratangential to the ovum (Pl. II., Fig. 17.), while in other cases it is perpendicular to the egg-surface (Pl. II., Fig. 18.). All intermediate angles have been observed. I at first thought it possible that the angle changed with the stages of the polar mitosis, but on looking over the preparations I found that such was not the case. It is hardly necessary to repeat here that the nucleolus had disappeared before the mitosis took place. At the anaphases the centrosphere and centrosomes become invisible.

After the second polar body is cast off eight refringent rod-shaped chromosomes are found in a clear space, which probably represents dense nuclear fluid (Pl. II., Figs. 16, 19.). From this number the chromosomes in somatic cells are conjectured to be *sixteen*.

The egg-nucleus acquires at the same spot a distinct nuclear membrane. Chromatin is distributed as very fine granules which come to occupy the nodes of the nuclear reticulum (Pl. II., Fig. 20.). The path along which the egg-nucleus makes its way toward the centre of the ovum is clearly made out by the funnel-shaped intrusion of the vacuolar layer into the yolk part (Pl. II., Fig. 20).

That a micropyle is present piercing the vitelline membrane is almost certain, although it has not as yet actually been determined; for it will be remembered that the vitelline membrane is formed prior to fertilization and even before the formation of the polar bodies.

The spermatozoön enters as a rule from a place diametrically opposite the second polar mitotic figure, and at the metaphase of the latter (Pl. II., Fig. 18.). The spermatozoön in the yolk part of the ovum can always be recognized by the accompanying sperm-asters; were it not for this feature, the head of the spermatozoön might readily be mistaken for a yolk granule, since it is only a little larger (2 μ) than the latter. The spermatozoön while still near the periphery of the egg resolves itself into eight refringent chromosomes floating in clear dense fluid (Pl. II., Fig. 19.). At this stage both the germ-nuclei, still wanting the nuclear membrane, cannot at first sight be distinguished the one from the other, but upon a more careful study it is found that the egg-nucleus lies on the whole nearer the periphery than the sperm-nucleus (Pl. II., Fig. 19.). The sperm-nucleus comes next to be provided with a thick nuclear membrane as in the egg-nucleus. It then travels towards the centre of of the egg, and there meets (Pl. II., Fig. 21.), and becomes apposed for a time to, the egg-nucleus. The nuclear membrane dissolves away at the place where the two nuclei come into contact with each other, so that at one stage the cleavage nucleus thus formed assumes the form of an hour-glass (Pl. II., Fig. 22.). In living ova the two germ-nuclei can be seen as clear spots by the aid of low powers (50–60 diameters); by higher magnification, on the contrary, they cannot be made out owing to the necessarily small depth of the objectives.

V. 2-CELL STAGE UP TO BLASTULA.

On the fading away of the membrane of the cleavage nucleus the mitosis begins, whose figure lies in a somewhat clear space stained faint blue by iron-hæmatoxylin. The chromosomes are thread-like during the pro- and earlier ana-phases (Pl. II., Figs. 23, 24.), but in the later anaphases they are found to shorten into small rods and to occupy either end of the spindle, at this time centrospheres and centrosomes becoming invisible (Pl. II., Fig. 25).

Next a constriction goes on to some extent around the egg, but it must not be taken as a part of the cleavage phenomena. In the course of about half an hour the ovum regains its original spherical form. I afterward found that the same thing occurs also among Mollusks. It is the second constriction which now appears that divides the egg into two blastomeres. At the beginning of the first cleavage the yolk layer seems to be concentrated about the nucleus of each blastomere, the vacuolar layer entering between the two blastomeres. Meanwhile the constriction becomes deeper and deeper until the egg is divided into two, the cell membrane being formed between them (Pl. I., Fig. 2; Pl. II., Fig. 26.).

The nucleus is vesicular with a thick and strong looking membrane: chromatin is very small in quantity, diffusely distributed on the nuclear reticulum. These features of the nuclei last for a long time until histological differentiations take place.

As the egg is quite spherical, the yolk is distributed equally, and moreover as the polar bodies are soon absorbed, it becomes impossible to orient the first cleavage plane with reference to the future body axes.

The second cleavage plane appears at right angles to the first (Pl. I., Fig. 4.). When the cleavage is finished there is sometimes found a space at the centre of four blastomeres, but this is by no means always the case. There are some cases in which each quadrant elongates peripherally so that the egg assumes as a whole the shape of a cross or four leaved clover. From this cleavage onward all imaginable irregularities creep into the manner of cleavage. In some eggs the second cleavage is finished in one blastomere while the other remains without constriction (Pl. I., Fig. 3.). In the cleavage of the egg of *Cistella* SHIPLEY ('83) gives a figure (Taf. XL., Fig. 22.) of just the same case. The retardation of cleavage in a blastomere does not seem to depend upon the difference in size of the latter, but upon some unknown causes. It should here be noted that from the second cleavage on, the chromosomes appear as rods unlike those in the first (Pl. II., Figs. 28. 30.).

The third cleavage cuts perpendicularly to the preceding two, thus giving rise to eight blastomeres (Pl. I., Fig. 5.). A section at this stage is shown in Pl. II., Fig. 27 where we can distinctly see the segmentation cavity at the centre. The dissolving of yolk-granules proceeds in each blastomere from the outer surface inward, so that only the yolk-mass remains near the surface of the segmentation cavity. This behavior of the yolk can be noticed up to young embryos. Toward the end of the stage each blastomere becomes slightly compressed, so that the egg as a whole is flattened in one direction as we can readily see on comparing Pl. I., Fig. 6 and 7. In these figures the indication of the cleavage plane next to appear is noticeable.

The fourth cleavage takes place in two parallel planes which pass through the centre of each octant (Pl. I., Fig. 8.). The

relative position of the above planes—whether parallel or at right angles—to each of the preceding cleavage planes could not be determined; for there is nothing which can serve as an accurate landmark, such, for example, as the polar bodies or the difference in size of the blastomeres. The direction of this cleavage is indicated in Pl. II., Fig. 28. where the mitiotic figure still lies in the yolk portion. At this stage these blastomeres enclose a spacious segmentation cavity (Pl. II., Fig. 29.). This type of cleavage is considered normal, but there are not wanting many cases out of the usual course. Among them the 12-cell stage is rather frequent, the fourth cleavage having been retarded in four octants. In these instances, however, there result blastulæ as perfect as those resulting from the eggs which undergo normal cleavage.

The fifth cleavage appears in two planes perpendicular to the fourth, thus a lenticular embryo results, composed of two tiers of cells, each tier being made up of 16 blastomeres (Pl. I., Fig. 9.; Pl. II., Fig. 30.[1]). It should here be stated, however, that the embryos which develop as regularly as this are not many. It is worth while to call attention to an interesting coincidence in the manner of cleavage up to the 32-cell stage of *Lingula* with that which goes on in eggs of several species of marine Bryozoa studied by Barrois, Vigelius *et al.*

The cleavage processes as just described were not made out by following any one single egg but by observing a number of eggs under cover slips for 12 hours consecutively and by comparing what was seen in different eggs. For fear that the pressure caused by cover slips might have acted as a mechanical stimulus

1. This is a section through an egg not exactly at the 32-cell stage but through one in which the cleavage processes have departed a little from the normal course.

upon the cleavage-plane, I re-examined the preserved eggs and found that the things above mentioned held true in them.

As the cells continue to increase in number there results a typical cœloblastula (Pl. I., Fig. 10.; Pl. II., Figs. 31, 32.). In this a spacious segmentation cavity can be seen upon applying a slight presure to the cover glass (Pl. I., Fig. 11.). All the cells are not of equal height; those at one pole are a little higher than the remaining ones (Pl. I., Fig. 11.; Pl. II., Figs. 31, 32.). The cells are prism-shaped, pentagonal or hexagonal in section (Pl. II., Fig. 31.) and are somewhat vaulted on the outer as well as on the inner surfaces. Histological differentiation has not as yet taken place; all the cells show the same structure. As already stated the dissolution of yolk-granules proceeds from outside inward in every cell. This can best be seen on comparing the thickness of the yolk layer in Pl. II., Figs. 29–32. During early stages the nuclei are found lying in the yolk layer (Pl. II., Figs. 29, 30.), but later they are found outside the yolk layer, as the latter is reduced as in Pl. II., Fig. 32.

Of abnormal development I found many interesting cases. One or two blastomeres at the 2-, 3-, or 4-cell stages were observed to continue normal cleavage processes after other blastomeres had degenerated and gave rise to dwarf blastulæ, gastrulæ or even embryos. This must have been caused by some external stimuli such, for instance, as an increase of salinity of the water by evaporation, or want of oxygen. Experiments similar to those recently made on the eggs of the sea-urchin, Amphioxus, hydromedusæ, etc. were thus accomplished in our case without carrying them out on purpose. At degeneration of the eggs or blastomeres they, in a majority of cases, segment off into many

spherules according to the usual mode of plasmolyses, but sometimes they elongate so as to assume the form of a thread, reaching 10–15 times their original length.

VI. GASTRULA UP TO THE OLDEST EMBRYO THAT COULD BE REARED FROM THE EGG.

At the time when the section of a blastula consists of 30–40 cells an invagination commences at the pole where there are taller cells (Pl. I., Figs. 12, 13.; Pl. II., Fig. 33.). As the invagination goes on, the typical gastrula is formed. Now we see in a sagittal section that, as the result of the unequal growth of the invaginated area, the arms of the V-shaped entoblast are not equal in length, so that the tip of the entoblast is directed nearly horizontally (Pl. I., Fig. 13.). The mesoblast is rapidly proliferated from the entoblast walls (Pl. IV., Fig. 54.). The mesoblast cells, however, do not loosen themselves, but adhere closely to the entoblast, forming a compact cell-mass with the latter, which will be called hereafter the mes-entoblastic cell-mass (*ms. ent.*). The mass fills up almost the entire segmentation cavity (*sg. cv.*) as is seen in Figs. 34. (Pl. III.) and 54. (Pl. IV.). The archenteron is, at this stage, found sometimes completely obliterated, so that we only see the cell-mass hanging in the segmentation cavity from a pole of the ectoblast sac (Pl. III., Fig. 34.). In other cases, however, a tolerably spacious archenteric cavity persists within the mes-enteric cell-mass. The nuclei of the entoblast cells undergo some chemical changes, by which they show an increased affinity with nuclear stains, while those of the mesoblast and ectoblast cells retain their original properties (Pl. IV., Fig. 54.).

The description of the structure of the gastrula having been given, we may, for convenience's sake, next follow the external developmental changes up to the oldest embryo that I was able to rear from the egg. Then in the section following I shall enter into the more minute structural characters of the embryos during these stages.

a. External form.

In Fig. 34. (Pl. III.) an embryo a little more advanced than the gastrula is represented in side view. At the upper pole the blastopore is closed and at this place the mes-entoblastic cell-mass is connected with the ectoblast. This pole becomes the anterior face and the opposite end, which is lowest in the figure, the posterior face of the embryo of the ensuing stages. As the embryo at this stage is quite spherical or a little compressed antero-posteriorly, we cannot as yet determine which side represents the future dorsal or ventral side. With the growth of the embryo, the ectoblast begins to fold outward horizontally in all directions to form the ring-like mantle fold, until the embryo assumes nearly a disc-shape, flattened antero-posteriorly. An embryo at this stage is represented in two views; the one (Pl. III., Fig. 35.) from the anterior face, the other (Pl. III., Fig. 36.) in an aspect which is either ventral or dorsal. In the former figure we see the central area (*am. rg.*) elevated from the general surface into a hillock. This area I shall call the arm-ridge, for it subsequently gives rise to the arm-apparatus. The surface of the ridge has a warty appearance owing to the fact that the cells composing it are still large and have their ends greatly bulged outwards. Surrounding the arm-ridge there

is a ring-like area (*mt. fd.*) which I shall designate as the mantle fold, as it soon becomes the mantle. Between the arm-ridge and the mantle fold there is a shallow furrow. On what becomes the sides of the body there soon appears on each side, at the place marked with stars in the figure, a shallow depression on the mantle fold. By these two depressions we can now distinguish in the embryo the two sides. Fig. 36. (Pl. III.) represents the same embryo viewed from either the dorsal or ventral side. In this figure we see more clearly the relation of the arm-ridge, the mantle fold, and the shallow furrow between them. Moreover we see that the posterior face of the embryo shows a gentle vaulting.

An embryo a little more advanced than that just described is represented in Fig. 37. (Pl. III.) in the same view as in Fig. 35. (Pl. III.). At this stage an invagination begins to be marked at the future ventral median part of the arm-ridge. Proceeding dorsally and posteriorly it forms the stomodæum (*stmd.*). Interrupted on the ventral side by this invagination the arm-ridge comes to assume the form of the letter U whose curved portion is directed dorsally. Both the arms of the U are symmetrical.[1] The lateral depressions on the mantle fold (* *) become deeper than before, and are ready to cut the mantle fold into two lobes.

Next the stomodæum comes into communication with the archenteron. The former constitutes the anterior half of the œsophagus. Fig. 38. (Pl. III.) is an embryo of this stage, pressed by the cover glass from the antero-ventral direction and drawn on a much smaller scale than Fig. 37. The arm-ridge is pressed down dorsally and the depressions on the mantle fold

1. In the embryo drawn in the figure the symmetry is a little disturbed. This must not be taken as a normal case.

are obscured by pressure. Here we see distinctly the connection of the stomodæum (*stmd.*) and the archenteron (*arch.*).

The depressions on the mantle fold become deeper and are no longer confined to the anterior surface but pass around two sides. The ring-shaped mantle fold is thus cut up into two lobes. The cutting line finally extends all around the posterior wall of the embryo. Concurrent with this change the mantle fold which has thus been divided, and may now be called the mantle lobes, rapidly increases in size, especially at the anterior margin, so that the lobes come to assume a nearly circular form. Fig. 40. is a side view of such an embryo. In this a large central mass composed of the body proper and the arm-apparatus, and two widely separated mantle lobes are distinctly visible.

An embryo of the same stage is represented in a dorsal view in Fig. 39. (Pl. III.). Here we see a very large arm-apparatus with a rough surface; the dorsal mantle-lobe is in full view, while the ventral is concealed by the arm-apparatus. On applying slight pressure from the dorsal side upon another embryo of a similar stage (Pl. III., Fig. 41.) the tolerably spacious archenteron (*arch.*) and the segmentation cavity (*sg. cv.*) became visible. In this the arm-ridge[1] is found to be densely covered by cilia, whose slow movement can be distinctly seen through the vitelline membrane. The formation of the mantle lobes is thus accomplished by the cutting of the mantle fold and the posterior wall by a furrow, and by the anterior and lateral growth of the mantle. This process must not be confounded with the later growth of the circular mantle lobes in its diameter.

The next changes consist chiefly in the flattening of the

1. In the embryo the arm-ridge is exceptionally small. Normally it bulges out a great deal above the mantle lobe (*Cf.* Figs. 39 and 40).

arm-apparatus and the lateral growth of the mantle lobes, as is seen in Fig. 42. (Pl. III.) which represents an embryo at this stage seen from the ventral and slightly from the anterior side. The arm-apparatus (*am. ap.*) comes to assume the form of a disc, almost circular or elliplical in outline. Moreover from this stage onward the arm-apparatus is not situated just midway between the dorsal and ventral lobes of the mantle but approaches nearer the former than the latter, so that it appears to project from the dorsal lobe. The anterior free portion of the dorsal lobe is, therefore, shorter than that of the ventral lobe. This relation of the mantle and arm-apparatus will be referred to later. The cells composing the arm-apparatus have become gradually smaller, and consequently its warty appearance has vanished. On the ventral median plane of the arm-apparatus the mouth (*m.*) is seen. The mantle now comes to acquire a nearly semi-circular form, having the posterior straight edge corresponding to the diameter. The mantle increases greatly in size, especially at the anterior margin so as to nearly hide the arm-apparatus as is seen in an embryo of a similar stage drawn in a side-view (Pl. III., Fig. 43.). In another embryo the segmentation cavity was seen just inside the posterior body wall.

Two embryos a little further advanced are shown in Fig. 44. (Pl. III.) in the ventral view, and in Fig. 45. (Pl. III.) in the dorsal view. In these we percieve the following changes : the rupture of the vitelline membrane, the formation of the cirri and the tentacle, and the secretion of the shell. The period in which the bursting of the vitelline membrane takes place varies considerably ; in some cases it takes place at this stage, while in others it is delayed still later. The vitelline membrane bursts on the anterior end of the embryo ; this is probably caused by the

combined action of the anterior growth and the expansion of the arm-apparatus. It is not difficult to observe the process of bursting under a microscope. A little opening being made at the tip of the arm-apparatus the latter stretches out of the hole, which is thus enlarged by degrees. Now the cilia on the arm-apparatus, 12 μ in length on an average, move more rapidly than before. The vitelline membrane is sometimes found for a long time attached closely to the posterior half of the embryo. That the vitelline membrane has persisted without rupture until now shows on the one hand that the limit of elasticity of the membrane is great, and on the other that the embryo has increased but little in volume from the egg. This will best be seen on comparing Pl. I., Fig. 1., and Pl. III., Fig. 44. By the longitudinal growth of the arm-apparatus it becomes up-lifted on a short stalk, thus simulating a mushroom (Pl. III., Figs. 44, 45.). Concurrently with this change the arm-apparatus becomes triangular with rounded angles. The median angle is the *Anlage* of the tentacle (*tnt.*), and the lateral two those of the first pair of cirri (cr^I.). Reaching this stage the embryo begins to contract its arm-apparatus. That it is very sensitive is proven by the fact that it instantly retracts its arm not only at a shock made on the stage of the microscope, but even at a slight knock upon the table. Enfeebled embryos, on the contrary, do not retract their arm-apparatus at all. This may readily be observed even in far advanced larvæ. When contracted, the longitudinal axis of the arm-apparatus lies nearly parallel to that of the embryo, but when fully extended the former makes an angle with the latter. The semicircular form of the mantle becomes more pronounced, coming to possess a straight posterior edge. The mantle at this stage grows in all directions, that is, not only anteriorly and

laterally, but also posteriorly, as is seen in the figure (Pl. III., Fig. 44.), and comes to have a narrow space between the posterior edge of the mantle and the body proper. In consequence of the rapid growth of the mantle it becomes somewhat thinned out, leaving the thick part only along the margins. The shell is present at this stage as a very thin cuticle secreted on the entire external surface of the mantle. This is best seen in an enfeebled embryo, in which the mantle is frequently found contracted, the shell being left exposed. The body proper may now be distinctly seen through. On the lateral and posterior sides of the body proper there is seen the thin ectoblast layer separate and at places even having a little space between it and the inner mass. At the median portion of the body proper we see a thick walled sac which constitutes the œsophagus and the stomach[1] (*stm.*). In the œsophagus the canal is narrow while in the stomach the lumen is somewhat dilated. The space on either side of the alimentary canal, limited externally by the ectoblast layer just described, is filled up by the compact mesoblast cell-masses (*msb.*) which soon give rise to the coelomic sacs. Up to this time the embryo has rested on the bottom, or probably in nature has been carried to and fro by the currents ; now, however, it begins to swim about freely, widely opening its mantle and protruding its arm-apparatus.

A little later the arm-apparatus comes to take a nearly pentagonal form, a new pair of protuberances appearing between the tentacle and the first pair of cirri. These new elevations are the *Anlagen* of the second pair of cirri (cr^{II}.). An embryo of this stage is represented in two views : Fig. 46 (Pl. III.) seen from the anterior and ventral sides ; Fig. 47 from the left and a

1. *Vide* footnote under the heading *g* in Sect. X.

little from the anterior side. In the latter figure we see the tentacle has begun to bend dorsally. Fig. 48 (Pl. III.) is another embryo of a similar stage viewed from the anterior side. In this and in Fig. 47, the arm-apparatus arises more markedly from the dorsal mantle lobes. The shell has become so conspicuous that we can detect it without difficulty.

In consequence of the growth of the tentacle and cirri the arm-apparatus becomes distinctly five-lobed as is seen in Fig. 49 (Pl. III.) which represents an embryo from the dorsal side, and in Fig. 50, which is a ventral view of another embryo a little older than that drawn in Fig. 49. The lumen in the arm-apparatus, known as the arm-sinus (*am. sn.*), can clearly be seen. At the beginning of this stage the part immediately posterior to the mouth is elevated ventrally and unites the two projected ventral ends of the arm-apparatus, so as to form a continuous sheet, so that the mouth is taken into, and placed nearly at the centre of, the arm-apparatus. This process will best be understood by comparing Fig. 46 (Pl. III.) with Fig. 50 (Pl. III.). The tentacle is bent dorsally: this is seen especially well when it is projected out of the mantle cavity, but may also be observed in preserved material, as in Fig. 51 (Pl. III.) which is drawn from the right side. A comparison of this figure and Fig. 43 will show in what way the embryo has been developed in the interval. The tip of the tentacle at this stage is sometimes a little flattened (Pl. III., Fig. 50.). This character is peculiar to the tentacle and is never met with in the cirri. The shape of the mouth (*m.*) changes according to the degree of contraction; sometimes it assumes a crescent form, while at others it becomes triangular. A little anterior to the mouth a shelf-like transverse ridge makes its appearance on the arm-apparatus. This repre-

sents the epistome (Armfalte) (*epst.*). When the arm-apparatus contracts the œsophagus is bent nearly at right angles, thus for a short distance running dorso-ventrally from the mouth and then taking a vertical course to the stomach. When, on the contrary, the arm-apparatus stretches, the œsophagus becomes almost straight. When the animal contracts the lumen of the stomach is seen as a transverse slit, its anterior and posterior walls being closely apposed on each other, giving the deceptive appearance of the coelomic sacs being formed as enterocoelic diverticula, as KOWALEVSKY ('73) has observed in the embryo of *Cistelia*. The mesoblast cell-masses which are placed one on each side of the alimentary canal acquire a distinct lumen (coelom) (*cl.*). Concurrently with the thinning of the walls the coelomic sacs come to extend all around the alimentary canal, as is seen in Fig. 50. (Pl. III.). As to the mantle, no special change takes place except growth in size.

At the next stage (Pl. III., Fig. 52., and Fig. 53.; both ventral views) the third pair of cirri (cr^{III}.) arise between the second pair (cr^{II}.) and the tentacle (*tnt.*) as low protuberances. Later on new pairs of cirri are always added between the tentacle and the cirri next to the latter. This mode of intercalation has been noted by KOWALEVSKY ('73), BROOKS ('78) and BEECHER ('93). When the arm-apparatus stretches, the tentacle and the first pair of cirri are directed dorsally. In the contracted state the position of the cirri is constant: the first pair of cirri face anteriorly; the second pair posteriorly (Pl. III., Fig. 52.). The stomach (*stm.*) is dilated into a spacious sac, especially when the animal contracts. The walls of the coelom become thinner and thinner until they are hardly visible. A pair of muscles, which I shall call the ventral muscles (*m. vt.* Fig. 53) makes their first

appearance. The muscle originates on the ventral body wall at the right and the left of the posterior end of the œsophagus, and runs forward along the latter, disappearing in the arm-sinus at about the same level as the mouth. It is indeed due to the contraction of these muscles that the arm-apparatus is retracted. It is frequently observed that an embryo when fatigued does not contract its arm, and this may be due to the fact that these muscles partly lose the power of contraction. These muscles attain a high degree of development in later free-swimming larvæ, and afterwards gradually degenerate. The mantle and shells show a rapid growth as compared with the body proper and the arm-apparatus. The marginal parts of the mantles are somewhat thickened owing to the large cells composing them and to the presence of the marginal lacuna (Randlacune) within. At this stage both ends of the straight posterior edge of the shell terminate in rounded angles, not in teeth as in embryos a little older.

I have thus far made no mention of the general appearance and color of the embryos. It is, therefore, not superfluous to touch next upon those points.

The embryo in general is transparent, but some thicker portions have a slight opacity. In young embryos the cells are so large that we can distinguish them individually. Hence they look very granular. The mantle even in advanced embryos has a mottled appearance in consequence of the unequal thickness of the cell layers. In older embryos the arm-apparatus, especially the tip of the cirri, is rich in refracting granules. The color of the embryo is a light yellow with a greenish tone; the thicker part, of course, being more greenish and darker in color (Pl. III., Fig. 53.). In some of the oldest embryos which I was able to

rear from the eggs, the tips of the cirri were tinted a very light yellowish-brown.

β. Internal structure.

1. Alimentary Canal.

Fig. 54 (Pl. IV.) is an embryo of nearly the same stage as the one represented in Fig. 34 (Pl. III.), cut obliquely through the blastopore. In this section we see that the entoblast is arranged epithelially and constitutes the inner part of the mesenteric cell mass. The entoblast cells already show a stronger affinity for stains than the mesoblast. As the embryo grows the blastopore is closed, the entoblast forming a sac (Pl. IV.). The stomodæum is next formed at the spot where the blastopore was present, and consists of the cells which in their behavior toward stains are similar to those of the arm-apparatus.

The entoblastic sac, just mentioned, elongates and is differentiated into two parts: the anterior half becomes the posterior part of the œsophagus, retaining its great thickness, while the posterior part becomes stomach, its walls becoming thin. Fig. 62 is a frontal section of a slightly advanced embryo. In this there is seen the posterior half of the œsophagus, as also the stomach highly compressed and appearing as if it were giving off a pair of diverticula. At first sight this appearance may give the impression that the *Anlagen* of the coelom are being formed after the enterocoelic type. But a closer examination reveals that it is nothing but the collapsed stomach (Pl. IV., Figs. 62, 65.). Such an appearance is not found in the embryos fixed in a stretched condition.

It should here be noted that in the early stages both the ecto- and ento-blast cells in the part facing the segmentation cavity retain their original character in having yolk-granules as in the blastula. This is best shown in Fig. 59 (Pl. IV.) which is a portion of the ectoblast from the same section represented in Fig. 58 (Pl. IV.).

2. Mesoblast, Body Cavity and Muscles.

As has already been pointed out in the study of surface views, the mesoblast cells are proliferated from the lateral walls of the entoblast, and form with the latter a compact mesento-blastic cell mass. Afterward the mesoblast cells group themselves in two masses closely apposed to, but with a distinct line of demarkation from the alimentary canal (Pl. IV., Fig. 62, 63, 65, 68. *msb.*). As the masses are placed on the right and left of the alimentary canal, the latter comes in direct contact with the ectoblast on both the dorsal and ventral surfaces. It should be stated that SHIPLEY ('83 p. 511) observed the alimentary canal laid against the dorsal ectoblast in the embryo of *Cistella*. The mesoblast cell mass soon hollows out and the cells are flattened, coming gradually to line the entoblast on one side and the ectoblast on the other. The lumen thus formed is the body cavity (Pl. IV., Figs. 63, 65. *cl.*). In *Lingula*, therefore, the body cavity arises after the schizocoelic type.

Anteriorly a part of the mesoblast cells becomes loose and mesenchymatous (Pl. IV., Figs. 58, 60, 61, 62, 65, 66, 67, 69. *ms.*). These cells find their way into the arm-apparatus, and finally fill the arm-sinus loosely, leaving much space between cells (Pl. IV., Fig. 69. *ms.*). Even after the mesenchyme cells

come to line the arm-sinus, the spacious lumen thus formed communicates directly with the body cavity.

The *M. ventralis* is seen as a few fibres imbedded among the mesenchyme cells of the arm-sinus. It is probable that these fibres have been formed out of mesenchyme cells (Pl. IV., Fig. 69 *m. vt.*).

3. Arm-apparatus.

The arm-apparatus appears as a fold (arm-ridge) of the ectoblast as has been stated in the foregoing section (Pl. IV., Figs. 55, 56, 57.). The ridge gradually increases in size, protruding a great deal out of the mantle. The nuclei in the ridge are spindle shaped and arranged in a single row (Pl. IV., Fig. 57.). They are compact and their staining capacity is equal to that of the nuclei in the stomodæum and exceeds that of the nuclei in other parts. As mentioned above, the inner cavity of the arm-apparatus (arm-sinus) is loosely filled with mesenchyme cells (Pl. IV., Figs. 65, 69).

That the arm-apparatus is attached almost entirely to the dorsal mantle is best shown in Fig. 58 (Pl. IV.) ; in later stages, however, it gradually separates from the dorsal mantle. (Pl. IV., Figs. 61, 64.).

On the dorsal side of the arm-apparatus, directly embracing the œsophagus there is seen a very thin epithelium, whose nuclei do not differ in any respect from those of the other ectoblast cells (Pl. IV., Figs. 61, 64.). This epithelium is, therefore, considered not as a part of the original arm-ridge, but as derived from the ectoblast dorsal to the ridge.

4. Mantle.

In the embryo the mantle is formed at a very early period as a single ring-shaped fold or ridge, around the arm-ridge (Pl. IV., Figs. 55, 56.), and is afterward cut up into two lobes as has been described before. Since it is formed as a *fold* of the ectoblast the mantle consists of two layers, each layer being one cell thick. The inner layer is very thin while the outer attains a considerable thickness (Pl. IV., Figs. 60, 61, 64–68.). The mantle lobes grow not only at the anterior and lateral, but also at the posterior edge, where growth takes place in two parallel ridges, leaving a shallow furrow between them (Pl. IV., Figs. 60, 61. *fr.*). The epithelium forming the above furrow becomes very thin (Pl. IV., Fig. 64.). We perceive in the course of development that the mantle increases in size faster than the body proper and the arm-apparatus (*Cf.* Pl. IV. Fig. 61. and Fig. 70.), and becomes thinner and thinner as it grows, retaining its original thickness only along its margin—a peculiarity which is due to the presence of comparatively large cells and of the spacious marginal lacuna (Pl. IV., Fig. 70.).

In passing, I should like to state here that the ectoblast constituting the lateral body wall is thicker than the inner layer of the mantle, and each cell is highly rounded and vaulted toward the exterior (Pl. IV., Figs. 65, 68.).

5. Shell.

A little prior to the rupture of the vitelline membrane a thin cuticular layer is secreted on the dorsal and ventral surfaces of the mantle. The shell is formed at first as a circular lamella

folded double along one of its diameters, and is secondarily divided into two valves along the posterior edge. In the furrow between the two posterior mantle folds the shell is at first secreted just as in other regions, but it is soon separated from the epithelium of the mantle furrow (Pl. IV., Fig. 64.). This is apparently due to the rapid growth of the posterior mantle-folds. The cuticula thus separated must retain its original thickness, as no new secretion can be added. But in the oldest embryos I was able to rear from the eggs the division into two valves had not yet taken place (Pl. IV., Fig. 70.).

VII. RECAPITULATION.

Let me recapitulate the principal developmental process which took place during the growth of eggs :—

1). The eggs and sperm are discharged early in the morning. The fertilization thus takes place outside the body.

2). The egg is holoblastic, the cleavage being total and equal.

3). The blastomeres are so arranged at the 32-cell stage as to form two layers, each consisting of 16 blastomeres as in the Phylactolæmata.

4). The cœloblastula is formed.

5). The gastrula is next formed.

6). The blastopore is closed.

7). The archenteron persists as the cavity of the alimentary canal.

8). The entoblast gives rise to the posterior half of the œsophagus and to the stomach.

9). The mesoblast arises from the entoblastic walls, and has two different destinations :—

α). Giving rise to the coelomic sacs.

β). Filling up the arm-sinus as mesenchyme cells.

10). The body cavity is formed after the schizocœlic type.

11). The ectoblast gives rise to four structures:

α). The mantle.

β). The outer epithelium of the arm-apparatus.

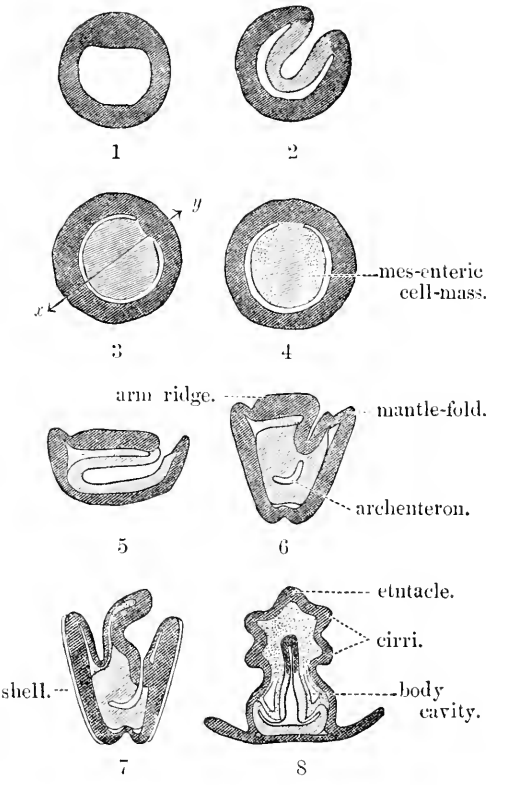
γ). The anterior half of the œsophagus.

δ). The ectoderm of the lateral body wall.

12). The mantle is formed at its first appearance as a ring-like fold, which afterwards is divided into two mantle-lobes.

13). The shell is secreted as a circular lamella folded double along one of its diameters.

14). The arm-apparatus is formed at first as the arm-ridge, which on increasing in size comes to be provided with the tentacle and pairs of cirri.



Ectoblast-crossed.
Mesoblast and Mesenchyme-dotted.

1. Blastula.
2. Gastrula.
3. The same further advanced.
4. The same cut along *x y* (The archenteron obliterated).
5. Embryo at the beginning of the mantle-fold formation (Sagittal section).
6. Embryo, in which the stomodæum is formed. (Sagittal section).
7. Embryo with shells. (Sagittal section).
8. The same. (Frontal section).

15). A new pair of cirri is always added next to the tentacle.

As is seen from the above summary of the developmental processes, the general structural scheme of *Lingula* has by this stage already been attained. No remarkable metamorphosis takes place after this. It is only upon this scheme that the new organs are gradually built up during the post-embryonal stages which will be dealt with in the ensuing sections.

As for the points recapitulated above, a comparison of the accompanying diagrams will make my meaning clear.

B. POST-EMBRYONAL STAGES.

During the post-embryonal stages new pairs of cirri are constantly added at the base of the tentacle. I will divide the larvæ to be described hereafter into several stages according to the number of the cirri, thus :

Free-swimming larvæ.
- 3 p. c. stage[1] = Protegulum stage.
- 4 p. c. stage.
- 5–6 p. c. stage.
- 7–9 p. c. stage.

Sedentary larvæ. 10–15 p. c. stage = larvæ with protruded peduncle.

As development goes on gradually, no sharp lines of demarkation between these stages can, of course, be drawn. But the increase in number of the cirri appears to me to be the point that best indicates the age of the larvæ. Their size, for instance, is not a reliable criterion, for that is a function of nutrition both in nature and in confinement. When the food supply is specially abundant the larvæ at the 7 p. c. stage, for

1. p. c. = pairs of cirri.

example, attain almost the normal size of those of the 12–13 p. c. stage; while the larvæ at the 15 p. c. stage when imperfectly nourished would not exceed in size well-fed larvæ of the 8–9 p. c. stage.

VIII. STAGE OF 3 PAIRS OF CIRRI.
(PROTEGULUM STAGE.)

During the past three seasons (1899–1901) the larvæ of this stage were found in tow-nets only on one occasion (July 28=21 of the lunar calendar). The larvæ, as far as I could judge from my experience, were five or six days old. This being the case, the eggs must have been laid at the preceding spring tide. As *Lingula* in confinement laid eggs on July 27, there is a good reason to believe that in captivity spawning was delayed for about five days by the unnatural conditions of confinement.

On the single occasion when the larvæ of this stage were collected, there were unfortunately so few of them that I was not able to obtain satisfactory sections. I shall, therefore, refer chiefly to the structures which could be determined in surface view.

The larvæ at the stage (Pl. V., Figs. 73, 74.) differ but little from the oldest of those I succeeded in rearing from the egg, and thus there is fortunately no gap in the materials obtained from the two sources. In the larvæ under consideration the most striking feature is the great increase in size of both the mantle and the shell. The latter attains the typical form of the Protegulum, as defined by Beecher ('91 p. 344). It is a little higher than a semicircle, the ratio of the height to the length of the posterior edge, being 3 : 5. The posterior straight edge,

measuring 280 μ on an average, does not increase in length throughout all the larval stages. This edge which subserves the function of a hinge for a long time even after the larvæ forsake the free-swimming life, I shall refer to as the hinge line—a term which may at first appear inappropriate for the Ecardine Brachiopods. At each extremity of the hinge line the shell is bent slightly outwards, and forms the tooth, which is characteristic of the Protegulum. Up to the time when these teeth are formed the shell grows equally on all the radii of the semicircle, and thus it results that on the hinge line which represents approximately a diameter, or the sum of two radii, the growth is twice that of the height which corresponds to only a radius. A comparison of the actual size of two larvæ (Pl. III., Fig. 53, and Pl. V., Fig. 74.) will best verify the above point :

	The oldest embryo reared from the egg. (Pl. III., Fig. 53.)	Protegulum. (Pl. V., Fig. 74.)	Difference.
Height.....	138 μ	176 μ	38 μ
Hinge line.....	216 μ	295 μ	79 μ

The ventral valve presents a greater curvature than the dorsal (Pl. IV., Fig. 71.). Both valves are still connected with each other by means of thin cuticula, which, however, will soon be torn. At the hinge line both valves increase in thickness (Pl. IV., Fig. 71.).

The lobes of the mantle have become so thin that it can hardly be seen except at its thickened margin, where there exist the marginal lacuna and the glandular cells, becoming later the glandular ridge (Drüsenwall) (Pl. IV., Figs. 71, 72.). The mantle

is more or less contractile; for when the larvæ are fatigued or thrown into a fixing fluid it is sometimes separated from the shell-margin by a strong contraction.

Though the cirri have not increased in number as compared with the oldest larvæ reared from eggs, they are elongated and in each a lumen becomes visible which I shall call the cirrial canal (*cr. cn.*). The lumen is a diverticulum of the central arm-sinus, and is formed by the flattening of mesenchyme cells to line the cavity of the ectoblast. The cirrial muscle (*m. cr.*) is already formed, the fibers being produced from mesenchyme cells (Pl. IV., Fig. 71.).

A pair of muscles, the ventral muscle, (*m. vt.*) (Pl. IV., Fig. 72, and Pl. V., Fig. 74.) which have been seen in the preceding stage, become more conspicuous. In parasagittal section the course of the muscles can be clearly followed. They arise from the ventral body wall a little posterior to the anterior edge of the stomach and run forward, passing one on either side of the œsophagus, and terminating on the dorso-lateral side of the latter.

In two fixed specimens, a pair of black spots, one on each side of the œsophagus, were seen by reflected light from the ventral side; by transmitted light each spot was resolved into 2–3 transparent and highly refractile crystall-like granules. Whether they are the *Anlagen* of *Occlusores Anteriores* or not I cannot as yet decide owing to the lack of materials.

At this stage there occurs a notable thickening along the ventral margin of the arm-apparatus. This thickening forms the ventral ridge of the arm-apparatus, and persists up to the 5–6 p. c. stage as is seen at the point marked * in Fig. 91 (Pl. VII.). Afterward the part is greatly thinned out.

While the œsophagus shows no special changes, the stomach

now becomes a voluminous sac. Its walls, however, have not as yet differentiated histologically, the mid-gut and the liver-lobes not having appeared. The stomach fills up the greater part of the body-cavity, and when it is fully distended, or when the arm-apparatus is stretched, there is no space left between it and the body walls.

Coincident with the growth of the stomach and of the body cavity a distinct change takes place in the coelomic wall. Its parietal and visceral layers which have hitherto been composed of large rounded cells, now assume the characters of a true epithelium.

The ectoblast of the body wall ventral to the neck remains in the condition of a thin epithelium, and no further differentiation occurs at this stage. It is here, however, that in the 5–6 p. c. stage the nerve tissue arises.

IX. STAGE OF 4 PAIRS OF CIRRI.

As materials of this stage proved very scanty, it is deemed best, not to enter into its more intimate changes.

The most conspicuous change is the great growth of the shell. The enlargement of the shell (secondary shell) has taken place only along the anterior and lateral margins of the Protegulum and not at all on the hinge-line. This is best seen in the outline drawing of Fig. 76 (Pl. V.). At this stage the length of the shell has become somewhat greater than the length of the hinge line. It will be recalled that in the Protegulum these two measurements stood to each other in the ratio of 3 : 5.

The third and fourth pairs of cirri are formed between the tentacle and the second pair of cirri. The thin central part of

the arm-apparatus swells out dorsally, as is seen in Fig. 78 (Pl. V.).

In the œsophagus is a valve-like structure which reduces its calibre (*œs.*) (Pl. V., Fig. 75.). The stomach has differentiated into two parts: the so-called liver, and the mid-gut. The former occupies the main part of the stomach and is filled with a number of unicellular algæ which are taken into the liver cells as food. The mid-gut is represented at this stage as a small ciliated area. The intestine has already made its appearance.

X. STAGE OF 5–6, AND 7–9 PAIRS OF CIRRI.

No account of the foregoing stages has hitherto been published, but the later larvæ, as we noted, have been studied in detail. The work of BROOKS ('78) which is based upon materials whose earliest stage was the larvæ of 5 p. c. stage (the sixth pair was about to be formed), leaves little to be desired as far as a knowledge of surface changes and the outlines of organology is concerned. As sections, however, were not employed in the work of the American author,[1] there are, of course, many details lacking in the history of the later development, and in my present account I shall endeavor to describe some of the detailed changes not yet touched upon by any previous writer.

The 5–6 p. c. stage is of a special interest, since it is at this stage that the *Anlagen* of many important organs are laid. It would, of course, be desirable to give a detailed account of these larvæ, and afterward of the next stage, but, as there are no sudden changes between the 5–6 p. c., and 7–9 p. c. stages, I

1. BROOKS ('87) promised that he would describe sections of *Lingula*-larvæ in other places (p. 90), but he, so far as I know, has not fulfilled his promise.

shall deal with them together so as not to interrupt the course of description.

a. Habits.

The free-swimming larvæ of *Lingula* occur abundantly in the plankton collected in the inlet of Moroiso just in front of the Marine Station at Misaki. They are taken with tow-nets from the latter part of July until the first half of September *i.e.* during about two months. On rainy or windy days, however, they are invariably absent, since doubtless they sink to the deeper water. More of them are collected at rising than at ebb tide. This fact coupled with another, viz., that stages earlier than the 7 p. c. stage are seldom met with, make it possible that the embryos are carried out of the bay at very early stages, and it is only on their return to the region of the mud flats, that they are taken by the collector. The larvæ are captured at the surface as well as at the depth of a few feet. But how deep they can sink, and how far off the shore they are carried, I have not yet tried to determine.

The larvæ do not move about to any great extent among the plankton collected with a tow-net, and are generally found quite immobile in the bottom sediments which are composed of Radiolarians, Diatoms, etc. Taking advantage of this peculiarity I generally obtained the larvæ by searching for them only in such sediments. After they with a small quantity of water have been transferred to a slide the only movements I have noted are occasional contractions of the arm-apparatus, by which the body cavity is dilated. It may be noted that the arm-apparatus contracts suddenly, and then again slowly extends until it fills up

the mantle cavity. When the larvæ are taken out of the plankton sediments and placed in a spacious vessel of fresh sea water, they first begin to swim about in their own peculiar manner (Pl. VI., Fig. 88, *a. b.*). It reminds one of the figure of the swimming *Discinisca*-larva given by FRITZ MÜLLER ('60), but there are some differences. From between the wide gaping shells the cirri are gracefully protruded out of the shell to almost two thirds of their length. All the cirri are directed anteriorly with a gentle curvature outward; on the ventral or dorsal aspect therefore the cirri look like the ribs of a fan. In this way the larvæ swim very slowly, keeping their body axis nearly always vertical. Sometimes they stand on the widely expanded arm-apparatus upon the bottom of the vessel as if they were looking for food. During the act of swimming the breadth of the body proper is much reduced.

Swimming larvæ, when even slightly alarmed, retract their cirri very rapidly into the mantle cavity, and sink to the bottom. In this position they remain for a considerable length of time. Larvæ which give evidence of not having fed for a time are sluggish and do not draw in their cirri, even when roughly disturbed. They appear to have almost lost contractility. Such larvæ, therefore, can be brought upon a slide without danger of causing the retraction of their cirri.

Diatoms and other unicellular algæ constitute the chief food of the larvæ. Among diatoms the genus with drum-like test (*Ethmodiscus*) predominates.

Before giving detailed anatomy of the free-swimming larvæ a comparison of the figures of larvæ, respectively with 5 p. c. (Pl. V. Figs. 77, 78.), 6 p. c. (Pl. V., Fig. 79.), 7 p. c. (Pl. VI.,

Fig. 85.), 8 p. c. (Pl. VI., 84.), 12 p. c. (Pl. VI., Fig. 87.) and 15 p. c. (Pl. VI., Fig. 86.) will afford a general idea of developmental changes, and show how our *Lingula*-larvæ differ from those figured by Brooks in having the narrower arm-apparatus, a broader body proper and a shorter peduncle.

b. **Shell.**

During the 6–7 p. c. stage the shell is almost circular. It, however, soon, increases more rapidly in length than in breadth, and at the 8–9 p. c. stage it has become oblong :

	Length.	Breadth.
5 p. c. stage	313 μ.	386 μ.
6 ,, ,,	411 μ.	444 μ.
7–8 ,, ,,	663 μ.	615 μ.

The ratio of the hinge line to the length of the shell at different stages is as follows :

3 p. c. stage = Protegulum	1 : 0.60
4 ,, ,,	1 : 1.00
5 ,, ,,	1 : 1.25
6–7 ,, ,,	1 : 1.47
8–9 ,, ,,	1 : 2.52
10–15 ,, ,,	1 : 3.00

Here the hinge line is taken as 1, its actual length being 280 μ on an average. It should here be noted that in the young *Lingula* of 5 mm. (in shell length) the ratio is 1 : 18.51.

While at the 5–7 p. c. stage the shell is transparent and colorless (Pl. V., Figs. 77, 78, 79., and Pl. VI., Fig. 83.), at the 8–9 p. c. stage the posterior and lateral margins of the shell acquire a light brownish tint, and near the hinge line the

superficial layer of the shell becomes bright green (Pl. VI., Fig. 84., and Pl. VII., Fig. 97.). The colors do not dissolve out in alcohol or in any other reagent.

The Protegulum remains as such in the shell. In the dorsal valve it is faintly visible, while in the ventral it is always outlined by a definite ridge. On transverse section the lateral margins of the Protegulum slightly project from the general surface of the shell. BROOKS on the contrary, observed the Protegulum on the dorsal valve only ('78 p. 39.).

The shell is of medium thickness throughout, thinning, however, toward the margins. Near the hinge line it thickens with age. Here the ventral valve (*vt. sh.*) (Pl. VII., Fig. 97.) ends in a thin lamella, which is the remnant of the early connecting cuticula. This lamella bends over dorsally so as to overlap, and lock with the thickened edge of the dorsal valve. The latter thickens a little anterior to the hinge line where it thins out and is provided with a horizontal ridge directed outward (Pl. VII., Fig. 97., *r.*).

Referring now to the extremities of the hinge line in surface view (Pl. VII., Fig. 96.), the hinge line of the ventral valve is somewhat longer than that of the dorsal, as BROOKS states ('78 p. 38). While the ventral valve (*vt. sa.*) terminates at either end with a pointed tooth, whose sharp edge forms part of the margin of the Protegulum (*prt.*), the dorsal valve (*dr. sh.*) spreads out into a thin plate whose margins are rounded and sinuate.

The secretion of the shell goes on uniformly all over the dorsal and ventral walls of the body proper as well as on the outer surface of the mantle lobes, but the most active organ of secretion is the mantle margin. This fact is best confirmed in the posterior mantle where the growth is early arrested on account

of the presence of the hinge line, but the secretory activity continues uninterrupted, and thus results the thickening of the shell in this region. In other parts, on the contrary, the mantle increases continually and no special thickening of the shell takes place.

The microscopical structure of the shell can best be studied in longitudinal sections (Pl. VIII., Fig. 130.). The shell is composed of cuticular substances secreted in layers. Outside this is a special layer, the periostracum (*pr. ost.*), which measures about 1 μ in thickness, exhibits a slight difference in refractive index from the main cuticular layer, and is of a very light yellow color.

c. Mantle and Setæ.

It need hardly be remarked that the mantle which gives rise to the shell keeps equal pace with it in growth and therefore corresponds with it in outline. Along the mantle margin there is a thickened zone bearing a yellowish brown pigment (Pl. V., Figs. 77–79., and Pl. VI., Fig. 83.). It is indeed only by this zone, that the presence of the mantle can be recognized, since in other regions it is transparent and invisible.

Along the margin of the mantle, it should be recalled, a lacuna (Randlacune) is seen in the oldest embryo which was reared from the eggs (Pl. IV., Fig. 70.) (*vide* p. 27.). In the larvæ of the 5–6 p. c. stage the marginal lacuna is found to be loosely filled with mesenchymatous connective tissue cells which have probably been proliferated from the ectoblast. Later on the lacuna extends toward the body proper. From the 7 p. c. stage on there come into view the muscle fibres (Pl. VI., Figs. 84,

85.) which run toward the body proper, two or three of them uniting in their course (*Cf.* Pl. VIII., Fig. 131., *r. m.*). The presence of such muscle fibres has been observed by KOWALEVSKY ('83) in the larvæ of *Cistella* (p. 64.).

The inner epithelium of the mantle margin facing the mantle cavity is composed of columnar cells. It changes, however, into the gland zone, (Pl. VII., Fig. 98, *gl. cll.*) at a little distance from the margin and just proximal to the pigment zone. In *toto* preparations the gland zone is seen to be composed of large polygonal gland cells (Pl. VII., Fig. 85, *gl. cll.*), as SIMROTH figures ('97 Fig. 5.). The formation of these cells is begun at the postero-lateral corner of the mantle and gradually spreads forward (Pl. VI., Fig. 85, *gl. cll.*) until they cover the entire circumference of the mantle. The gland cell is filled with secretion granules which stain intensely with hæmatoxylin or are colored a light violet with carmalum. It is quite invisible in fresh materials. The nuclei are pushed aside by the granules (Pl. VII., Fig. 98, *gl. cll.*). The gland cells, it may be noted, give rise to the interesting gland-ridge (Drüsenwall) of the adult. Proximal to the thickened margin the inner epithelium remains very thin and it becomes almost impossible to determine the line separating the inner and outer layers of the mantle. In this region the nuclei are infrequent and scattered here and there (Pl. VI., Fig. 85.).

As to the outer epithelium of the mantle there is nothing worth mentioning except that it increases slightly in thickness at the mantle margin.

Setæ are first seen at the close of the 7 p. c. stage along the entire margin of the mantle. At the 8 p. c. stage nearly all the setæ are still so short that they hardly reach the shell margin,

but several planted at the postero-lateral corners of the mantle are tolerably long, extending for a short distance beyond the shell margin (Pl. VI., Figs. 84, 85., *st.*). On a careful study of *toto* preparations elongated nuclei arranged along a seta seem to support the view that the seta has been secreted by the ectodermal invagination as in the adult. This feature is however, very difficult to ascertain in sections.

d. **Pallial Sinus.**

By a careful adjustment of the microscope the pallial sinus can be seen in living larvæ as four finger-like processes of the body cavity which stretch into the mantle at the antero-lateral corners of the body proper. They are quite invisible both in fixed larvæ and in stained preparations, owing to the fact that in preserved specimens the thin walls of the sinus become closely pressed against each other, so that the lumen is entirely obliterated. They appear at the beginning of the 8 p. c. stage. As shown in Fig. 84. (Pl. VI., *pll. sn.*) the outline of the sinus appears as an interrupted line. It should be stated that each dot in this line is intended to represent a single flattened cell. Further notes regarding the sinus need not be given, since they only confirm the descriptions of Brooks.

e. **Body Cavity and its Walls.**

In surface view, the outline of the body cavity is circular in most cases, but the diameter changes a great deal according to the degree of contraction of the parietal muscle. Fig. 84. (Pl. VI.) shows it in a medium sized condition.

At an early stage the cavity communicates only with the

arm-sinus, but later five more diverticula of the body cavity are developed; *viz:* two pairs of the pallial sinuses, and an unpaired peduncular cavity. Here I will confine myself to the description of such parts of the wall of the body cavity as have no definite relations with such organs as the ganglia, otocysts, nephridia, pallial sinuses, or peduncle. The body wall is composed of two layers, as already stated, the inner being the parietal layer of the mesoblast, and the outer the ectoblast.

The lateral body wall remains in its original state in the larvæ of the 5 p. c. stage, but in the 6 p. c. stage the cells of the peritoneum (parietal layer of the mesoblast) proliferate special cells outward just beneath the epidermis. From these cells are formed the muscle fibres which now come to constitute the parietal muscles (Pl. VI., Fig. 85., and Pl. VII., Figs. 93, 94., *m. pr.*). These fibres run longitudinally along the lateral body wall, bending toward the median plane near the hinge region. Even at the 8 p. c. stage the muscle layer remains very thin, being but a few fibres thick. The ectoderm is extremely flattened throughout.

On the dorsal and ventral faces the body wall is for the most part so exceedingly thin that it becomes very difficult to make out the two layers of which it is composed.

To describe first the outer layer. Notwithstanding its thinness, it maintains the power of secreting shell substance throughout life, to which alone the increase in thickness of the shell is attributable. At the insertions of muscles this ectoblastic layer is modified into muscular tissue (Haftzellen, Blochmann), a character which will be referred to more fully in the section on the muscular system.

The inner layer, or peritoneum, is equally thin. This layer

does not directly turn into muscular tissue, but proliferates cells between itself and the outer layer. In the cells thus formed the muscle fibres (shell muscles) are produced. The peritoneal layer, on the other hand, remains throughout life as the sheath of the muscles. VAN BEMMELEN ('91) thought, however, that the peritoneum itself changes into the muscular tissue. Between the otocysts the peritoneum produces a thickening which according to BROOKS represents a nerve string connecting the otocysts, but this conclusion cannot, I believe, be accepted. The thickening is clearly composed of enlarged epithelial cells filled with highly refractive granules. There is no evidence determining the true nature of this thickened ridge. I am, however, led to believe that it is an area whose function is excretion.

The supporting substance which as a rule is found between the epidermis and the peritoneum must certainly be present in the larvæ now under consideration, but it is still too thin to be readily determined. As to the formation of the supporting substance, though there is no positive proof in *Lingula,* I believe that it is secreted not only by either or both of these two layers, but also by the cells which are proliferated by either of the layers and are found in the adult imbedded in the supporting substance.

f. Blood Corpuscles.

A few blood corpuscles were first noted floating about in the body cavity at the end of the 7 p. c. stage. The corpuscles rapidly increase in number and at the 8–9 p. c. stage several hundreds of them are seen (Pl. VI., Fig. 84.). At this time only the ordinary kind of corpuscles is found, the leucocytes and

spindle bodies occurring first in more advanced larvæ. The early corpuscles measure 8 μ in diameter; they are much smaller than those of the adult (16–20 μ), although very much like them in form. At the centre of the corpuscle there is a relatively large, compact and round nucleus. The later development of the blood cells consists chiefly in the increase of the cytoplasm. The refractive, probably nutritive, granule found in the adult corpuscle is not as yet visible. When blood corpuscles are crowded together they present a light red color, as in the adult.

In life the corpuscles accumulate at the posterior end of the body cavity, and I at first thought they were proliferated there. Sections, however, showed clearly that in this region there is no sign of the production of corpuscles. On the other hand, in transverse sections of some larvæ, a mass of polyhedral cells on the ventral side of, and just posterior to, the ventral ganglion was seen which insensibly passed over to the ordinary epithelial cells of the peritoneum on one hand, and to the blood corpuscles on the other. These cells gave every evidence of having been produced from the peritoneum and of being in stages of transformation into blood corpuscles. Afterward I was able to observe in a living larva of the 8 p. c. stage that the posterior face of the ventral ganglion has a jagged appearance, as if from this region the blood corpuscles had recently come off and that some corpuscles were still adhering (Pl. VII., Fig. 99, *b.c.*).

In the larvæ of the 6 p. c. stage a curious element is found floating in the body cavity. It is not very common. It is a flat disc of an irregular outline; sometimes it assumes a crescent shape; sometimes a spindle shape. On the flat surface there are found about ten parallel depressions stopping short at the margin

of the disc. It takes a strong orange-G stain. No nucleus is present in it. One of these curious bodies is represented in Fig. 82, Pl. VI., (coloured yellow, half orerlapping the right *obl. int.*). In larvæ older than that of the 6 p. c. stage I could not detect this element at all. Its nature is quite unknown to me.

g. Alimentary Canal.

During the 4 p. c. stage the alimentary canal has differentiated into several parts (*vide.* p. 39.) *viz:* the œsophagus, liver (=oral part of the stomach, Brooks '78), mid-gut (=Mitteldarm, Blochmann; '00=intestinal part of the stomach, Brooks), and intestine (=Enddarm, Blochmann). From the 5 p. c. stage onward the chief changes concerning the alimentary canal consist in the constriction of the liver into lobes.

In life the œsophagus and intestine are of a very light yellow color, while the liver is tinted a little darker brownish yellow. The mid-gut, on the contrary, is transparent and almost colorless.

The œsophagus (Pl. V., Figs. 77–80, Pl. VI., Figs. 81, 83–85, and Pl. VII., Figs. 91–95, *œs.*) in its first part takes a nearly horizontal course, and bending at almost a right angle, it reaches the stomach a little posterior to the ventral ganglion (*vt. gn.*). It is composed of tall columnar epithelial cells throughout its whole length : in these the nuclei are compact, spindle shaped and are situated near the base of the cells (Pl. VII., Fig. 100). In somewhat advanced larvæ, at about the 9 p. c. stage, the walls of the œsophagus contain uncellular glands here and there. In life the movement of cilia on the œsophageal walls is very distinct, even when the animal is at rest, and a constant current

can be seen passing from without into the stomach. In section we can see a well stained layer of the basal pieces of the cilia.

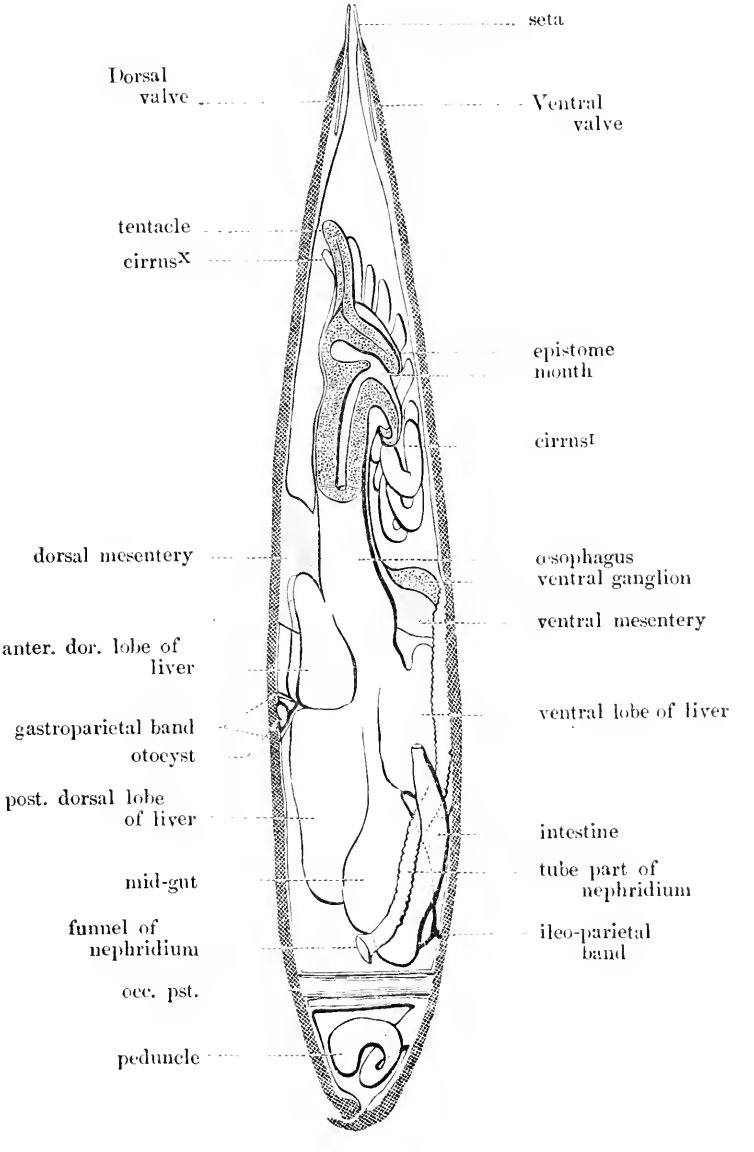


Diagram of a larva of the 10 p. c. stage:—Right side view, the shells, the mantle, the body walls, and the anterior part of the body being sagittally cut off.

The wall of the œsophagus, it should here be noted, comes in the dorsal-most portion, in close relation with the integument of the neck. The latter, a thin epithelium, is here actually apposed to, and wraps around, the thick columnar cells of the œsophagus (Pl. VII., Figs. 91, 93, 95).

Near, but not quite at, the end of the œsophagus is a valve-like elevation of the wall, which reduces the calibre of the canal. This indicates the boundary between the parts formed of the ectoblast (=the stomodœum.) and entoblast (Pl. V., Figs. 77, 79, and Pl. VII., Fig. 93). In living larvæ cilia appear to be planted differently, as they point away from one another, (Pl. V., Fig. 80), those that are anterior to the boundary being directed anteriorly, and those posterior to it posteriorly. The current is, however, the same in both parts, being toward the liver. In section the difference between these two parts is striking: the nuclei of the anterior part are longer than those of the posterior part and, moreover, the direction of the nuclei of these two parts is different, inclining away from the boundary (Pl. VII., Figs. 93, 105).

The liver is derived from the main part of the stomach[1] and is the chief seat of digestion. This part I shall refer to as the liver, although its function is obviously not equivalent to that of the vertebrate organ of the same name. The walls of the liver consist of a thick glandular epithelium (Pl. VII., Figs. 93, 94, 103, 104) whose cytoplasm is highly glandular and whose nuclei are spherical, closely applied to the base of the cells. In this

1. Hitherto I have used the term "stomach" in quite a different sense from the same term employed in the adult anatomy. I have used the term for the spacious sac posterior to the œsophagus. This "stomach" has now differentiated into the liver and the mid-gut, and the "stomach" as such has, therefore, vanished. Still I shall call the central cavity the stomacal cavity. The adult stomach is formed *de novo* (*vide infra*).

region cell boundaries become invisible. It is certain that digestion is intracellular. Diatoms and other unicellular algæ, as a rule, are found imbedded in the liver cells (Pl. VII., Fig. 101). It may be remarked that this mode of digestion goes on throughout later life: in the adult diatom tests are found even at the blind end of the finely branched liver lobules. While it is clearly the fact that intracellular digestion occurs, I have no reason to deny the presence of extracellular digestion. Yet I believe that it is more profitable and more economical to digest food particles within the cell than outside it, as the stomacal cavity is widely open to the exterior and admits freely the water from outside. Cilia are not found on the liver cells. CALDWELL ('82) states that in *Actinotrocha* intracellular digestion prevails in ciliated cells, but this difference may readily be ascribed to the difference in the types. Besides food particles a great number of highly refracting globules of various sizes are found in the liver cells (Pl. VII., Fig. 102). That these globules are oil drops, as BROOKS states, is proved by their solubility in alcohol and by their being blackened by osmic acid. These globules, therefore, are indicated by the presence of vacuoles in preparations treated in the usual way.

As above stated, the liver at the 5–6 p. c. stage comes to be constricted so as to form three lobes. The two diverticula on the dorsal side are to be known respectively as the anterior dorsal lobe and the posterior dorsal lobe; the one on the ventral side is the ventral lobe (Wood-cut p. 51.).

The anterior dorsal lobe is the last of the three lobes to develop. At the 6 p. c. stage the *Anlagen* of the lobe appear as a pair of out-pocketings of the liver, one on each side of the œsophagus (Pl. VI., Figs. 81, 82, *an. dr. lv.*). They afterward

approach the median plane until they fuse partially, assuming the same shape as in the 7–8 p. c. stage. At the latter stage the lobe (Pl. VI., Fig. 84, and Pl. VII, Figs. 91, 93, 94) is situated at the centre of the shell and is the smallest of all the lobes. It is nearly triangular in shape: posteriorly it reaches the line connecting the otocysts: laterally it is limited by the *occlusores anteriores*. In dorsal aspect the lobe is bifurcated in the anterior half of its length, the gap between the two horns being occupied by the dorsal mesentery. Eventually this lobe becomes the so-called salivary gland of CUVIER; it does not, of course, differ structurally from any other liver-lobe.

The posterior dorsal lobe is the lateral outgrowth of the liver wall and hence it is the oldest of all the lobes. In larvæ of the 5–6 p. c. stage it is almost circular in outline (Pl. V., Figs. 77–80, and Pl. VI., Figs. 81, 82, *pst. dr. lv.*). By the 7 p. c. stage (Pl. VI., Figs. 83, 84, and Pl. VII., Figs. 93, 94., *pst. dr. lv.*) it gives off anteriorly two cœca which pass as far forward as the gastroparietal band (*gst. bd.*), just beneath the otocysts (*ot.*). Posteriorly it extends as a pair of posterior cœca as far as, or a little beyond, the posterior end of the mid-gut (*md. gt.*). This part of the liver is the broadest part of the alimentary canal, being so especially at the anterior cœcum, and gradually decreasing in breadth posteriorly. It should here be noted that the size of the liver lobes varies considerably according to the quantity of nutriment which it contains. Whether the larvæ have recently fed can at once be judged from the breadth of the liver, and indeed from that of the posterior dorsal lobe alone, for this part of the liver is bulged out laterally and can readily be seen. The median dorsal portion of the liver shows but little swelling, though histologi-

cally it does not appear different from the other parts of the liver (Pl. VII., Figs. 91, 93, *pst. dr. lv.*).

The ventral lobe of the liver is faintly seen at the 5 p. c. stage (Pl. V., Fig. 77, *vt. lv.*). From this time it increases in size, and by the 8 p. c. stage it (Pl. VI., Figs. 83, 85, and Pl. VII., Figs. 91, 93) becomes a cup-like out-bulging of the liver walls, which communicates by a broad opening with the stomachal cavity. The opening can readily be seen in a dorsal as well as in a ventral view. As the result of a more rapid growth in the posterior direction, two lateral swellings arise in that part, the lobe thus assuming an inverted heart-shape. While anteriorly it extends somewhat further forward than the anterior cœca of the posterior dorsal lobe, posteriorly it does not extend as far as the middle region of the same. Between this lobe of the liver and the mid-gut a narrow space remains for the *M. obliqui medii*, which here freely decussate. In some, especially older, larvæ the epithelium between the ventral and posterior dorsal lobes is found ciliated. This is the beginning of the formation of the stomach of the adult.

The mid-gut which appeared at the 4 p. c. stage as a slightly concave area of the larval "stomach" (Pl. V., Fig. 75, *md. gt.*) has now attained a moderate length, slightly bending toward the ventral body wall (Pl. V., Figs. 77–80, Pl. VI., Figs. 81–85, and Pl VII., Figs. 91, 93, 94, *md. gt.*). It consists of high columnar cells distinctly bearing cilia. The cells are so closely apposed to one another that they appear as a group of fine threads. The nuclei of these cells are situated at different levels and stain so intensely that in sections they appear near the base of the epithelium as a dark zone of five or six nuclei in thickness (Pl. VII., Fig. 93). On the ventral side the mid-gut extends

as far forward as the ventral lobe of the liver, while on the dorsal side it is greatly restricted since the greater part of this region is occupied by the dorsal lobe of the liver. In subsequent changes this part of the alimentary canal attains a greater length. It should be noted that in the larva referred to as No. 3, SIMROTH ('97) has apparently mistaken the mid-gut for the *Anlage* of the liver (p. 6.).

From the 6 p. c. stage onward to the oldest sedentary larva (15 p. c. stage) which I was able to study, a pit is found at the bottom of the mid-gut (Pl. V., Fig. 80, Pl. VI., Figs. 83, 84, and Pl. VII., Figs. 91, 93, *pt.*). A narrow passage way into the intestine is seen at the bottom of the pit (Pl. V., Fig. 80). The fate of this pit is as yet unknown to me.

The intestine seems to arise at the 4 p. c. stage by the constriction of the liver. No difference in structure can be noticed between the intestine and the liver (Pl. V., Figs. 75, 80, and Pl. VII., Fig. 103., *int.*). Afterward it comes to be composed of a thin epithelium (Pl. VII., Fig. 104, *int.*). It increases in length until it meets the body wall about in the middle of the latter. Then the ectoblast actively proliferates cells at the place of contact, and these soon cause the tip of the intestine to become attached there (Pl. VI., Fig. 85, and Pl. VII., Fig. 104). The anus opens to the exterior at the 8 or 9 p. c. stage.

The whole digestive tract just described is covered on the outside by a thin flat epithelial layer, which is the visceral layer of the mesoblast.

In larvæ of the 6 p. c. stage an œdematic swelling of the visceral layer of the mesoblast at the posterior portion of the alimentary canal is often met with in fresh material (Pl. V., Fig. 80, and Pl. VII., Fig. 118, *) as well as in stained

preparations (Pl. VI., Fig. 82, *). This structure cannot be an artefact; it perhaps forms a part of the ileo-parietal band.

h. Mesenteries.

In the free-swimming larvæ all the mesenteries found in the adult are present, *viz.:* two mesenteries *sensu stricto* (dorsal and ventral), the gastro-parietal band, the ileo-parietal band and the intestinal mesentery. The last two are especially difficult to make out in well fed larvæ, for in such cases the liver and muscles occupy the whole body cavity. In poorly fed larvæ, on the contrary, the alimentary canal is greatly reduced in size, and hence the mesenteries are fully extended. Such specimens should, of course, be selected for study.

The ventral mesentery (Pl. V., Fig. 77, Pl. VI., Fig. 82, and Pl. VII., Figs. 99, 105, *vt. mes.*) is a median septum, which extends between the posterior part of the œsophagus and the anterior part of the ventral body wall. While it is connected anteriorly with the posterior concave face of the ventral ganglion (*vt. gn.*), its posterior border is unattached. This mesentery consists of two layers closely applied to each other. In a sagittal section we can determine the spindle shaped nuclei of the component cells.

The dorsal mesentery (Pl. VI., Fig. 87, and Pl. VIII., Figs. 122, 125, 126, *dr. mes.*) is a very narrow septum, which can be seen in a sagittal section. In examining transverse sections we find that it bisects the anterior out-bulging of the body cavity (Erker of Blochmann), connecting the œsophagus with the dorsal body wall for a distance of 80–90 μ. Posteriorly it reaches the point of bifurcation of the anterior dorsal lobe of the

liver. The same feature just described is also seen in fresh specimens and in *toto* preparations, when carefully examined.

These two mesenteries are probably the remnants of the septa formed in early stages by the apposition of the wall of two cœlomic sacs.

The ileo-parietal band (Pl. VI., Figs. 81, 82, Pl. VII., Figs. 93, 106, 115, and Pl. VIII., Figs. 133, 134, *il. pr. bd.*) has a transverse position as is best seen in the accompanying cut, and passes nearly parallel to the frontal plane of the body. Ventrally it becomes narrow and is attached to the ventral body wall near the *M. occlusor posterior*. Dorsally it comes to attach itself to the ventral side of the mid-gut. Laterally it is drawn out into two narrow wings: the left wing forms the ventral

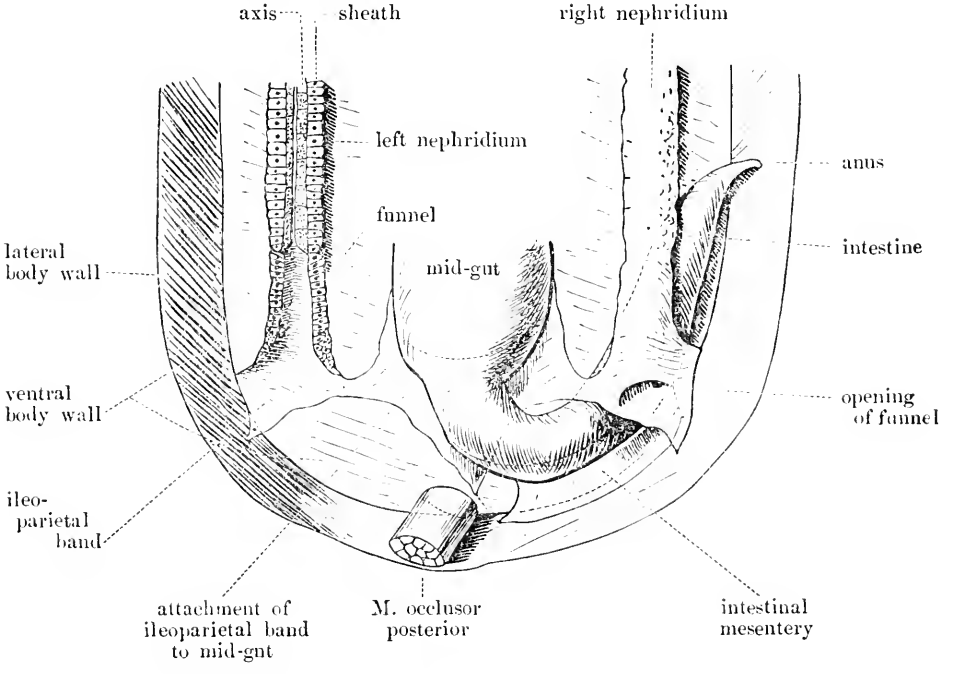


Diagram showing the relations of the ileo-parietal band, the intestinal mesentery, and the nephridia (dorsal view). (The dorsal half of the left nephridium is cut off).

part of the nephridial funnel and finally reaches the body wall in the angle where the lateral and ventral body walls meet. The right wing takes a little different course from the left, owing to the fact that the posterior part of the nephridium is separated from the ventral body wall by the intestine. The wing at first runs dorsal to the intestine and thence passes over to the ventral lip of the nephridium. It is finally attached anteriorly to the place where the ventral body wall meets the lateral one, while posteriorly the attachment is shifted a little dorsally by the presence of the intestinal mesentery.

The intestinal mesentery (Pl. VI., Fig. 87, Pl. VII., Figs. 106, 115, *int. mes.*) suspends the intestine from the lateral and posterior body wall for a short distance.

The latter two mesenteries seem to be formed, respectively by the folding of either the visceral, or the parietal layer of the mesoblast, though I have not been able to study their formation. (*Cf.* p. 56–57.).

The gastro-parietal band is of quite a different nature from any other mesentery. Strictly speaking it is not a mesentery at all, but only a lifting up of the peritoneum, in which some tendon-like cells have been produced. These cells are easily recognized by their stronger refractivity. In dorsal view (Pl. V., Fig. 80, and Pl. VI., Figs. 83, 84, *gst. pr. bd.*) the band appears to divide the body cavity into the small anterior, and the large posterior, parts, though in reality both parts communicate freely with each other, as the band is still a low ridge. The band begins from the ganglion, turns backward and, passing along the anterior part of the otocysts, stops short at the median part which is occupied by a highly glandular epithelium. I will here lay stress upon the fact that the band is quite independent of the otocysts. This

band appears to have been mistaken by BROOKS for a nervous element ('78 Pl. II., Fig. 3, and Pl. III., Fig. 6). BLOCHMANN ('00) is of the same opinion as myself, stating clearly the absence of a nerve ring around the œsophagus (p. 124.).

In connection with the mesenteries I shall describe the primary germ-cells. In the 8 p. e. stage I was able to detect what are supposed to be the germ-cells. They, though few in number (Pl. VII., Fig. 107, *gm. cl.*), form part of the epithelial cells which constitute the ileo-parietal band. These cells greatly exceed their fellows in size : the nucleus is vesicular with a single well difined nucleolus, and the cytoplasm is highly granular. They greatly resemble the germ-cells of young *Lingula* in position as well as in cytological features.

i. **Nephridia.**

BLOCHMANN ('98 and '00) considers the otocysts described by FRITZ MÜLLER ('60) and BROOKS ('78) as the nephridia, but these vesicles, as far as my observations go, have been rightly interpreted by the earlier writers. I may here refer to a pair of string-like organs which I discovered in the larvæ of *Lingula :* structures which I do not hesitate to consider as the *Anlagen* of nephridia. They certainly answer all the conditions of an excretory organ—moreover there are no other organs in the adult which appear to be developed out of them, if we except the nephridia. The reasons given on the following pages, I believe, will be found adequate to justify my determination of the string-like organs. It is indeed remarkable that so conspicuous an organ as the structure in question should have entirely escaped observation by former writers.

The nephridium is very conspicuous in the advanced larvæ (7–15 p. c. stage). But at the 5–6 p. c. stage it is quite invisible in life from without, and its presence is only detected in stained *toto* preparations or in sections. I shall first describe the organ in the 5–6 p. c. stage and then in the 7–9 p. c. stage.

On a ventral view of the larvæ at the 5–6 p. c. stage we see a pair of cell strands (Pl. VI., Fig. 82, *nph.*) whose posterior part forms the ileo-parietal band. In transverse sections of the larvæ of the same stage we see at the level of the *M. occlusores anteriores* a pair of small protuberanes on the lateral body walls near the ventral shell valve (Pl. VII., Figs. 92, 108, *nph.*). From the protuberance backward there is found in the body wall a cell rod, which pushes aside the peritoneal epithelium from the epidermis. The rod is composed of highly glandular cells. Somewhat later a narrow lumen appears in it, its cells coming now to be so arranged as to form a tube, which in a transverse section is seen to consist of two or three cells. Judging from the length attained at the present stage we can infer that the organ had made its appearance at the 4–5 p. c. stage. Lack of material at this stage, however, prevented me from ascertaining the exact origin of the organ.

At the next stage (7–9 p. c. stage) the nephridium becomes so conspicuous that we can easily detect it in fresh specimens. In an examination of *toto* preparations we find that the differention of the nephridial tube into the tube-part and the funnel has already taken place (Pl. VI., Fig. 85, and Pl. VII., Fig. 107). Thus, little by little, the orginal tube becomes converted into a structure which can easily be reduced to the conditions of the adult nephridium. Fig. 109 (Pl. VII.) shows a longitudinal section through the axis of a larval nephridium. We see that in

its anterior two thirds the organ is made up of two kinds of cells; the tube formed of large glandular cells constituting the axis (*ax.*), and smaller cells forming a sheath (*sth.*) around the above mentioned axial tube. The axis is identical with the cell tube found at the preceding stage; the cells are entirely filled with excretion granules and, in life as well as in preserved specimens, they look highly refractile. Nuclei are inconspicuous, having less affinity for stains. The canal in the tube is hardly visible in this section, but in others it appears as a tolerably wide canal (Pl. VII., Fig. 110). The sheath is a layer of mesoblast cells, which are almost cubical or exceedingly rounded toward the body cavity. In surface view accordingly these cells give a warty appearance to the nephridium. The nuclei are comparatively large, spherical, and have a great affinity for stains. It is certain that these mesoblast cells have been derived from the peritoneum, as the nephridium shifted away from the body wall, and that they increased in number by subsequent division.

The nephridium in its posterior one third (Wood-cut p. 58, Pl. VII., Fig. 109, and Pl. VIII., Figs. 133, 134, *fn.*) consists of peculiar mesoblast cells, forming a funnel closed anteriorly but open dorsally at the posterior end. The cells are arranged epithelially and the nuclei stain intensely, as in those of the sheath. Posteriorly these cells become a thin plate which forms a part of the ileo-parietal band (*vide* p. 58.). The cells of the ileo-parietal band and of the tube-part may contribute to the formation of the funnel, but I have no evidence as to its origin. At the end of the 9 p. c. stage the funnel does not yet open anteriorly.

By study of transverse sections the relation just referred to will be more clearly understood. The nephridial tube which

was enclosed in the body wall at the 5–6 p. c. stage, is now constricted off from, but on one side is still apposed to, the body wall (Pl. VII., Fig. 110). We see that the size of the cells forming the sheath varies considerably according to their position. In a section of the funnel a ring of cells with deeply stained nuclei is seen (Pl. VIII., Fig. 134). If traced posteriorly, the funnel will be found to open dorsally to the body cavity; the ventral lip of the funnel is produced, forming part of the ileo-parietal band. The left nephridium is usually closely apposed to the ventral body wall, though no cellular connection with the latter exists, as is proved by the fact that the nephridium is sometimes found widely separated from the body wall. The right nephridium, on the other hand, is separated in its posterior half from the body wall by the intestine. And we find that it passes posteriorly closely apposed to the dorsal surface of the intestine (Pl. VII., Fig. 104, and Pl. VIII., Fig. 134).

During free-swimming larval life the axis in the tube part of the nephridium is, I think, the active organ in excreting the waste material which is finally ejected to the exterior through the central canal. In excretory function the sheath must also play an important role in accumulating and transmitting the waste material to the axial tube. In what manner the sheath secures the waste products is by no means certain, although the following explanation of the process may be advanced:—the sheath-cell can, of course, absorb a considerable quantity of waste products directly from the surrounding cœlomic fluid, which is constantly set in motion by the contraction of muscles, etc., but the cells can also obtain those materials from the blood corpuscles. The latter having collected the waste products in the course of their circulation are drawn to the sheath-cells, and there become

attached to them, yielding up the waste material by a process of osmosis. It is certainly a fact that blood corpuscles are found attached in numbers to the sheath (Pl. VI., Fig. 86).

j. Otocysts.[1]

In the year 1860 FRITZ MÜLLER ('60) found in the larvæ of *Discinisca* structures which he identified as organs of hearing. These he found on both sides of the stomach just beneath the dorsal valve and described them as "zwei ansehnliche Gehörbläschen von 0.04 mm. Durchmesser, in denen man 20–30 Otolithen (von etwa 0.002 mm.) in lebhafter tanzender Bewegung erblickt" (p. 77).

In his next paper ('61) on the *Discinisca* larvæ, he discusses the changes in the soft parts and states: "the previously spherical auditory vesicles were shrunken into longish sacs, closely surrounding otoliths. In somewhat older animals there were no traces of the organ of sense....." (p. 565).

MORSE ('81) made observations on Japanese *Lingula* and discovered the auditory capsules. From the abstract of his paper read before a meeting of the Boston Society of Natural History, I can only learn that "their [otocysts] position and general appearance recall the auditory capsules as figured by CLAPARÈDE in certain tubicolous Annelids" (p. 157). This is the only description hitherto published of the presence of the otocysts in the adult Brachiopods.

In the same year BROOKS ('78) studying *Lingula*-larvæ

1. In the present section I have employed the term "otocyst" from the anatomical similarity to the organ of the same name found in other animals, not from the physiological point of view. The vesicles must, I believe, subserve as an organ of stability as ascertained in other cases by YVES DELAGE, ENGELMANN, VERWORN, BEER, etc.

confirmed MÜLLER's observations. In the larvæ of the close of the 7 p. c. stage, he saw on the dorsal side that nerve fibres turn backwards from the ventral side, and "terminate in a pair of large dilatations, * * each of which contains an elliptical cavity, within which are numerous vibrating otoliths" (p. 63). On the fate of the otocysts he adds: "if the otocysts had been present [in the sedentary larvæ], and no larger than those of the latter figure [10 p. c. stage], they would not have been visible with any available magnifying power. There is no reason, therefore, to suppose that they disappear during development" (p. 64).

Just twenty years later BLOCHMANN ('98) found the auditory vesicles discovered by FRITZ MÜLLER in the larvæ of *Discinisca*, but he considered them nephridial funnels. "Sie liegen dicht unter der Dorsalschale und wenden ihre erweiterte Oeffnung dorsalwärts. Den Anfang ihres Ausführungsgangs sieht man leicht (Fig. 1). An einem Exemplar konnte ich diesen bis zu seiner Mündung an der Vorderwand verfolgen. Er verläuft zwischen den Oc. anterior und dem lateralis. Die 20–30 Körnchen, die FRITZ MÜLLER für otolithen hielt, waren jedenfalls nicht weiter als Exkretionskörnchen, die durch die Wimpern des Trichters in Bewegung gehalten werden. Ich kann auch die von BROOKS für die *Lingula*-larve beschriebenen Otocysten nicht als solche anerkennen....." (p. 422). In his "Untersuchungen über den Bau der Brachiopoden (Zweiter theil)" ('00) he advances the same view as in his former paper (p. 124).

If we examine the detailed structure of these vesicles in the larvæ of *Lingula*, I think, we can safely conclude with FRITZ MÜLLER and BROOKS that they must be regarded as the otocysts. In the dorsal view of living larvæ older than the 5 p. c. stage we can readily see a pair of very conspicuous vesicles (*ot.*) posterior

to the *M. occlusor anterior* (*occ. ant.*) and just dorsal to the gastro-parietal band (*gst. pr. bd.*) and the upper cœcum of the posterior dorsal lobe of the liver (*pst. dr. lv.*) (Pl. V., Fig. 80, Pl. VI., Figs. 81, 83, 84, and Pl. VII., Fig. 111). The otocyst is in some larvæ almost circular, in others slightly compressed longitudinally, and in still others nearly triangular. It measures 45–55μ in diameter on an average. It is completely enclosed by thick but transparent walls, which are thicker at both lateral and median sides than at other points. It contains in its cavity a fluid which appears light red by transmitted light. The tint may be due to a refraction phenomena of light which enters the fluid through the protoplasmic layer of the body. We meet with this light red color in many other cases : for example, in the contents of the contractile vacuoles of Protozoa, or in Noctiluca.

During the 5 p. c. stage the otocysts remain in the state just described ; that is, nothing can be found in them. But at the 6 p. c. stage there are found in the fluid contents of the vesicle a few refractile concretions, otoliths, which are in rapid motion, each particle being a little apart from others (Pl. V., Fig. 80). At the 7–8 p. c. stage the otocyst increases in size and the otoliths (Pl. VII., Fig. 111) become over 40 in number, each granule having also been enlarged. They are now found passively grouped into a central mass which all together is in a constant dancing motion. This is doubtlessly caused by cilia on the inner face of the otocysts, though they were not made out either in living specimens or in preparations.

I shall here describe an anomalous case of the otocyst. In a larva of the 8 p. c. stage which Mr. Hayata picked out of the plankton and kindly placed at my disposal (Pl. VII., Fig. 112) the right otocyst was divided into two chambers by a vertical

partition ; in the left of these compartments about twenty otoliths were present, while in the right there were only three.

By a study of the *toto* preparation of a larva at the 5 p. c. stage (Pl. VI., Fig. 81) we see that the walls of the otocyst are composed of a small number of cubical or slightly flattened cells. If we trace posteriorly a series of transverse sections of a larva of this stage, the anterior edge of the otocysts is found in the angles formed by the lateral and dorsal body walls in the same section in which the nephridia appear, (Pl. VII., Fig. 92, and Fig. 108, which is a portion of the section next to Fig. 92, drawn under a higher magnification). In the latter figure we see that the otocyst is composed of a layer of epithelial cells. As far as the origin of the otocyst is concerned, I think, we can assume that it arises as an invagination of the lateral epidermis of the body wall, and attains its definite position by pushing aside the peritoneum. It is certain that the cells composing it show no difference from the neighboring epidermal cells.

By the 7–9 p. c. stage (Pl. VII., Fig. 94) the otocyst has increased in size. Its walls are now composed of a number of cubical epithelial cells, whose nuclei stain intensely. As the body walls bulge out, the vesicles appear to be shifted toward the median plane, but in reality they remain in their original position, that is in the angles of the body walls, as we can see in transverse sections. The dorsal wall of the otocysts is, as a rule, very thin, and here one is apt to mistake an artefact for a normal opening. I should note that I have not observed otoliths in fixed preparations nor in sections. Of the chemical composition of the otoliths, it must be confessed that little is definitely known.

It has now been shown that the vesicles in question fulfill all the characters of otocysts, and on the other hand we may

conclude that they cannot be nephridia, since they do not possess the most essential features of an excretory organ, such as 1) the duct, 2) an opening (nephrostome), and 3) excretory cells.

The otocyst, it should be noted, persists as such throughout life, showing no sign of degeneration. As to the otocysts in the adult I shall fully describe them in another paper (See Art. 5, this volume).

k. Ganglia.

My results on the nervous system of the larvæ of *Lingula* differ very much from those of Brooks, the chief point being that I have failed to discover the presence of a complete nerve-ring around the œsophagus, containing the ventral and lateral ganglia, as has been maintained by Brooks.

At the 5–6 p. c. stage the epidermis of the anterior and well rounded-out body-wall, ventral to the neck, becomes marked out into a definite area, and here arise three eminences, one central and two lateral. The central eminence becomes the ventral ganglion (Infra-œsophageal ganglion) (Pl. VI., Fig. 82, *vt. gn.*) while the two other smaller eminences, which are situated more dorsally, one on either side of the ventral ganglion, become the lateral ganglia (*lt. gn.*). The relative position of the lateral and ventral ganglia will be best understood by comparing two sections, one transverse (Pl. VII., Fig. 113) and the other longitudinal (Pl. VII., Fig. 94). As the lateral ganglia are formed in direct continuity with the ventral ganglion, they cannot, as Brooks believed, be connected by means of nerve fibres.

At the 7–9 p. c. stage the ventral ganglion becomes more prominent (Pl. VI., Fig. 85; Pl. VII., Figs. 93, 105). It is best

compared to a hollow hemisphere compressed dorso-ventrally. The middle part is thickest, and from this region it thins out gradually in every direction. Its ventral edge is prolonged further posteriorly than the dorsal. The ganglion is not an eminence projecting inward into the body cavity from the anterior body wall as a posteriorly directed thickening, as BROOKS figures in his well known diagramatic sagittal section of a *Lingula* larva (Pl. VI., Fig. 16), but it is a thickening directed and projecting anteriorly. The lateral ganglia at this stage extend for a little distance forward along the neck.

As to the histogenesis. As is seen in a longitudinal section of a larva at the 6 p. c. stage (Pl. VII., Fig. 114), the epithelium, which is to become the ganglion, at first increases in thickness and the nuclei, each with one nucleolus, acquire a vesicular character. The nuclei then travel peripherally, the cytoplasm being drawn out gradually taking the form of nerve fibres. At the 7–9 p. c. stage (Pl. VII., Fig. 105) the fibrous layer increases in thickness and the nuclei come to occupy a thin peripheral layer. The fibres in frontal as well as in transverse sections present the typical granular appearance peculiar to a section of fibres, while in a sagittal section almost the whole course of the fibres is seen. It can thus be determined that a majority of the fibres run dorso-ventrally. In the nuclear layer placed peripheral to the fibrous one in the ganglion a differentiation has already taken place at this stage: the distal nuclei epithelially arranged in one row have become rather compact, acquiring a stronger affinity for nuclear stains; a layer of nuclei (3–4 thick) proximally placed, on the other hand, retains its original vesicular character. The former is composed of the ordinary epithelial cells while the latter of ganglion cells (Pl.

VII., Fig. 105). The nerve fibres must be given off by ganglion cells at first proximally and horizontally, but in what manner they are produced and what course they subsequently take I could not determine, since the preparations of the larvæ made according to the methyl-blue method were unsuccessful. I hope to re-examine these points by other methods, such as silver or gold impregnation, or hæmatoxylin-methods. The posterior thread-like prolongations of the most peripherally situated epithelial cells just mentioned are attached to the supporting lamella already secreted on the posterior face of the ganglion. The prolongation is very fine and several of them come together in their course to the supporting lamella, so as to form a bundle. In a transverse section, therefore, the bundles come to look like islands arranged with some regularity among the sections of nerve fibres. The cells referred to as small ganglionic cells by BLOCHMANN, which are found in the adult scattered in the fibrous layer, have not as yet made their appearance.

Besides the central nervous system above mentioned the *N. obliquorum* (Pl. VI., Fig. 82; Pl. VII., Fig. 115, *n. obl.*) can be seen in larvæ of the 6–9 p. c. stage. It is a very fine cell strand, a few nuclei appearing like the nodes of the fibre. It spans the space between the body wall and the *M. obliqui medii* (*obl. md.*). In some larvæ of the 8 p. c. stage the *nervus pedunculari*s (*n. pd.*) is seen in the ventral view as a pair of white lines (Pl. VII., Fig. 116) converging posteriorly to a point a little to the right of the *M. occlusor posterior* (*occ. pst.*). BROOKS ('78) has described (p. 60) and figured (Pl. III., Fig. 6) the nerves, but he has apparently mistaken them for muscles (retractor muscles).

7. Muscles.

Since the parietal muscle layer has been described in connection with the body walls (p. 47), it will not be necessary to touch here upon this structure, and the muscles found in the arm-apparatus and the peduncle will, for reasons of convenience, be dealt with in later sections. I shall, therefore, in the present confine myself to a description of the shell-muscles.

The muscles of Brachiopods bear different names according to different authors. Among them I shall follow throughout the present paper the terminology of Blochmann ('00). The following table will be found necessary to avoid confusion :—

Blochmann.	Brooks (after Davidson).
lateralis...	... ... *j*.
occlusor anterior	... ... *h*.
occlusor posterior...	... ... *g*.
obliquus internus...	... ... *k*.
obliquus externus...	... ... *l*.
obliquus medius[1]	... ... *i*.

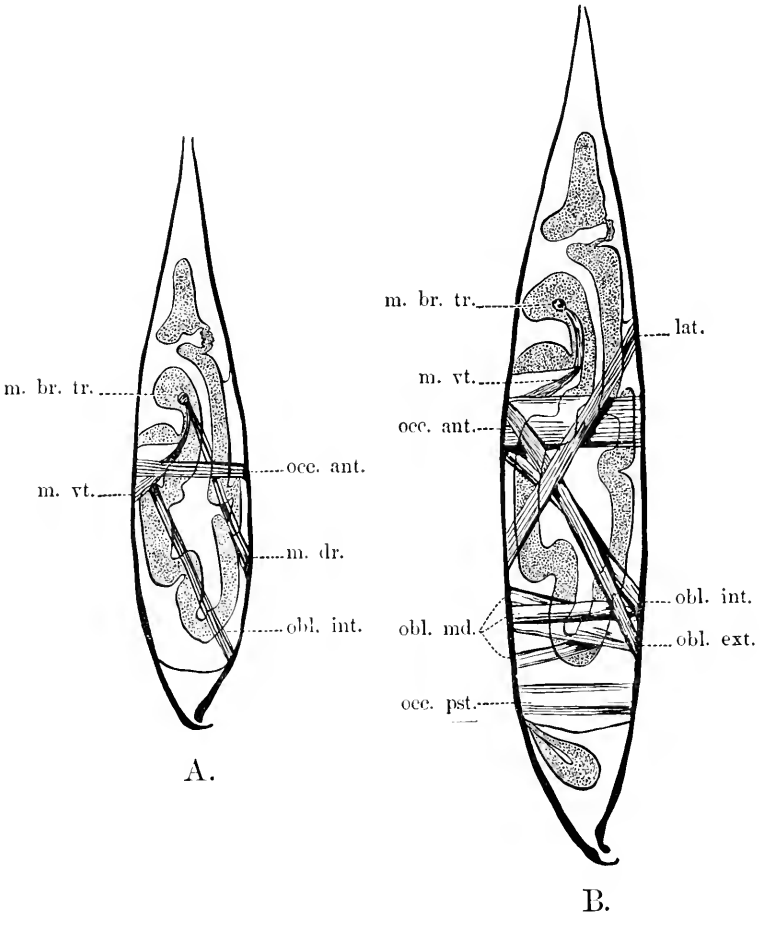
Before entering into the description of the particular muscles I shall describe here the finer structure of a muscle, taking as a representative the *occlusor posterior* in a larva of the 9 p. c. stage (Pl. VII., Fig. 117). The muscle is non-striated and consists of rigid looking fibres. The nuclei which are scattered here and there

1. It is worth noting that Blochmann in his "Untersuchmegan ü. d. Bau d. Brachiopoden 2ter Theil" ('00) describes the *posterior adjuster* of Hancock as the *M. obliquus medius* in the text (p. 107), while throughout his atlas (*e.g.* Tafel XV., 21.) the *M. obl. med.* is marked with the abbrebiation *obl. int.* and *vice versâ*.

among the fibres, are of a long spindle shape, compact, and rich in chromatin which is in the form of fine granules. The peritoneal lining around the muscle (sheath) cannot be seen readily as in the adult, but the presence of it is certain. In all cases I have observed that no cell layer intervenes between the muscle and the outer layer of the body wall. The latter, the ectoblast cells, (*h. c.*) undergo at the insertion of the muscle a peculiar change and become muscle cells. They become elongated dorso-ventrally, and their nuclei are displaced and attached to the cell wall. These cells are named by BLOCHMANN "Haftzellen" ('91). The area covered by the "Haftzellen" is a little larger than the insertion of the muscles. In a surface view of a larva each of these cells is seen as a polygonal disc, its diameter greatly exceeding that of each muscle-fibre bundle. In the *occlusor posterior* some thirty "Haftzellen" are present (Pl. VIII., Fig. 128). Physiologically considered the "Haftzellen" must play an important *rôle*. If the cells should remain unchanged as in the original epithelium, the violent contraction of the neighboring muscle would cause the detachment of the muscle from the shell. The "Haftzellen," therefore, act as the tendon of mesoblastic muscle-fibres, attaching themselves firmly to the entire muscle on the one hand and to the shell on the other.

Now we shall undertake the description of the shell-muscles found in the larvæ of the 5–6 p. c. stage. As the muscles are very fine at this stage it is difficult to detect them in living specimens. They are best studied in *toto* preparations (Pl. VII., Figs. 81, 82) and in sections (*Cf.* the Wood-cuts p. 73). At this stage four pairs of muscles are found, *viz.* the *occlusor anterior* (*occ. ant.*), the *obliquus internus*, (*obl. int.*), the *dorsalis* (*m. dr.*) and the *ventralis* (*m. ct.*). It should be noted that the last two

pairs are the larval muscles, which degenerate in the course of development. The *obliquus externus* (*obl. ext.*) appears at a stage a little older than that of the Wood-cut A (Pl. VI., Fig. 82).



Musculature seen from the left side.
A. 5–6 p. c. stage.
B. 7–9 p. c. stage.

The *occlusores anteriores* (Pl. VI., Figs. 81, 82, Wood-cut A, *occ. ant.*) are composed of a few bundles of muscle fibres, situated at the anterior corners of the body cavity close by the anterior

body wall, and running across the body cavity dorso-ventrally. They are probably formed from the dorsal body wall. In a living larva of the 6 p. c. stage I observed that the *occlusores anteriores* are formed at first as a group of short rods attached to the dorsal body wall.

The *ventrales* have already been met with from the 3 p. c. stage (Pl. III., Fig. 53) onward. These muscles arise on the ventral shell, a little mediad of, and posterior to, the origin of the *occlusores anteriores* (Pl. VI., Figs. 81, 82, Wood-cut A, *m. vt.*). Running forward they are inserted on the antero-ventral side of the œsophagus, where they unite with the transverse arm-muscle (*m. br. tr.*). It should be noted that the insertions of the muscles have greatly shifted ventrally, travelling half around the œsophagus.

The *dorsales* (Pl. VI., Fig. 81, Wood-cut A, *m. dr.*) are the only muscles which have their origin on the dorsal shell. They are originate at the same level as the otocysts, but nearer the median plane. They then proceed forward diverging, passing just mediad of the *occlusores anteriores* until they join the transverse arm-muscles. The above two pairs of muscles, *ventrales* and *dorsales*, subserve the retraction of the arm-apparatus as a whole. These pairs are not present in the adult and are to be regarded as larval muscles.

The *obliqui interni* (Pl. VI., Figs. 81, 82, Wood-cut A, *obl. int.*) show but little difference from those of the stage next to be described. Their origins lie on the ventral shell very near the median plane at the same level as the *occlusores anteriores*. They then run dorsad and posteriorly, diverging as they go backwards, and finally are inserted on the dorsal shell. When the body wall contracts, these muscles often appear as if their posterior

ends projected out of the body wall, but in reality the outline of the latter can readily be seen covering the ends of the muscles (Pl. VI., Figs. 81, 82).

Although the *obliqui externi* (*obl. ex.*) are found in the late 6 p. c. stage (Pl. VI., Fig. 82) they may conveniently be described in connection with the musculature of the next stage.

I shall now take up the description of the musculature of the larvæ at the 7–9 p. c. stage. With minor exceptions my results agree with those of BROOKS. As the arrangement of the muscles to be described agrees essentially with that of the 10–15 p. c. stage, the reader will adequately understand the course of the muscles by reference to the wood-cut B, and to Fig. 128 (Pl. VIII.).

The OCCLUSOR POSTERIOR (*occ. pst.*) (Wood-cut B, Pl. VIII., Fig. 128).

This unpaired muscle is formed at the beginning of the 7 p. c. stage, arising from the posterior body wall (Pl. VII., Fig. 119). Subsequently it is entirely separated from the body wall. In some cases, however, this muscle lies so closely apposed to the body wall that one might mistake it for a part of the latter. At its first appearance it is composed of a few fibres, but as the larvæ grow, the fibres increase both in number and in size. At the 7–9 p. c. stage on surface view it has the shape of an ellipse whose major axis lies in a transverse direction; it is sometimes crescent-shaped with its concavity directed forward (Pl. VI., Fig. 84, Pl. VII., Fig. 116, and Pl. VIII., Fig. 128).

The OCCLUSORES ANTERIORES (*occ. ant.*) (Wood-cut B, Pl. VIII., Fig. 128).

In a ventral view we see the origin of these muscles a little posterior to the ventral ganglion, one on each side of the œsophagus. In a dorsal view we see the insertions of these muscles antero-lateral to the anterior dorsal lobe of the liver, a little anterior to the gastroparietal band and the otocyst (Pl. VI., Fig. 84). In fairly advanced larvæ each of these muscles comes to be made up of two halves, median and lateral (Pl. VIII., Fig. 128) as in the adult. The lateral half is supposed by Brooks to be the ventral end of the *obliquus externus* (*l* in his terminology). But I found that in larvæ very much younger than those he refers to (Fig. 7, Plate IV.=young sedentary *Lingula*), the origin of the *obliqui externi* is distinctly seen a little exterior to the lateral half of the *occlusores anteriores*. It, however, seems probable that a small part of the anterior occlusor gives rise to the *obliquus externus* at the very outset. The *occlusores anteriores* are divided into two segments, the upper and the lower, equal in length, by the supporting lamella, which projects posteriorly from the dorsal edge of the ventral ganglion (Pl. VII., Fig. 94).

The Ventrales (*m. vt.*) (Wood-cut B, Pl. VII., Figs. 93, 94).

These muscles arise from the ventral body wall, on the median side of the *occlusores anteriores*, and are made up of a small number of fibres. Running forward along the œsophagus they finally join the transverse arm-muscle. They degenerate toward the end of this stage.

The Laterales (*lat.*) (Wood-cut B, Pl. VIII., Fig. 128).

The fan-like expanded origins of these muscles are situated a little anterior to the origins of the *obliquus medius* on the

ventral body wall. The muscles run forward converging toward each other, and, at about the middle of their course, are attached to the ventro-lateral body wall at the posterior dorsal edge of the ventral ganglion, where the fibres of these muscles seem to be broken, so that the part anterior to this point can hardly be regarded as the continuation of the part posterior to it. From this interruption, the muscles continue to proceed forward forming an angle with the posterior limb, and tending still more toward the median plane. They finally secure their attachment on the dorsal body wall at the anterior end of the "Erker," (Pl. VII., Fig. 93, Pl. VI., Fig. 87). Brooks states that "[the *laterales*] appear to unite with the dorsal ends of the muscle "h," [*occ. ant.*] as no independent ends of similar muscles were visible in a dorsal view" (p. 58); but at such stages as Brooks' figures 3, 5 and 6, I find that the muscle has the independent dorsal insertion described above.

The Obliqui externi (*obl. ex.*) (Wood-cut B, Pl. VIII., Fig. 128).

These muscles arise from the ventral body wall at the same level as, and on the external side of, the *occlusores anteriores*. They run between the *obliquus internus* and the *laterales* posteriorly and dorsally until they are inserted on the dorsal body wall a little posteriorly and ventrally to the *obliquus internus*. In younger larvæ the muscles unite ventrally with the *occlusor anterior* and it is probable the *obliquus externus* have at the outset been separated from the *occlusor anterior*.

The Obliqui interni (*obl. int.*) (Wood-cut B, Pl. VIII., Fig. 128).

These muscles attach themselves to the ventral body wall very near the median plane a little posterior to the *occlusor anterior* and at the same level as the anterior end of the otocysts. They diverge as they go backward and pass the furrow between the posterior dorsal and ventral lobes of the liver. They finally secure their insertion at the angle made by the lateral and the dorsal body walls.

The Obliqui medii (*obl. med.*) (Wood-cut B, Pl. VIII., Fig. 128).

These are composed of three stout muscles peculiar to *Lingula*. They have their origin on the ventral body wall on either side of, and not far from, the median plane, and from these portions they decussate right and left securing their attachment to the dorsal valve near the insertion of the *obliquus internus*.

I was unable to discover in living specimens or in sections a thickened muscular ridge on the dorsal side of the stomach which Brooks supposes "to connect the stomach with the lining of the dorsal valve" (p. 61).

m. **Peduncle.**

The peduncle makes its appearance at the 6 p. c. stage (Pl. V., Fig. 79, *pd.*) as an elevation of the inner layer of the ventral lobe of the posterior mantle, a little to the right of the median plane of the body. The elevation is elliptical, measuring 20 μ in length and 30 μ in breadth (Pl. VII., Figs. 118, 119). Into this elevation there leads out from the body cavity an evagination which gives rise to the peduncular cavity. At the 7 p. c. stage the peduncle is circular (Pl. VI., Fig. 83). With age it increases

in length, attaining at the 7–9 p. c. stage the form of a twisted sausage (Pl. VI., Fig. 84). As far as the external appearance of this organ is concerned, BROOKS' description leaves little to be desired: so I shall discuss in the following pages only the inner structure. In passing, however, I might add that the peduncle is colorless in early stages but gradually assumes a light green tint (Pl. VI., Fig. 84). In all the larvæ of this stage examined, the peduncle (Pl. VI., Fig. 84) has its attachment a little to the right of the *occlusor posterior*; thence it is bent forward to the right, then it turns dorsally, is twisted again to the left, takes an almost horizontal course and finally terminates with a dilated end. This agrees with BROOKS' description and figures: in his figure 11, however, the peduncle is shows as turning ventrally.

As the peduncle is nothing more than an outgrowth of the posterior end of the body cavity, it consists of two layers: the outer ectoblast and the inner peritoneum. As is seen in sections (Pl. VII., Figs. 93, 94) the outer layer is an epithelium composed of high columnar cells, whose nuclei are of a long spindle shape and have a strong affinity for stains. At the base of the peduncle this epithelium passes over to, and forms, the inner lamella of the posterior mantle. The inner peritoneal layer, on the contrary, is made up of loosely placed cells, with attenuated bases and enlarged tips, in the latter of which a round compact nucleus is present. In some places, however, the layer becomes somewhat epithelium-like. This layer produces externally a layer of longitudinal (slightly oblique) muscle, which at this stage is only one fibre thick. The muscle layer must be considered as of the same category as the parietal muscles. BROOKS has given an entirely different interpretation: "between this [outer layer of of the peduncle] and the ciliated epithelium is a

circular layer of muscle continuous with the muscle *r* [*occ. post.*]" (p. 65–66). One might naturally come to this opinion if surface preparations alone were studied. Moreover BROOKS ascribed the wrinkled appearance of the peduncle to "the contraction of the circular muscles of its wall at somewhat regular intervals" (p. 66). On examining sections we can soon find that such circular muscles are not present at all. Within the inner layer of the peduncle there is a narrow canal which can be shown to be a prolongation of the body cavity. It communicates with the latter through an elliptical opening compressed dorso-ventrally, a little to the right of the *occlusor posterior.*

It will be seen from the above description that the peduncle of *Lingula* is quite different from that of the Testicardines in its structure as well as in its mode of development.

n. Arm-apparatus.

I shall employ the term, arm-apparatus (BLOCHMANN), to indicate collectively the lophophore and the tentacles of BROOKS. The entire apparatus is of a very light yellow or brownish color (Pl. V., Figs. 77–79, and Pl. VI., Figs. 83, 84). In younger larvæ the tips of the tentacle and the cirri are colored yellowish-brown. The arrangement of the cirri when the larvæ are at rest is to a certain extent definite, as BROOKS has stated : the ventral three or four pairs of cirri form the ventral group, while the rest constitute the dorsal. When the arm-apparatus is fully extended it takes the form of a funnel compressed dorso-ventrally, as already described in connection with the habits of the free-swimming larvæ (Pl. VI., Fig. 88, *a*, *b*).

I shall now refer first to the tentacle and next to the cirri.

The tentacle (Pl. V., Figs. 77–79, and Pl. VI., Figs. 83–85, *tnt.*), which is wanting in the larva of *Cistella*, appears to represent one of the larval sense organs. It is usually shorter but broader than a full grown cirrus and terminates bluntly. It contains a central lumen (tentacular canal) (Pl. VII., Fig. 120, *tnt. cn.*) which is loosely lined with epithelial cells. In some of these cells the longitudinal muscle fibres can be detected. The muscle I shall call the tentacular muscle (*m. tnt.*). It is through the contractility of these muscle fibres that the shortening and bending of the tentacle is effected. The tentacle is often bent elbow-like at a place midway of its length (Pl. V., Figs. 75, 77). The wall of the tentacle (Pl. VII., Fig. 121), as seen in a transverse section, is composed of a thick epithelium, whose nuclei are on the ventral side (*v*) similar to those of the cirri in shape, size and affinity for stains, being, however, not so closely grouped as those of the cirri. On the dorsal side (*d*), on the contrary, there are arranged near the periphery one, or at the most a few layers of vesicular nuclei, while in the more central part the section presents a granular appearance corresponding to the sections of nerve fibres. The above vesicular nuclei are certainly those of sensory cells, while the compact nuclei situated on the ventral side must be those of the ordinary ciliated epithelial cells. Dorsal to the tentacular canal the supporting substance (Pl. VII., Fig. 121, *sp. l.*) forms for a short distance a vertical plate which at the outer end bifurcates into a pair of diverging septa terminating among the epithelial cells.

The epistome (Armfalte) (Pl. VI., Fig. 85, *epst.*) which made its first appearance at the 3 p. c. stage, has now become a conspicuous shelf-like ridge, overhanging in front of the mouth. It is a fold of the epithelial layer whose component cells do

not show any difference from those of the cirri (Pl. VII., Figs. 91, 93).

The cirri are constantly budded out on each side of the base of the tentacle, or more strictly speaking, on the ventro-lateral sides of the latter. In the larvæ now under consideration only nine pairs are formed,—an insignificant number compared with the several thousands that are found in the adult. At its first appearance a cirrus is like a little hillock, which elongates with age, until it attains a length of about 30 μ. In the position of rest it is bent upon itself and the more ventrally a pair is situated, the nearer the point of bending comes to the base of the cirri. Structurally each cirrus is a hollow tube, its diameter being in most cases less than that of the tentacle (Pl. VI., Fig. 84), and its walls being formed of a thick epithelium, whose nuclei are arranged compactly and take an intense stain. The latter are most numerous on the inbent side of the cirrus, while on the outer curved side they occur sparingly (Pl. VII., Fig. 121). In life the cirrus is covered uniformly with long cilia, those at its tip being the longest. The lumen in the cirrus (cirrial canal) (*cr. cn.*) runs nearer the inner side than the outer, and stops short at the tip, leaving there for a short distance a solid part (Pl. V., Figs. 77–79). The cirrial canal is lined with a thin epithelium as in the tentacular canal (Pl. VII., Fig. 121). On the inner side of the cirrus, between the outer epithelium and the inner lining, a layer of muscle fibres, which run along the cirrus to the arm-sinus, constitutes the cirrial muscles (*m. cr.*). Sometimes a septum is seen in the cirrial canal, spanning the space between the inner or muscular, and the outer or non-muscular side (Pl. VII., Fig. 121). In the canal blood corpuscles are found.

Between the epistome and the mouth there occurs a recess

(Pl. V., Fig. 78, and Pl. VII., Fig. 91, *rc.*) which represents the thinnest part of the arm-apparatus, and bulges out dorsally when the animal contracts. It disappears when the arm-apparatus is fully extended, helping to give a great length to the latter. This recess is gradually reduced as the larvæ grow, and in those of the next stage this structure disappears altogether, leaving no trace.

Ventral to the mouth there is an area which forms the ventral part of the arm-apparatus. In this portion the cells are of the same nature as those of the œsophageal epithelium : the cell-layer has become greatly reduced in thickness at the 7–9 p. c. stage, as compared with the 3–6 p. c. stage (Pl. VII., Figs. 91 93, ⁎).

In the region of the arm-apparatus where the dorsal group of the cirri are planted, there occur a pair of well marked ridges projecting dorsally and recurving a little towards the median plane. Between this and the œsophagus which (*œs.*) forms a median longitudinal out-swelling of the body wall (Pl. VII., Fig. 95) there occur a pair of tolerably deep grooves (Pl. VII., Fig. 95, ⁎ ⁎).

The cavity in the arm-apparatus (arm-sinus) is directly continuous with the body cavity at this stage, the blood corpuscles freely flowing from one to the other. The communication takes place by means of a pair of canals situated along the ventral side of the œsophagus (Pl. VII., Fig. 95, † †). The arm-sinus in its turn gives off the tentacular and cirrial canals. The sinus is partly filled with trabecular mesenchymatous cells and with complicated musculature. As the larvæ advance, these trabecular cells turn into the epithelial lining of the arm-sinus. Between this lining and the outer epithelium there comes to be found a strong layer

of supporting substance, its thickness varying greatly in different parts. The great arm-sinus of the adult has not as yet appeared even in the larva of the 9 p. c. stage. The arm-sinus of this stage is, however, certainly not the small arm-sinus as stated by BLOCHMANN ('98) in the larvæ of *Discinisca* (p. 422): it is potentially rather the small arm-sinus *plus* the great arm-sinus, because the latter does not appear *de novo*, but is later divided off from the early sinus by the growth of a septum.

In the arm-apparatus there are two kinds of muscles: the one being the longitudinal arm-muscles, and the other the transverse arm-muscles. The former (Pl. VII., Fig. 95, *m. br. lg.*) is composed of the cirrial muscles and the tentacular muscles. The cirrial muscles of the dorsal group of cirri joined by the tentacular muscles run ventrally and posteriorly like the ribs of a fan. The cirrial muscles of the ventral group of cirri, on the other hand, are each divided into two slips, one of which comes to unite with a similar slip from the cirrus adjacent on one side, and the other with that on the other side. The united slips of all the cirrial muscles of the ventral group of the cirri come together with the muscles from the dorsal group of cirri, and run posteriorly along the ventral wall of the anterior prolongation of the body cavity († †), forming the anterior part of the *m. ventralis* (Pl. VII., Fig. 93, *m. vt.*). The transverse arm-muscles lie transversely along the ventral margin of the arm-apparatus, being attached at their extremities to the cirrial muscles of the lateral and posteriormost pair of cirri (Pl. VI., Fig. 81, *m. br. tr.*).

XI. STAGE OF 10-15 PAIRS OF CIRRI. (SEDENTARY LARVÆ).

In the foregoing chapter we have discussed in some detail the anatomy and histology of the free-swimming larvæ (the 5–9 p. c. stage). We will next describe the developmental changes which take place in the sedentary larvæ (the 10–15 p. c. stage).

On keeping the larvæ of the 7–9 p. c. stage in a vessel for several days we find that they cease to swim about and attach themselves to the bottom of the vessel by the tip of the protruded peduncle. The larvæ assume a vertical position and move their valves laterally against each other with a somewhat gliding motion as in the adult. The attachment of the peduncle is now so firm that the larvæ cannot be displaced by even a tolerably severe jerk. From this stage onward, therefore, the water of the vessel may be changed without danger of losing specimens. The protrusion of the peduncle takes place in captivity at the *beginning of the 10 p. c. stage*, and immediately thereafter the larvæ secure their attachment, but at what stage the peduncle is extended from the shell under natural conditions, I was not able to determine. Acceleration or retardation may easily occur in confinement. According to my observations, it took about one month and a half from the outset of development for the larvæ to attain the 15 p. c. stage.

Shell.—The shell increases in length and thickness in the same manner as in the preceding stages. At the 15 p. c. stage it assumes the shape of an ellipse, the ratio of the length to the hinge line being 3 : 1 (Pl. VI., Figs. 86, 87). A larva drawn in Fig. 86 (Pl. VI.) measures 800 μ in length and 636 μ

in breadth. The margin of the anterior half of the shell (Pl. VI., Fig. 86, and Pl. VIII., Fig. 131) is dentated or sinuated, owing to the uneven growth of the inner layer of the shell. Morse found in the transparent plate of *Glottidia pyramidata*, a young shell with a margin finely notched ('73, p. 260).

The periostracum comes to view as a more distinct layer than that in the preceding stage, measuring 1.5 μ in thickness (Pl. VIII., Fig. 130, *pr. ost.*).

As the part of the peduncle lying between the valves is very much flattened, there is no perceptible difference in the space separating the valves at the hinge line, whether the peduncle is protruded or not (Pl. VIII., Fig. 123). In either case the hinge may be serviceable; but in reality it has becomes of less use, because the shells no longer gape at the anterior end as widely as in the free-swimming larvæ, and the arm-apparatus is likewise not protruded out of the mantle cavity.

Mantle and Setæ.—In the mantle the amount of brown pigment which was present in but a small quantity in the preceding stages has increased in a zone slightly proximal to the margin (Pl. VI., Fig. 86, and Pl. VIII., Fig. 131). The large gland cells have also increased in number, forming a distinct layer of a tolerable breadth all around the mantle margin a little inside the pigment zone just described, and constituting the gland ridge (Drüsenwall), which becomes more prominent in the adult. This zone is best seen in stained *toto*-preparations (Pl. VI., Fig. 87, *gl. cll.*). The mantle muscles become more distinct, running radially through the marginal lacuna and in life they present a silky appearance. The fibres branch at their entrance into the thickened border of the mantle and terminate as fine fibres (Pl. VIII., Fig. 131, *r. m.*).

At this stage with the change in the mode of life from the free-swimming to the sedentary condition, the setæ undergo a striking change. Not only a rapid increase in length but also an unequal growth takes place among them. The longest setæ measure 250–300 μ in length and 2–3 μ in diameter, and are planted along the anterior margin and two latero-posterior corners of the mantle: in other portions the setæ are of a smaller size (Pl. VI., Figs. 86, 87). In a general way it may be said that the difference in the length of these structures is correlated with the sedentary life in the mud: the circulation of water in the mantle cavity commences, and, at the entrance of the water current, the setæ subserve a function similar to that of our eyelashes. This difference in length of the setæ becomes more pronounced in the adult. On a careful examination we may at once convince ourselves that the seta shows longitudinal striation and regular segmentations, each segment measuring 8–10 μ (Pl. VIII., Fig. 132).

Pallial sinus.—The pallial sinuses increase in length, but they present no noticable changes in form. Even at the 15 p. c. stage the posterior branches, (Nebenstämme) have not as yet made their appearance.

Blood corpuscles.—By this stage the blood corpuscles have greatly increased in number (Pl. VI., Fig. 86). The spindle bodies,[1] a curious modification of the ordinary blood corpuscles, are now to be noted for the first time. Though few in number, the spindle bodies occur in both peduncular and body cavities (Pl. VIII., Fig. 129, *sp. b.*). In length they measure 10–12 μ

1. *Vide* N. Yatsu:—Notes on the histology of *Lingula*, etc. Art. 5, This Vol.

and are, therefore, much smaller than in the adult. As far as their origin is concerned, they are produced between the peritoneum and the outer ectoblast of both the dorsal and the ventral walls of the body cavity. As they have a great affinity for erythrosin it is not difficult to detect them in sections. As to the formation of the spindle bodies the reader is referred to my paper on the histology of *Lingula* (*loc. cit.*).

Alimentary canal.—No noticeable change occurs during the stage of sedentary larvæ which I was able to study, except that the constriction of the liver lobes has become a little more marked. The posterior cœca of the ventral lobe of the liver show a greater increase in length posteriorly than in the preceding stages (Pl. VIII., Fig. 128). We can now therefore notice four cœca, two dorsal and two ventral, occurring in the same transverse section. In some larvæ the pit at the bottom of the mid-gut is still visible.

Mesenteries.—The mesenteries show but minor changes from the conditions of the preceding stage. The only one which requires special description is a portion of the ileo-parietal band which is situated at the entrance of the peduncular cavity. This portion of the mesentery elongates to become connected with the intestinal mesentery as is seen in the adult.

Nephridia.—The nephridia are more conspicuous in this than in the 7–9 p. c. stage. They are readily seen as a pair of moderately thick strands filled with refractile excretion granules; mixed with them oil globules are found, being blackened in the osmic preparations (Pl. VI., Figs. 86, 87, and Pl. VIII., Fig.

128, *nph.*). The opening of the nephridium to the exterior is shown in Fig. 135 (Pl. VIII.).

Otocysts.—The otocysts become more conspicuous than before. In *toto*-preparations we see these structures as distinctly closed sacs whose walls are made up of a thin epithelium (Pl. VI., Fig. 87, *ot.*). The otocysts persist throughout life as will be seen in my paper on the adult histology (*loc. cit.*). This observation does not agree with that of FRITZ MÜLLER in the larvæ of *Discinisia* ('61). But this discordance must be due to the generic difference.

Nervous system.—As to the ganglia there occurs no perceptible change except the increase in size. That part of the ventral body wall which is situated between the points of origin of the *occlusores anteriores* and gives the attachments to the *obliqui interni*, forms a recess directed posteriorly. On the proximal wall of this recess there are seen a pair of longitudinal thickenings which, without doubt, constitute the *Nervus pedunculari*s. The above recess is in all probability transformed into the closed sac covered with a thick layer of supporting substance which is found at the insertion of the *obliqui interni* in the adult.

Muscles.—During the 10–15 p. c. stage the shell muscles tend more and more to assume the adult form and undergo no important change except the disappearance of the *M. ventrales*, the *M. dorsales* having vanished prior to the 7 p. c. stage. The *M. ventrales* attained their maximum growth during the preceding stage and now, concurrently with the change in mode of life, become very inconspicuous and eventually disappear. In one

specimen which I was able to observe, the remnants of the muscles were still visible attached to the *occlusor anterior* as shown in Fig. 86 (Pl. VI., *m. vl.*).

Peduncle.—After the peduncle becomes protruded from the posterior mantle cavity it undergoes a noteworthy change. Its epithelium seems to react to the new stimuli and commences to secrete a superficial gelatinous layer (Pl. VI., Fig. 87, and Pl. VIII., Fig. 123). This secretion seems to be at first very viscous and soft, but hardens by degrees. The power of secretion is evidently greatest at the end of the peduncle, where the epithelial cells are of a higher columnar shape and here the gelatinous layer is naturally thickest (Pl. VIII., Fig. 123). In longitudinal sections radiating lines are seen in the gelatinous layer at the tip of the peduncle (Pl. VIII., Fig. 137) as EKMANN ('96) has observed in the adult. The mass between these radiating lines appears to correspond to the product of secretion from one cell of the peduncular epithelium. On this point BLOCHMANN ('00) is of the same view as myself (p. 104). It may be added that these radiating lines are not seen in transverse sections of the adult peduncle. This fact indicates that the mass secreted from each cell is fused to form a ring.

Chemically the gelatinous secretion is quite different from the shell. Treated first with borax carmine and afterwards with picric acid as a counter stain, the shell takes a yellow color, while the secretion stains red as in the case of cytoplasm. This reaction toward stains persists in the adult. KRUCKENBERG ('85) studying the adult *Lingula*, says: "die elastische Hülle des fleischigen Stieles besteht fast ganz aus ihn [chitin]," (p. 413). As to the chemical composition of the shell the above author and

SCHMIEDEBERG ('82) proved it to be chitin. EKMANN also says ('96): "Ihr [Lingula-Stiel] Chitin wird von Kalilauge nur wenig beeinflusst, löst sich aber fast vollstandig in siedender Salzsäure," (p. 233). The gelatinous substance secreted from the peduncle is, therefore, either another kind of chitin or some sort of albuminoid material.

In most parts of the peduncle a distinct canal is found, lined with a cylindrical epithelium, while in some other portions the peritoneal epithelium is somewhat mesenchymatous, the lumen being almost obliterated (Pl. VIII., Figs. 136, *a*, *b*, 137).

Arm-apparatus.—Striking changes take place as well in the external appearance as in the internal structure of the arm-apparatus. When free-swimming life comes to an end the arm-apparatus loses its locomotive function and acquires a new one, for it then becomes an organ for causing the circulation of water in the mantle cavity. Correlated with this change the neck region and the longitudinal axis of the arm-apparatus become relatively much shortened, and the two lateral extremities of the latter are bent dorsally, foreshadowing the arm of the adult.

The tentacle becomes greatly reduced as in the case of *Discinisca* (FRITZ MÜLLER) and of *Glottidia* (BROOKS). It remains for a time as a prominence on the top of the epistome (Pl. VI., Fig. 86), but it gradually diminishes in size until at the 15 p. c. stage no trace of it can be seen even in longitudinal sections (Pl. VIII., Fig. 122). The tentacle, therefore, is only a larval organ.

As to some changes affecting the cirri. In the preceding stages the only portion in which new cirri arose was at the base of the tentacle. At the 10–15 p. c. stage, however, a new pair

of cirri appeared on the most ventral part of the arm-apparatus, that is, ventral to the first pair of cirri (Pl. VI., Fig. 86,[1] and Pl. VIII., Figs. 122, 123, 128, *cr*^{n}.). Since this was the last pair of cirri that appeared in the larvæ which I was able to rear, it is quite impossible to decide whether the cirri which appear afterward continue to arise in the ventro-median portion of the arm-apparatus. As the result of the dorsal reflexion of both extremities of the arm-apparatus the cirri situated at the tip the of future arm begin to curve outward in such a way that their convex sides are turned toward the median plane (Pl. VI., Figs. 86, 87, and Pl. VIII., Fig. 128). This is the *Schizolophus* stage of the arm-apparatus of BEECHER ('97).

The epistome (Armfalte) has been referred to at the preceding stage as a shelf-like thickening occurring near the ventral basal part of the tentacle. At the present stage (10–15 p. c.) it attains an enormous size, eventually becoming the lip along the arm. In the oldest larva I have been able to examine, this region had assumed the shape of a depressed pentagon, overhanging in front of the mouth : it here reached almost the ventral region of the arm-apparatus (Pl. VI., Fig. 86, and Pl. VIII., Fig. 128, *epst.*).

To turn to the internal structure of the arm-apparatus. In earlier stages there appeared an arm-sinus communicating directly with the body cavity. It was lacunar in character partly filled with muscles and mesenchymatous cells. In the sedentary larvæ a strong septum now appears and shortly divides the arm-sinus into two : of the cavities thus formed one elongates and becomes a canal, while the other undergoes but little change. The former is still widely open into the body cavity ; it will in far advanced larvæ be cut off from the body cavity and known as the great

1 By mistake marked *cr*^{II}. in Fig. 86.

arm-sinus (*gr. am. sn.*). In a surface view the sinuses can be seen to pass anteriorly along the ventro-lateral side of the œsophagus; they then diverge from each other and terminate blindly (Pl. VI., Fig. 86, and Pl. VIII., Fig. 128, *gr. am. sn.*). The remaining cavity, small in comparison with the great arm-sinus is entirely separated from the body cavity and gives off the cirrial canal. It is referred to as the small arm-sinus (*sm. am. sn.*). It should be mentioned that this sinus later comes into communication with the body cavity by means of a pair of canals, though this did not take place during the stages I was able to examine. The walls of the sinus at the 10–15 p. c. stage consist of stout supporting substance with a lining of thin epithelium; the latter has certainly been formed by the transformation of the mesenchymatous cells found in the arm-sinus in the preceding stages. The muscles in the arm-sinus which attained their elaborate development in the free-swimming larvæ, undergo regressive changes in the course of the 10–15 p. c. stage, and become the insignificant brachial (*m. br.*) and cirrial muscles, which subserve the function of protruding the arm slightly out of the shell. The retrogressive changes affecting the arm-apparatus must clearly be due to functional changes at the beginning of the sedentary life. The foregoing relations of the sinus and muscles will be more clearly understood by reference to Figs. 124–125, Fig. 123, and Fig. 138 (Pl. VIII.).

The lacunar system found in the adult around the œsophagus (Periœsophagealkammer) and in the epistome (Faltensinus) do not as yet make their appearance.

Nerve tissue (*n.*) is now seen as the thickening of the epithelium which covers the ventral wall of the œsophagus; anteriorly it appears in a more lateral, and still more anteriorly

in a dorso-lateral position, along the dorsal furrow formed by the reflected part of the arm-apparatus and the œsophagus.

Differences between the larvæ of the 15 p. c. stage and the young *Lingula* (4.5–9 mm. in shell length).—In order to complete my present studies I made sections of several young specimens of *Lingula* (4.5–9 mm. in shell length); one of the median sagittal sections is diagrammatically represented in the accompanying cut. A glance at the cut shows on one hand that the young differs but little from the adult, and on the other that it has undergone not a little change since the oldest stage (15 p. c. stage) I was able to rear from the free swimming larvæ. I shall, therefore, briefly enumerate the chief changes that have taken place during the intervening stages:—In the young *Lingula* (4.5–9 mm.).

1). The shell has come to be composed of two or three layers of alternate cuticular and calcareous parts.

2). The pallial sinuses have greatly developed in the mantle. The setæ have increased both in number and in size.

3). In the lateral body walls the parietal muscle layers have greatly thickened. Both on the ventral and dorsal body walls we can see the specialized portions from which the spindle bodies are formed.

4). The blood corpuscle and spindle bodies have increased in number. The leucocytes have made their appearance.

5). The mid-gut and the intestine have elongated exceedingly; the latter turns dorsally and after making a loop terminates in the anus. The liver lobes have been constricted and drawn out into the hepatic ducts, the stomach being formed.

6). The gastroparietal band has increased in breadth, and strong muscle fibres have been laid. The ileo-parietal band has

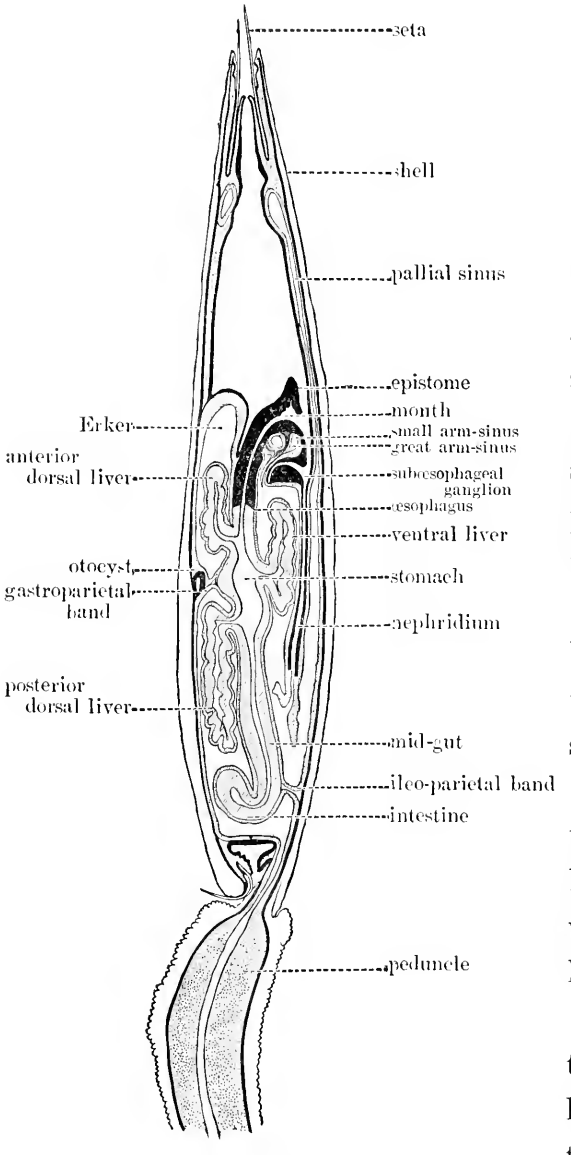


Diagram of median sagittal section through young *Lingula* (9 mm.). Organs not median are represented. ×12·5.

exceedingly elongated accompanying the elongation of the alimentary canal.

7). The axis cells of the nephridium have increased in thickness and are filled up with secretion granules; the funnel has increased in size and been separated partly from the ileo-parietal band.

8). The otocyst has become enclosed in the thick layer of supporting substance.

9). In the peduncle the muscle layer has become extremely thick while the epithelial layers have thinned out.

10). Both extremities of the arm-apparatus have begun to coil owing to the rapid growth of those portions; the cirri have increased in size and in number. The small arm-sinus has made its way to the body cavity by means of a pair of fine canals, while the great

arm-sinus has lost its communication with the body cavity; the latter has given off into the arm-apparatus a pair of finger-like processes; the lacunar system around the œsophagus and in the epistome has made its appearance.

XII. CONCLUSION.

a. **Observations of Previous writers.**

To show how far our knowledge of the development of Brachiopoda has advanced up to the present day, it will be, I think, not superfluous to devote the following few pages to a brief history of the embryology of Brachiopoda. I then shall enter upon a comparison of the developmental processes of our *Lingula* with other forms.

The first investigator to describe the "embryos" of *Lingula anatina* Brug. was Richard Owen ('35) ('53, p. 387), but judging from his figures it is very obvious that what were taken for embryos of the animal by Owen must have been blood corpuscles.

The next author is Oscar Schmidt ('54), who gave an account of an embryo of the Norwegian *Terebratula*. It is interesting that in this paper there is given for the first time a description of a true Brachiopod-embryo.

Shortly after this Gratiolet ('60), in a somewhat extended memoir on the Brachiopod appears to have mistaken the problematical elements of the coelomic fluid known as "Spindle-bodies" for young *Lingula* developing in the body cavity as the result of self-fertilization.

In the same year FRITZ MÜLLER ('60) reported from Desterro, Brazil, his excellent observations on the larval form of *Discinisca* —a contribution valuable not merely as the first description of free-swimming larvæ of the Brachiopoda, but also as a mine of information so minute and precise that even to-day a zoologist can find in it but little to correct. The latter fact is the more remarkable since at that day but few naturalists, finding so curious a larval form in so remote a region as Desterro, could have determined even the group to which it belonged.

Next in the fall of 1859, or in the spring of 1860, MCCRADY ('60) found a larval Brachiopod off Sullivan's Island in Charleston Harbor. This larva is said to have been that of *Glottidia*.

At about the same time or probably a little earlier than MCCRADY'S discovery, CARL SEMPER ('61) states in his "Reisebericht" written on the 30th of November 1859, from Zamboanga in Mindanao, that "ein einziges Mal habe ich eine junge *Lingula* getroffen.....der Stiel fehlt noch," (pp. 103–104).

In the same year FRITZ MÜLLER'S second paper ('61) appeared, in which he fully described the habits of the *Discinisca*-larvæ.

LACAZE-DUTHIERS ('61, *a*, '61, *b*) studied several stages of the embryos of *Lacazella* (*Thecidium*) *mediterranium* RISSO. He was the first to observe the developmental changes in embryos.

E. S. MORSE ('73*c*[1]) dredged young *Terebratulina septentrionalis* COUTH., from the harbor of Eastport, Maine, and studied carefully the structure and developmental changes of the arm-apparatus and of the shell.

The next year MORSE ('70) examined young *Discina* and pointed out resemblances between this form and *Terebratulina*.

Another study by MORSE on the embryology of *Terebratulina*

1. Read in '69.

septentrionalis COUTH. ('73 *a*) is most important, as it is based on a fairly complete series of developmental stages.

In the same year KOWALEVSKY ('73, '83) published his epoch-making work to which we owe our most precise and valuable information regarding the early history of Brachiopod development. His well known observations cover the embryonic development of four genera; *viz.* *Cistella* (*Argiope*), *Lacazella* (*Thecidium*), *Terebratula* and *Terebratulina*.

Five years later BROOKS ('78) studied the free-swimming larvæ of *Glottidia pyramidata* STIMP. His material was taken in the vicinity of Fort Wool in the Chesapeake Bay during the summer of 1877. As already stated this is the only work on the embryology of *Lingula*.

VAN BEMMELEN ('83) in his anatomy of the Testicardines, expresses the opinion that internal fertilization cannot occur among Brachiopoda and that the egg shows no trace of yolk, being holoblastic and the cleavage being regular.

SHIPLEY ('83) obtained various developmental stages of *Cistella neapolitana* SCACCHI in Naples, and studied them in connection with the adult anatomy. In the main his results verify those of KOWALEVSKY.

SCHULGIN ('84) confirmed KOWALEVSKY's observations in *Cistelia neapolitana* SCACCHI (=*Argiope kowalevskii* SCHULGIN) on the formation of the mesoblast (p. 124). Further he remarks (p. 138) that the seeming segmentation in the larva of *Cistella* is not a true one, but is due to mere folds of the skin.

JOUBIN ('86) gives a brief account of the embryonic *Discina* in two places in his paper. He found an egg (p. 264) of the 2-cell stage between the cirri. The cleavage was unequal. At another time he saw a small body in the oviduct, which he with some doubt referred to as an embryo. It is in my opinion very

doubtful whether it was really so. If it was an embryo, it was at the 4-cell stage (measuring $220\mu \times 120\mu$).

JOUBIN ('87) remarks that he has repeated and confirmed MORSE's observations on *Terebratulina* at Roscoff, and KOWALEVSKY's on *Cistella* at Banyuls-sur-Mer, but he gives no further account of his studies.

BEECHER ('91) introduced the term "*Protegulum*" for the common form of shells at embryonic stages (p. 344).

The same author ('93) studied three specimens of *Terebratalia obsoleta* DALL in dry condition (0.3 mm., 0.65 mm. and 1 mm. in length).

EKMANN ('96) while studying the sections of an adult *Cistella* observed a larva with three regions in each of the oviducts.

SIMROTH ('97) described four free-swimming larvæ, of which two were collected during the Plankton Expedition, and the other two through VON SCHAB. The larvæ referred to as "No. 1" and "No. 2," are supposed to belong to *Discina* or an allied form. "No. 3" taken from the west coast of Africa, was supposed to be a *Lingula* larva by BLOCHMANN and SIMROTH. "No. 4" is identified by the former as belonging to *Crania* ('98, p. 426).

BLOCHMANN ('98) investigated the anatomical structure of the larvæ of *Discinisca* collected at Rhio, the capital of the island Bintang, south of Singapore. He studied them from sections as well as in surface view, and added many details to the early observations of FRITZ MÜLLER.

From the above references[1] a table may be compiled giving in systematic arrangement a list of Brachiopods, whose young forms or early embryonic stages have been studied.

1. Many papers dealing with the phylogenetic and ontogenetic development of the hard parts of the Brachiopoda have been omitted.

Forms studied.	Embryonic.	Larval.
Testicardines.		
Cistella neapolitana Scacchi	Kowalevsky (**'73**) Shipley (**'83**) [Schulgin (**'84**)][1] [Joubin (**'87**)] [Ekmann (**'96**)]	Kowalevsky (**'73**) Shipley (**'83**)
Lacazella mediterranea (Risso)	Lacaze-Duthiers (**'61**) Kowalevsky (**'73**)	
Terebratula sp.? „ *minor* Philip.	[Schmidt (**'54**)] Kowalevsky (**'73**)	
Terebratulina septentrionalis Couth. „ *caput-serpentis L.*	Morse (**'73***a*) Kowalevsky (**'73**) [Joubin (**'87**)]	Morse (**'73***a*, **'73***c*)
Terebratalia obsoleta Dall.		Beecher (**'93**)
Ecardines.		
? *Crania*		[Simroth (**'97**)]
Discinisca atlantica King. „ ? „ ?	[Joubin (**'86**)]	Müller (**'60**, **'61**) Morse (**'70**)
? *Discinisca atlantica* King.		[Simroth (**'97**)] Blochmann (**'98**)
Glottidia pyramidata Stp. *Lingula anatina* Brug. *Glottidia pyramidata* Stp. ?		[McCrady (**'60**)] [Semper (**'61**)] Brooks (**'78**) [Simroth (**'97**)]

1. Mere fragmental notes on the development are put in brackets.

b. Comparison of the Results.

As we can see from the above table the early developmental history of Brachiopoda has been studied only by LACAZE-DUTHIERS, KOWALEVSKY, MORSE and SHIPLEY. The researches of these authors, moreover, dealt with but little else than external changes; in fact KOWALEVSKY and SHIPLEY appear to have been the only investigators who have attacked the more intimate problems. And it must accordingly be evident that our knowledge of the embryology of Brachiopoda is far from being ripe enough to enable us to draw general conclusions either upon the scheme of development or upon the affinities of this class. Attempts to build genealogical trees out of the materials at hand have of course been made, but the results are admitted to be wholly tentative. As far, therefore, as the present studies are concerned, I think, it will be best to confine my summary to a comparison of the leading features of the development of *Lingula* with those of other Brachiopods.

Reviewing the early developmental characters of *Lingula* we can justly conclude that they do not agree with those of any Brachiopod hitherto studied. On the other hand, I would call attention in this connection, to the striking resemblance in the mode of cleavage up to the 32-cell stage of *Lingula* to some species of Phylactolæmata.

The next stage in the development of *Lingula*, that is, the formation of a cœloblastula certainly resembles the conditions in other Brachiopods. A comparison of the gastrula, however, cannot be made, since this stage has been observed in but one other form, *Cistella*. I think we can state with certainty that in

Lingula the archenteron is in many cases obliterated, while in other cases it persists as a wide cavity. This difference is of special interest on account of the fact that the developmental mode of *Lingula* is intermediate between that of *Cistella* and of *Lacazella*. The difference cannot be considered as one of kind but only of degree owing to the rapidity of cell increase at various parts.

The closure of the blastopore occurs both in *Lingula* and *Cistella*.

In *Lingula* the mesoblast is formed as a pair of cell masses proliferated from the lateral walls of the archenteron and the body cavity by a splitting of the same cell masses. *Lingula, therefore, is a deuterocœlier* (ZIEGLER), *whose body cavity is formed after the mode of schizocœl*[1] (HUXLEY, ROULE). The formation of the mesoblastic cell masses of *Lingula* closely resembles that of *Lacazella;* in the latter type, however, it was not observed that the mesoblast was proliferated from a thickening of invaginated entoblast walls. KOWALEVSKY ('83) states merely: "bientôt des cellules provenant probablement des premières remplissent cette cavité," (p. 69). From this statement some authors conclude that the entoblast and mesoblast in *Lacazella* are formed by the process of delamination. It is more probable that the process is a polar in-growth (Einwücherung). At any rate the result of the mesoblast formation, expressed by KOWALEVSKY in the sentence: "l'interieur se partage alors en trois lobes" has not a little similarity to that of *Lingula*.

The formation of the body cavity after the enterocœlic type

1. The term "schizocoel" has been employed in two quite different meanings. HUXLEY ('75) means "a perivisceral cavity formed by a splitting of the mesoblast" as in Annelids, while the HERTWIGS ('81) use the term as a synonym of "pseudocœl" (p. 13). (*Cf* ZIEGLER '98 p. 27 and p. 34).

was clearly observed by KOWALEVSKY in *Cistella*. The authors who insist from anatomical grounds that there is a close relationship between the Brachiopoda, Polyzoa and *Phoronis*, or who acknowledge that the Brachiopoda form part of the "Trochozoaires" from the similarity in structure of their larvæ, have always been perplexed by the enterocœlic type of the mesoblas, formation as stated by KOWALEVSKY. From this single instance of *Cistella* the HERTWIGS ('81) maintain that the Brachiopods have the nearest affinity to the Chœtognaths and their view has been supported with several facts by VAN BEMMELEN ('83). R. HERTWIG is still of this opinion (*Cf.* '00 p. 298). ROULE ('94) indeed goes so far as to doubt the observations of KOWALEVSKYT citing his similar observations in *Phoronis* to show how apt we are to fall into mis-interpretation when we study "ses transformations si passagères" only from surface views. But it is, I think, better not to doubt the facts witnessed by KOWALEVSKY until we have more exact observations of this point in the same animal. At any rate, according to my opinion there is as little difference between the schizocœl and enterocœl types of the formation of the body cavity (deuterocœl—ZIEGLER), as between gastrulation and polar ingrowth (Einwücherung). It is therefore nothing to be wondered at if these two modes of body-cavity formation prevail in the nearly allied forms.

In *Lingula* the mouth is formed at the place where the blastopore was closed, as in *Cistella* and *Lacazella*. While in *Lingula* the œsophagus and the stomach come into direct contact with both the ventral and dorsal ectoblast; in *Cistella*, according to SHIPLEY, they touch only the dorsal wall.

In all Brachipod-forms whose embroys have been studied the larvæ are divided by constrictions into three regions or "segments,"

which have been called the cephalic, thoracic and peduncular. In the larvæ of *Lingula* this division does not occur, but, if we attempt to divide them in this way, we may say the larva is made up of two "segments," the arm-apparatus representing the cephalic "segment" and the remaining part constituting the thoracic. The peduncle of the Ecardines cannot be homologized with that of the Testicardines from the morphological nor from the embryological point of view.

The upward reflexion of the mantle which at first covers the peduncular region in the larvæ of *Cistella* is not seen at all in the course of development in *Lingula*. In the latter the mantle grows anteriorly *ab origine* as in *Terebratulina* (Morse).

In *Lingula* the cirri are formed at an early stage and later on become the active organs of locomotion, while in *Cistella* the head region takes the form of an umbrella and serves temporarily (*i.e.* for a few hours) as a swimming organ. In *Discinisca* and *Lingula*, on the other hand, there is an evident adaptation to a long free-swimming life. The cirri appear in *Lingula* as hillocks in a position which corresponds to the cephalic region, while in *Cistella* they are formed at the submarginal part of the mantle, the tentacle being absent in that genus. We may, therefore, distinguish in the formation of cirri two methods: in one they are formed on the mantle (*Cistella*), and in the other on the cephalic region (*Terebratulina*, *Lingula*). As far as this point and some external changes are concerned there is a striking resemblance between *Lingula* and *Terebratulina*. Comparing, moreover, Morse's figures 46–77, 90–91 ('73 *a*) with my own (Pl. III.) we can at once perceive a striking similarity in the formation of the mantle, shell, etc.

In *Terebratulina* development does not deviate at all from

the straight course tending toward the adult form, and consequently no larval organs are produced. In *Lingula*, likewise, notwithstanding the considerable length of the free-swimming life the development is almost direct. The arm-apparatus may be considered as the only departure from the rule; but even this structure persists as such in the adult, only the tentacle and a part of the muscle undergoing retrogressive changes. In *Cistella*, on the contrary, there is a great deviation from the direct line of development and a true metamorphosis occurs. As true larval characters must be considered the umbrella-shaped head and the posteriorly directed mantle lobes.

XIII. A LARVA OF *DISCINA*.

Lying among the larvæ of *Lingula* I found on one occasion a specimen which attracted my attention by its circular shells. A closer examination made it evident that it was nothing else than a larva of *Discina*. This has proved to be the only specimen I have been able to secure up to to-day, and my description of it must, I fear, be inadequate, but it seems best to me to record whatever notes I have at hand, if for no better reason than that no living larva of *Discina* has been observed since 1861, when FRITZ MÜLLER secured his material at Desterro. I give a figure of the larva (Pl. VI., Fig. 89), as a drawing in natural colors has not as yet been published.

Although *Discina* has not yet been collected off Misaki or in the Sagami Bay, it will surely be discovered there by future explorations, judging from the wandering of this larva into the bay in front of the station.

In general appearance (Pl. VI., Fig. 89) the present specimen

resembles FRITZ MÜLLER's figure far more closely than BLOCHMANN's. The dorsal shell, 410μ and 468μ in longitudinal and transverse diameters respectively, is of a pale yellowish brown and is a little darker around the margins. The ventral shell is much smaller than the dorsal and of the same color. The mantle a little proximal to its margin is tinted with somewhat large brownish pigment granules, while the rest of it is almost colorless. The arm-apparatus is yellowish and the body proper is of a similar but lighter color.

The largest setæ, 318μ, are longer than those in the larva figured by BLOCHMANN, and almost equal in length to those from Desterro. These setæ (Pl. VI., Fig. 90 *b*) end in a short hookless portion different from the larvæ examined by BLOCHMANN. The tips of the setæ next to the last pair are also provided with hooks. The anteriormost pair are segmented at the lower third of their length, as shown, but not described in the right posteriormost seta of BLOCHMANN's figure (Taf. XXXI., Fig. 1). Other setæ belonging to the dorsal valve terminate, in an inwardly directed hook. FRITZ MÜLLER describes and figures a pair of eye spots (13μ and 75μ in diameter). BLOCHMANN could not make them out in his preserved material. But the apparent absence of these structures seemed to him of little important in comparing his specimens with those of FRITZ MÜLLER : "da sie voraussichtlich durch den Alkohol verschwunden sind," (p. 424). Though the larva I obtained was at the same stage as those from Desterro, yet I could find no pigment spots at all. Their presence or absence, therefore, probably depends upon the specific difference. The otocysts, it should be noted, are quite circular and situated much more posteriorly than those in FRITZ MÜLLER's figure. They are of the same structure as those found in the

larvæ of *Lingula*. Otoliths were also observed crowded together at the centre of the otocyst. The tentacle is not so cirrus-like as in *Lingula* and ends in a knob (Pl. VI., Fig. 30 *a*). Ventrally and a little posteriorly to the knob a very deep furrow passes posteriorly and stops short near the mouth. From the ventral margin of the knob a protuberance hangs over the furrow.

The position of the specimen in swimming suggests the figure by FRITZ MÜLLER, but the neck region was not so long as in his figure, and the proportion and length of the tentacle and cirri were somewhat different.

Curiously enough all larvæ of *Discina* hitherto studied[1] are exactly at the same stage *i.e.* the 4 p. c. stage. And there evidently must be some reason to account for this. It is possible that in *Discina* the free-swimming larvæ at a particular stage (the 4 p. c. stage), that is at a period shortly before the fixation, make their way toward the coast, as the *Lingula*-larvæ at the 7–9 p. c. stage.

Zoological Laboratory,
Tōkyō Imperial University.

Beginning of
October, 1901.

1. The specimens MORSE has studied ('70) must certainly have been much older than the 4 p. c. stage.

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XV. DESCRIPTION OF PLATES.

All figures are camera drawings with the exception of Pl. VI., Figs. 88, *a* and *b*. Magnification of each figure is noted in its explanation. Some figures are represented as accurately as possible in natural colors. In other cases it may be understood that the colors employed are of merely diagramatic value; thus:—

shell .....yellow
muscle .....red
nephridium .....green
and other parts .....gray.

The following abbreviations are used throughout the plates:

am. ap. .....arm apparatus.
am. rg. .....arm ridge.
am. sn. .....arm sinus.
ant. dr. lv....anterior dorsal lobe of liver.
arch. .....archenteron.
ax. .....axial tube of nephridium.
bd. cv. .....body cavity.
cl. .....cœlomic sac.
cr. .....cirrus.[1]
cr. cn......cirrial canal.
dr. mes......dorsal mesentery.
dr. int. .....dorsal mantle.
dr. sh. .....dorsal shell.
epst. .....epistome.
fn. .....funnel of nephridium.
gl. cll. .....gland cell of mantle.
gm. cll. .....germ cell.
gr. am. sn.....great arm sinus.
gst. pr. bd....gastroparietal band.
il. pr. bd. ...ileo-parietal band.
int. .....intestine.
int. mes......intestinal mesentery.
lat. .....M. lateralis.
lt. gn......lateral ganglion.
m. .....mouth.
m. br......brachial muscle.
m. br. lg. ...longitudinal arm muscle.
m. br. tr. ...transverse arm muscle.
m. cr......cirrial muscle.
m. dr......dorsal muscle.
m. pd......peduncular muscle.

1. The Roman numerals annexed show the order of the formation of the cirrus.

m. pr......parietal muscle.
m. vt......ventral muscle.
mg. l......marginal lacuna.
ms......mesenchyme.
ms. ent. .....mesentoblastic cell mass.
msb. .....mesoblast.
mt......mantle.
mt. fl. .....mantle fold.
nph......nephridium.
obl. ex. .....M. obliquus externus.
obl. int. .....M. obliquus internus.
obl. md......M. obliquus medius.
occ. ant......M. occlusor anterior.
occ. pst. .....M. occlusor posterior.
œs......œsophagus.
ot. ..... otocyst.
pd......peduncle.
pd. cv. .....peduncular cavity.
pll. sn. .....pallial sinus.
pr. ly......peritoneal layer.
prt......protegulum.
pst. dr. lv....posterior dorsal lobe of liver.
sg. cv......segmentation cavity.
sh. .....shell.
sm. am. sn....small arm sinus.
sp. l. .....layer of supporting substance.
st. .....seta.
stm......stomach.
stmd. .....stomodeal invagination.
tnt......tentacle.
vc. ly......vacuolar layer.
vit. mb. .....vitelline membrane.
vs. ly......visceral layer of mesoblast.
vt. gn......ventral ganglion.
vt. lv......ventral lobe of liver.
vt. mes. .....ventral mesentery.
vt. mt......ventral mantle.
vt. sh. .....ventral shell.
yk. ly......yolk layer.

PLATE I.

Plate I.

Fig. 1.—Mature egg in living state, in natural colors. × 310. *pl. bd.* = polar body.
Fig. 2.—2-cell stage, from life. × 310.
Fig. 3.—3-cell stage, from life. × 310.
Fig. 4.—4-cell stage, from life. × 310.
Fig. 5.—8-cell stage, from life. × 310.
Fig. 6.—Late 8-cell stage, from a preserved specimen. × 380.
Fig. 7.—Same egg as Fig. 6, in side view. × 380.
Fig. 8.—16-cell stage, from life. × 310.
Fig. 9.—32-cell stage, from life. × 310.
Fig. 10.—Blastula, from life. × 310.
Fig. 11.—Blastula slightly compressed, in optical section, from life. × 310.
Fig. 12.—Blastula, in which the gastrula invagination has commenced, slightly compressed, in optical section, from life. × 310.
Fig. 13.—Dwarf gastrula developed from a blastomere of the 2-cell stage, in optical section. × 370.

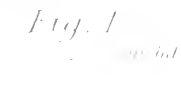
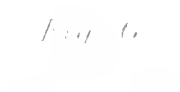


Fig. 1.



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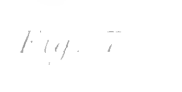

Fig. 7.

Fig. 2.

Fig. 3.

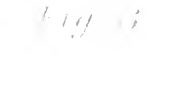

Fig. 9.

Fig. 4.

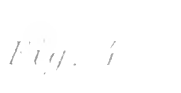

Fig. 10.

Fig. 12.

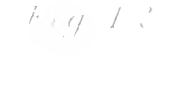

Fig. 11.

Fig. 5.

Fig. 13.

PLATE II.

Plate II.

Fig. 14.—Section through an ovum just laid. × 490.
Fig. 15.—Second polar mitosis at the metaphase. × 912 (immers).
Fig. 16.—Same, viewed from a pole. × 912 (immers).
Fig. 17.—Same, whose figure lies parallel to the surface of the egg. × 800.
Fig. 18.—Section through an ovum, into which a spermatozoon has entered. × 800.
Fig. 19.—Section through an ovum, in which the sperm (♂)- and egg-nuclei (♀) are still without their membrane. × 912 (immers).
Fig. 20.—Egg-nucleus travelling toward the centre of the ovum. A polar body is seen in this section. × 912 (immers).
Fig. 21.—Sperm and egg nuclei at the centre of the ovum. 912 (immers).
Fig. 22.—Sperm and egg nuclei fusing. × 500.
Fig. 23.—Segmentation mitosis in the early prophase. × 912 (immers).
Fig. 24.—Segmentation mitosis in the early anaphase. × 912 (immers).
Fig. 25.—Segmentation mitosis in the late anaphase. × 912 (immers).
Fig. 26.—Section through an egg at the 2-cell stage. × 490.
Fig. 27.—Section through an egg at the 8-cell stage. × 490.
Fig. 28.—Portion of a section through an egg at the 8-cell stage, in which the fourth cleavage mitosis has taken place. × 800.
Fig. 29.—Section through an egg at the 16-cell stage, in which the segmentation cavity is seen. × 490.
Fig. 30.—Section through an egg at nearly the 32-cell stage (slightly departed from the normal cleavage). × 490.
Fig. 31.—Blastula, from *toto* preparation (optical section). × 370.
Fig. 32.—Median section through a blastula. × 490.
Fig. 33.—Median section through an early gastrula. × 500.

Fig. 14

Fig. 15

Fig. 16

Fig. 17

Fig. 18

Fig. 19

Fig. 20

Fig. 21

Fig. 22

Fig. 23

Fig. 24

Fig. 25

Fig. 26

Fig. 27

Fig. 28

Fig. 29

Fig. 30

Fig. 31

Fig. 32

Fig. 33

to E. Nishida Tokyo.

PLATE III.

Plate III.

All the figures (Figs. 34–53), with the exception of Fig. 51, were drawn from life as if they were opaque bodies, (though in reality they were much more transparent), in order to show the external changes during these stages.

Fig. 34.—Embryo a little advanced from the gastrula, and with the mesentoblastic cell mass. (side view), from life. × 310.

Fig. 35.—Embryo with the arm-ridge and the mantle-fold (seen from the anterior face). × × Depressions on the mantle fold which are to double it into the two mantle lobes. × 370.

Fig. 36.—Same embryo, seen from the future dorsal or ventral side. × 370.

Fig. 37.—Embryo a little more advanced than that of Fig. 35. × × Depressions have become deeper. The stomodæum has appeared (anterior view). × 370.

Fig. 38.—Embryo at the same stage as Fig. 37 (seen from the anterior and ventral sides) slightly compressed. × 310.

Fig. 39.—Embryo a little older than Fig. 38, in which the mantle lobes have formed (dorsal view). × 370.

Fig. 40.—Embryo at nearly the same stage as Fig. 39 (side view). × 370.

Fig. 41.—Embryo at nearly the same stage as Fig. 39, compressed a little (ventral view). In this we see the arm-apparatus ciliated. × 310.

Fig. 42.—Embryo of the next stage with semicircular mantle-lobes (ventral view). × 370.

Fig. 43.—Embryo of a stage similar to that of Fig. 42, (side view). The mantle lobes have greatly increased anteriorly. × 370.

Fig. 44.—Embryo of the next stage with the *Anlagen* of the tentacle and the first pair of cirri. The mesoblast cell masses are clearly seen (ventral view). × 370.

Fig. 45.—Embryo of the same stage as the last figure. The rupture of the vitelline membrane is seen (dorsal view). × 370.

Fig. 46.—Embryo a little more advanced with the *Anlagen* of the second pair of cirri (seen from the anterior and ventral sides). × 370.

Fig. 47.—Same embryo (seen from the left side and ventral face). × 370.

Fig. 48.—Embryo of nearly the same stage as Fig. 46, showing that the arm-apparatus is attached to the dorsal mantle (seen from the anterior face). The ventral side is placed downward in the figure. × 370.

Fig. 49.—Embryo a little older than Fig. 46, with well developed tentacle and two pairs of cirri (dorsal view). × 370.

Fig. 50.—Embryo of a stage similar to that of the preceding figure. A pair of coelome sacs can be seen (ventral view). × 370.

Fig. 51.—Embryo of about the same stage, from a preserved specimen. × 380.

Fig. 52.—Embryo with three pairs of cirri,—the oldest of all the embryos I was able to rear from the egg (ventral view). × 370.

Fig. 53.—Embryo of the same stage as the last figure, whose arm-apparatus is stretched out and is about to swim. (in natural colors) × 370.

PLATE IV.

Plate IV.

Fig. 54.—Sagittal (non-median) section through gastrula. × 700.

Fig. 55.—Frontal section through an embryo at the stage of Fig. 35. × 490.

Figs. 56, 57.—Two sections from a series of sagittal sections of an embryo a little more advanced. Fig. 57 is median sagittal. In this embryo the formation of the mantle has been greatly retarded. × 700.

Fig. 58.—Median sagittal section through an embryo at the stage of Fig. 42. Here the arm-apparatus is seen as a fold of the dorsal mantle. × 596.

Fig. 59.—Portion of the ectoblast of the last section, showing the remaining yolk in the cells. × 912.

Figs. 60, 61.—Two consecutive sagittal sections through an embryo of the stage of Fig. 44; Fig. 61 being nearly median. *fr.* furrow between the two posterior mantle-lobes. × 700.

Figs. 62, 63.—Two frontal sections through an embryo like Fig. 44. Of these Fig. 63 is further dorsal than Fig. 62. × 700.

Fig. 64.—Nearly median sagittal section through an embryo of a stage similar to Fig. 46. *fr.* furrow between the posterior mantle-lobes. × 700.

Fig. 65.—Frontal section through an embryo of the stage of Fig. 46. The lateral diverticula of the stomach are seen. × 490.

Fig. 66, 67.—Two consecutive sections of a series of transverse sections through an embryo of nearly the same stage as Fig. 46. × 490.

Fig. 68.—Transverse section a little posterior to Fig. 67 from a series through an embryo of the stage of Fig. 50. × 532.

Fig. 69.—Portion of the arm-apparatus in transverse section through an embryo of the stage of Fig. 52. × 532.

Fig. 70.—Median sagittal section through an embryo of the stage of Fig. 52. × 532.

Fig. 71.—Sagittal section through a larva of the 3 p. c. stage. × 490.

Fig. 72.—Transverse section (slightly oblique) through a larva of the 3 p. c. stage. × 490.

PLATE V.

Plate V.

Fig. 73.—Larva of the 3 p. c. stage, dorsal view (from life). × 180.

Fig. 74.—Larva of the 3 p. c. stage, dorsal view (from a preserved specimen) × 210.

Fig. 75.—Larva of the 4 p. c. stage, ventral view (from life). × 215.

Fig. 76.—Larva of the 4 p. c. stage, dorsal view (from a preserved specimen). In this larva the secondary shell has formed in addition to the protegulum. The animal is shown only in outline. × 210.

Figs. 77, 78.—Larva of the 5 p. c. stage, ventral and dorsal views respectively (from life). × 220.

Fig. 79.—Larva of the 6 p. c. stage, ventral view (from life). × 220.

Fig. 80.—Body proper of a larva of the 6 p. c. stage, dorsal view (from life). × 215.

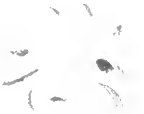

PLATE VI.

Plate VI.

Fig. 81.—Arm-apparatus and body proper of a larva of the 6 p. c. stage, dorsal view (from a stained preparation). × 220.

Fig. 82.—Body proper of a larva of the 6 p. c. stage, ventral view (from a stained preparation). × 500.

n. obl......nervus obliquorum.

*.....œdematic swelling of the visceral layer of the mesoblast.

***.....body wall.

Crescent-shaped body (colored yellow) seen over the right *obl. int.*...enigmatical element in the cœlomic fluid (see p. 50).

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Fig. 89.—Larva of *Discina*, ventral view (from life). × 110.

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Fig. 51

Fig. 52

Fig. 53

Fig. 54

Fig. 55

PLATE VII.

Fig. 91.—Median sagittal section through a larva of the 6 p. c. stage. × 210. *pit.* pit in the mid-gut. *rec.* recess between the epistome and the mouth.

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Fig. 106.

Fig. 107.

Fig. 108.

Fig. 109.

Fig. 110.

Fig. 111

Fig. 112.

Fig. 113

Fig. 114.

Fig. 115.

Fig. 116.

PLATE VIII.

Plate VIII.

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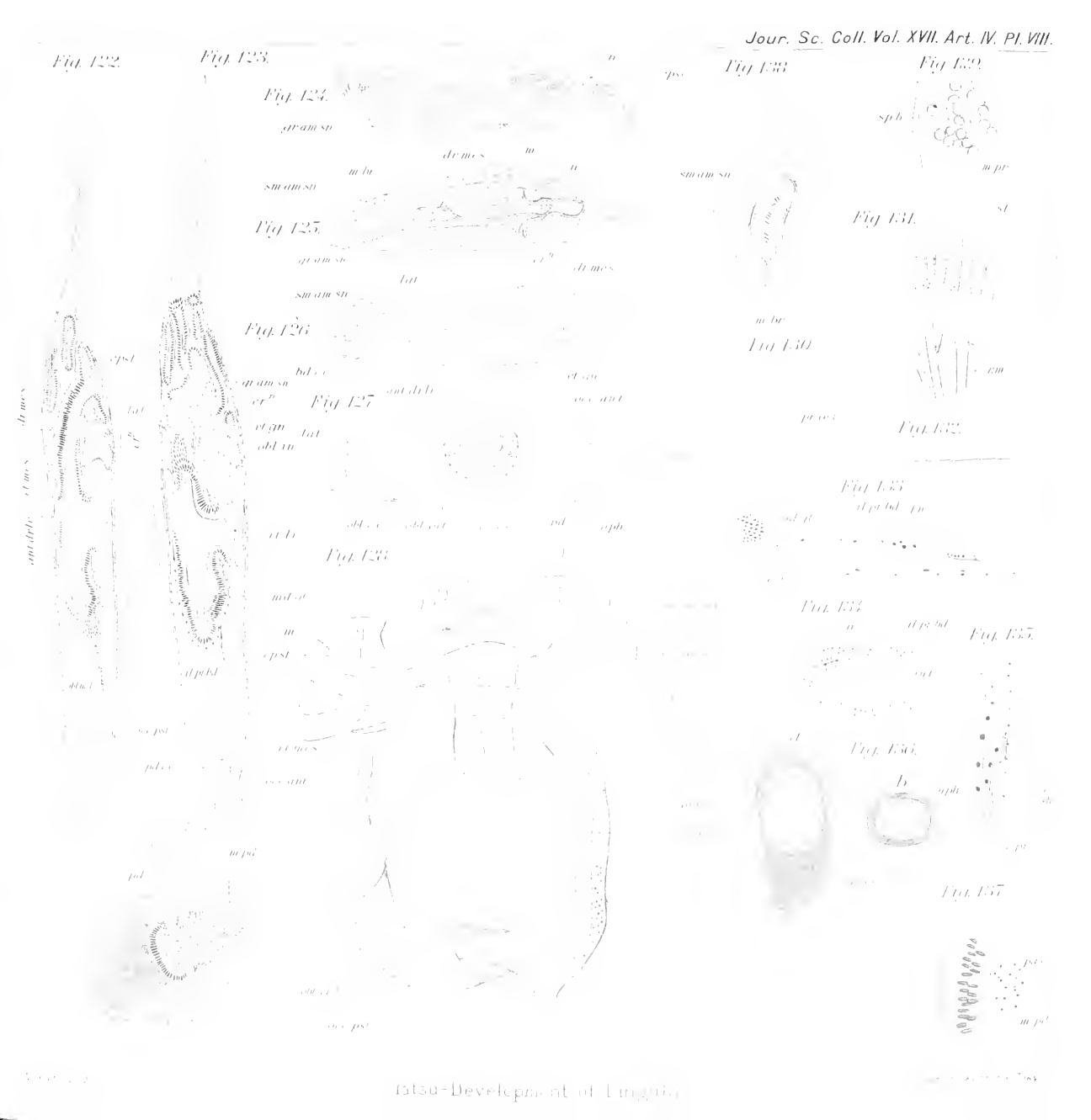
Fig. 122.
Fig. 123.
Fig. 124.
Fig. 125.
Fig. 126.
Fig. 127.
Fig. 128.
Fig. 129.
Fig. 130.
Fig. 131.
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Notes on Histology of *Lingula anatina* Brugière.

By

Naohidé Yatsu, *Rigakushi.*

With Plates I—II.

CONTENTS.

I. INTRODUCTORY.

Regarding the histology of *Lingula* there have hitherto appeared only two articles. One of them by Beyer ('86) is very far from being satisfactory, considered from the present state of our science. The other is the beautiful work of Blochmann ('00), thanks to which the main organization and some of the finer structures of *Lingula* have been made thoroughly clear. It must however be confessed that a greater part of the histology of this form still remains in the dark. The present piece of work, which has been undertaken in connection with my embryological studies, is thought, fragmentary though it be, to be worth publishing, as it may help to throw some new light upon the subject in question. As for other points I shall reserve them for a future occasion.

II. FORMED ELEMENTS OF THE CŒLOMIC FLUID.

In the cœlomic fluid of *Lingula*, which fills up the body cavity, the pallial sinus and the pedunculer cavity, and bathes all the viscera and muscles, we find three kinds of formed elements: viz. 1) blood corpuscles, 2) leucocytes, and 3) spindle bodies. Besides these there occur encysted sporozoa, and very rarely a *Distomum*, closely allied to those found in *Balanoglossus*, *Amphioxus*, and *Gephyrea*.

1. Blood Corpuscles.

The blood corpuscle (Pl. I., Fig. 1) exceeds in number all the other elements. It is colorless, transparent and somewhat

spherical in form, measuring 10–20 μ in diameter. It is usually depressed in one or two places. In section, therefore, it gives a crescent or biconvex shape. Just within the wall of the corpuscle there is a small refractive spherule, which is probably a nutritive oil drop (Pl. I., Fig. 2, *r. g.*). Treated with acidulated methyl green it swells up and appears like a hollow sphere (Pl. I., Fig, 2), for the cytoplasm is very transparent and is consequently invisible. When a section of blood corpuscles is stained with erythrosin the cytoplasm stains homogeneously red in well fixed specimens and looks rather compact. But under certain conditions of precipitation by the action of some fixing reagents the entire cytoplasm breaks up into coarse granules (Schollen) which also take a deep erythrosin stain. The nucleus is very compact, and the stain intense and uniform. The blood corpuscle has a well defined and rather thick membrane.

When the mantle of a young specimen (4.5–10 mm. in shell length) is examined under a low power, the circulation of corpuscles can be very clearly seen through the shell, as SEMPER ('61) has described. In every branch of the pallial sinus the observer can make out two opposed currents of blood corpuscles, although there is no septum which divides the sinus into two canals. The non-existence of a septum can be demonstrated in the living specimen, for it is seen that, when occasionally a corpuscle goes astray, it is quickly caught up by the opposed stream, as FRANÇOIS ('91) observed at Numea. The presence of the two currents is made possible by a low ridge (Epithelleisten of BLOCHMANN) which arises from the floor (next the shell) of the pallial sinus. The mantle of a young living *Lingula* is a good object for class demonstration of blood circulation. When sections are studied the corpuscles are sometimes found crowded

together in a pallial sinus and they here have the appearance of a fat-tissue, whose contents have been dissolved out.

2. Leucocytes.

Of the second element, the leucocyte, a small number are found in the cœlomic fluid, while a considerable number occur in the marginal lacuna (Randlacune). It is very probable that SEMPER ('61) observed the leucocytes in the lacuna, which he called the "Lymphraum." BROOKS ('78) on the other hand confounded this element with the ordinary blood corpuscles (p. 49). The leucocyte measures 13–20 μ in diameter, and is sometimes spherical in form with a wrinkled surface. It clearly shows amœboid movements giving off a few blunt pseudopodia and sometimes presenting fine sharply pointed, stiff pseudopodia which cluster together in twos or threes (Pl. I., Figs. 3 *a*, *b*, and 4). The leucocyte is always found filled with erythrophilous granules, therefore, and treated with erythrosin, gives a very beautiful appearance, as in Figs. 4 and 5 (Pl. I.). In some cases a swarm of leucocytes is attached along the epithelial ridge (Epithelleisten) of the pallial sinus (Pl. I., Fig. 5): not only do they attach themselves to it, but some of them also force their way into the ridge, probably performing the collection of waste products. This is proved by the following fact. The leucocyte is phagocytous in nature: the yellow pigment, of which more or less is found in the epithelial ridge, is carried out by the element, as shown in Fig. 5 (Pl. I.): in the epithelial ridge we often meet with a leucocyte taking up within itself a compact body, which will be described further on: in another case I was able to observe in the pallial sinus a leucocyte surrounding a spindle

body and a compact body (*vide infra.*) at the same time (Pl. I., Fig. 6).

3. Spindle Bodies.

The third element to be described is the enigmatical corpuscles, known as the spindle bodies. These bodies are so characteristic of *Lingula*, that they have been referred to in every work concerning this Brachiopod. I shall go somewhat in detail into the history and description of these bodies. But before doing so I may state in advance the main conclusion, that they are formed out of blood-corpuscles in two separate and independent places: one of these being the special areas on the dorsal and ventral body walls, and the other along the ridge which passes along the middle line of every branch of the pallial sinus.

A. Historical.

C. Vogt ('45) observed the proliferating ridge of the pallial sinus and figures it in his anatomy of *Lingula.* But he mistook the ridge for a blood vessel, an error, natural perhaps, since he did not study the ridge by means of sections.

A. Hancock ('59), in his beautiful monograph on the Brachiopods, considers the proliferating zones in the body walls as a part of the reproductive organs and indeed as the testes. He referred to these zones under the name of the "dendritic organ" and studied it in detail: ".....but further experience," he says, "has led to the conclusion that they are really a portion of the genital mass and that from the pressure of the valves, on their being closed, they become occasionally adherent to the

membrane.....the cells, however, in *Lingula* appeared to present different stages of development, varying much in size and form. Some were oval, others perfectly elliptical, the larger ones were pointed at both ends, and exhibited a double line in the centre, placed longitudinally; while the largest measuring $\frac{1}{180}$th of an inch [=141 μ] in length, were fusiform, with the extremities more or less sharply pointed. These corpuscles were filled with numerous delicate hair-like bodies, resembling spermatozoa. From these facts it can scarcely be doubted that the dendritic organ is the testis, and that the fusiform cells are fully developed spermatophora, containing spermatozoa" (p. 819). As far as the microscopical structure of the dendritic organ is concerned, his observations are correct in all essentials, but from the vague premise that the corpuscles contain "*hair-like bodies resembling spermatozoa,*" it was certainly a dangerous conclusion that the "*dendritic organ is the testis.*" It is strange that HANCOCK's erroneous conclusion passed over to BEYER's view, as will soon be seen. HANCOCK studied, on the other hand, the epithelial ridge and rightly saw the difference between it and the sexual organ found there in other Brachiopods, recognizing the difference in position dependent on the fact that the sexual organ hangs from the inner side of the pallial sinus while the epithelial ridge rises from the outer side (nearer the shell). But unfortunately as he did not study the ridge microscopically he was not able to ascertain that the ridge was the equivalent of the dendritic organ.

In the year following the publication of HANCOCK's memoir there appeared the work of GRATIOLET ('60) on the anatomy of *Lingula*. In this he expressed the curious view that the spindle bodies were young *Lingula*. These, he thought, were produced in the body cavity as the result of self-fertilization: he even

goes so far as to discover two valves in the spindle bodies! For these he has evidently mistaken surface depressions which occur not infrequently in the spindle bodies.

In BRONN'S Klassen und Ordnung des Thierreichs the question is raised: "ob nicht diese zellen, bei deren bildlicher Darstellung die Angabe von Vergrösserungsgrades vermisst werden, etwa für Prosospermien oder parasitische Pseudonavicellen zu nehmen worden" (p. 281). This interpretation seems *à priori* probable. But if the spindle body were a Sporozoon it should contain a nucleus or nuclei in some form or other. Such a structure is not found at any stage under any treatment.

MORSE ('73 *b*) noticed the presence of the spindle bodies and added that "these are amoeboid in their appearance, and may be seen bending and turning as they course through the more delicate ramifications in the pallial membrane" (p. 25). He advances no view as to the nature and orgin of this body.

BROOKS ('78) also observed the spindle bodies and states that when the bodies are crowded together they give an exceedingly faint violet tint. He noticed that "running out from one or both of the pointed ends of many of the corpuscles are long delicate filaments of variable length" (p. 49), and found the bodies, which he supposed to be on point of division.

BEYER'S investigation ('86) made known to us that the dendritic organ of HANCOCK and the epithelial ridge are equivalent structures. Like HANCOCK he came to the conclusion that the spindle bodies are the spermatophores. He does not seem to have himself studied the structure of the spindle bodies. In no part of his paper does he adduce the slightest evidence that these bodies contain spermatozoa. He refers, however, to the "Spermatogenesis" in a final paragraph. He also mistook the

the compact bodies (*vide infra*) for young ova. It seems hardly necessary to enter further into the criticism of his paper.

François ('91) observed the spindle bodies in the blood of *Lingula* at Numea. "Le sang qui circule dans l'axe du pedoncule, outre les globules sanguins ordinaires, contient des corpuscules fusiformes plus ou moins régulier, dont la taille varie de 20 à 100 μ de longueur. Ils present parfois quelques striés longitudinals. Faul-il voir là des globules transformés ou des fibres musculaires en formation qui se seraient détachés de la paroi ?" (p. 239).

In the same year Cori ('91) described the spindle bodies in his anatomy and histology of *Phoronis*. In this genus the body [=Spindelförmiger Körper (Cori)] was first described by Kowalevsky. It is formed in the "Gefässperitonealzellen," set free into the body cavity and then carried to the exterior through the nephridium. It measures 11–43μ, as I calculate form Cori's figures. In some cases near the middle part refractive granules are found, and sometimes it is enclosed by a membrane which presents a double contour. He concludes: "Die kleinen doppelt kontourirten Formen dieser Körper lassen mancher Merkmale erkennen, welche die Umbildung der rothen Blutkörperchen in diese wahrscheinlich machen könnten" (p. 557). My friend, Mr. I. Ikeda, who devoted himself during the past spring chiefly to the studies of the "Gefässperitonealgewebe" of the Japanese *Phoronis*, tells me that he has never met with the spindle bodies in his slides.

Blochmann ('00) observed the spindle bodies in the coagulated cœlomic fluid of *Lingula* and positively proves the absence of a nucleus in it (p. 118). But he gives no further account of the structure and origin of the bodies. It seems he was not

able to observe the spindle bodies proliferating from the epithelial ridge. The dendritic organ, HANCOCK's supposed testis, BLOCHMANN considers as merely the coagulum of the cœlomic fluid (p. 122).

B. OBSERVATIONS.

a. Form and Structure.

The spindle bodies assume all imaginable forms, ranging from the spherical to the greatly elongated. Sometimes they are triangular (Pl. I., Fig. 7 *b*), sometimes delicately spindle shaped (Pl. I., Fig. 19), often with one or two constrictions (Pl. I., Fig. 7 *c*); but in the majority of cases they are shaped like a spindle, or like a grain of rice; hence the name. The flagellum-like prolongation of the spindle body at one or both ends is often met with, as observed by BROOKS. This appearance is, I believe, probably due to an artefact, since the spindle body is very soft and plastic as MORSE has stated, and either end of it is, therefore, very easily drawn out. The doubly elongated spindle, which was supposed to be in a stage of division by BROOKS, was not infrequently observed. This I regard merely as a more elongated form.

The spindle bodies vary greatly in size, measuring 55 μ in length and 16 μ in breadth, on an average. The longest with a constriction often attains twice the usual length. On the other hand there are found specially small and slender ones (Pl. I., Fig. 11). Such considerable variations in size make it difficult for us to believe that they can have a definite structure in connection with a definite function.

The spindle body consists of a thin cell membrane with distinctly fibrous contents. The fibres themselves are in a majority of cases arranged in a single bundle parallel to one another, and converging toward either extremity (Pl. I., Figs. 10, 11), but in some instances there have been observed two bundles, so arranged as to form an angle with each other (Pl. I., Fig. 7 *a*) : or in rare instances three bundles are present, arranged like the sides of a triangle (Pl. I., Fig. 7 *b*) ; finally there are those in which several bundles appear running in various directions (Pl. I., Figs. 7 *f*, *g*). Sometimes a bundle of fibres is bent within the membrane (Pl. I., Fig. 7 *d*). Rarely only a fragment of a bundle is found (Pl. I., Fig. 7 *e*). The shape of the spindle bodies, as just described, depends upon the length and arrangement of the fibres. There are also modifications in the mode of combination of fibres with one another : when they are compact they assume the normal spindle shape, while when loosely arranged at one or both ends the bundle does not taper but the ends of the fibres stand widely apart (Pl. I., Fig. 7 *g*). The fibres, it should be stated, are best seen in fresh material just taken out of a living specimen, or in formalin preparations stained with acidulated methyl green. Under this treatment the fibers become somewhat loose and hence more distinct, staining light green with a darker tone in the middle. In favorable preparations treated in the same way, a series of fine easily stained refractive granules can be seen arranged on the equatorial surface (Pl. I., Figs. 8 *a*, *b*, *c*) as observed by Cori in *Phoronis*. These granules cannot be taken as nuclei, since they are not demonstrated by any other method. The fibres in the living state give evidence of a great plasticity : in fixed material, on the other hand, they appear stiff and brittle. Their behavior toward

the stains suggests that of a muscle. They are stained red with erythrosin, as the blood corpuscles usually are: in very favorable cases in hæmatoxylin-erythrosin-orange G preparations, they are differentiated from the blood corpuscles and compact bodies (*vide infra*) by their being stained red, while the latter two become yellow (Pl. I., Fig. 10). If the fibres were actually modified nuclei as BEYER maintains, they should be stained green with BIONDI-EHRLICH'S mixture, while as a matter of fact they become a homogeneous red.

b. Occurrence.

In the body cavity and the pallial sinus the spindle bodies are found sparingly intermingled with other corpuscles. Among the lobes of the testis they occur in numbers. In the peduncular cavity almost all the floating elements of the fluid are the spindle bodies, which seem to increase in number with age. The reason why they accumulate in this region may, I suppose, be that the spindle bodies are larger than the blood corpuscles and are heavier than the lattter. As to their fate, whether they are retained in the body or at times ejected is quite unknown.

c. Development.

During the larval life the spindle bodies seem to be produced from any part of both the dorsal and ventral body walls. In the adult, on the other hand, the proliferating regions are restricted to two independent places, as I have already stated. The one is the epithelial ridge and the other is the dendritic organ of HANCOCK.[1]

1. BEYER ('86) states that the spindle bodies are proliferated from the lateral body wall, but as this region is occupied by the parietal muscle layer, no proliferating zone can exist there. He perhaps means the dorsal and ventral body-walls instead of the lateral.

In young *Lingula*, or near the tip of the branches of the pallial sinus in the adult, the epithelial ridge is composed of tall columnar cells with enlarged tips and narrowed bases, showing no difference from the structure described by BLOCHMANN ('00). In older individuals, however, this region gives quite a different aspect. In sections of the mantle cut perpendicular to the epithelial ridge (Pl. I., Figs. 5, 9, 10), the following relations will best be seen. The boundary between the tall columnar cells forming the epithelial ridge vanishes as if the cells had been disorganized to a certain extent. The nuclei placed in the lateral portion of the ridge point away from the middle line of the latter. Near the base of the ridge there are found yellowish brown pigment granules. A number of the spindle bodies occur imbedded in the epithelial ridge, and, in a majority of cases lie in a cavity which has no membrane delemiting it from the neighboring part. What attracts special attention is the fact that the spindle bodies are found lying in any position in the ridge: sometimes they stand almost perpendicular to the surface, while in other cases they are placed nearly parallel. In an extreme case the spindle body is so elongated that its length exceeds the thickness of the ridge and appears to find hardly room enough for itself there (Pl. I., Fig. 11). From these facts alone one may consider that the spindle bodies do not belong to the epithelial ridge, but that they are introduced fronr without. That the spindle bodies, or at least their antecedents, are thus introduced will be shown directly. When they attain full growth they are set free into the pallial sinus. And very often there are seen holes which are formed after the escape of the spindle bodies. The sections through the middle line of the epithelial ridge also give very instructive figures, spindle bodies of dif-

ferent sizes being met with throughout almost the entire length of the section.

In young *Lingula* (4.5–10 mm. in shell length) at the posterior region of the dorsal and ventral body walls, where the dendritic organs of HANCOCK are to be developed, the peritoneal layer, a very thin epithelium (Pl. I., Fig. 14), is separated from the outer epithelium of the body wall, next the shell, and in the lumen thus formed many spindle bodies are found together with the leucocytes and the compact bodies. In preparations of the adult *Lingula*, especially in those fixed with sublimate, when all the viscera are taken out before or after the fixation, the dendritic organs come distinctly into view as three irregularly pinnated ridges (Pl. I., Fig. 12); the central being the longest, runs along the median plane; the lateral ones along the lateral body wall. Differing much from the structure seen in the young individuals, the organ in sections shows that it is composed of a many layered epithelium with vesicular nuclei and granular cytoplasm without cell-boundaries (Pl. I., Fig. 13). In this epithelium an enormous number of spindle bodies are found: a few of them are directly imbedded in the epithelium, while the rest, in groups of twenty or thirty lie in hollows. In the neighborhood of the cavity cytoplasm becomes disorganized as in the case of the epithelial ridge.

To the curious phenomenon that such enigmatical bodies of various sizes are produced in such a remarkable number from definite regions, I paid not a little attention, and at last I came to discover nearly *all stages of transformation from the ordinary blood corpuscles up to the spindle bodies.* Unfortunately the blood corpuscles were not found in the epithelial ridge, but in the dendritic organ of young *Lingula* they were distinctly seen

enclosed in the cavity (Pl. I., Fig. 14). As the first step of transformation the blood corpuscles become spherical : the cytoplasm shows a granular appearance, and the nucleus is pushed aside until it lies just inside the cell-membrane (Pl. I., Fig. 15). Then the nucleus seems to undergo degeneration though I was not able to obtain sections favorable for studying the karyolysis, and the granular cytoplasm changes into a very compact homogeneous and strongly refractive sphere. This I shall call the compact body (*c. p.*). Such bodies occur, in a tolerably large number, constantly imbedded directly in the epithelial ridge and the dendritic organ (Pl. I., Figs. 10, 13). They take a darker erythrosin stain than the spindle bodies and in all their behavior toward stains the compact bodies show no difference from the blood corpuscles. The bodies vary greatly in size (Pl. I., Fig. 13) although the blood corpuscles are of an uniform size. In what way such differences in size arise I do not understand : they may perhaps be caused by the fact that different portions of cytoplasm are used for the formation of the compact bodies. Sometimes, but not always, the compact bodies then undergo eccentric splittings, as the result of which a sickle-like space is left between the two portions in the section (Pl. I., Figs. 17 *a*, *b*). Sometimes a small spherical portion is formed near the periphery and this acts as the centre of splitting (Pl. I., Fig. 16). Next the fibres are formed at the expense of the compact metamorphosed cytoplasm ; at this time an enormous growth in length certainly takes place, as the fibres are not found coiled up from the outset of their formation. From each portion of the compact body thus split, a bundle of fibres is formed. Consequently all imaginable forms of spindle bodies are produced according to the degree and mode of splitting. In some cases I found spindle

bodies, in which one portion had already turned into fibres, while the other remained in its orginal state (Pl. I., Fig. 18); and in other cases lying beside the perfect spindle bodies, the remnants of compact bodies were found enclosed in the cell-membrane. Although from the fact just described it is obvious that the fibres are formed from the compact bodies, yet as to the manner in which the growth in length of the fibres takes place I cannot at present give any positive datum. Judging, however, from the fact that while the compact bodies are directly imbedded in the epithelium, and the spindle bodies are found in many cases lying in a cavity, it is probable that the spindle bodies in their growing stage emit a certain enzyme in order to dissolve the neighboring cytoplasm and to take it up in themselves. Owing to this nutrition, if it may be so called, the fibres of the spindle bodies increase in length, at the same time becoming more or less loose.

d. What is the Spindle Body?

As above stated the spindle body is a metomorphosed blood corpuscle, as Corr has observed in *Phoronis;* that is, it is distinctly *a cell, whose nucleus has degenerated and whose cytoplasm has been turned into a fibrous structure.* Since in every individual the spindle bodies are produced in such great numbers in definite regions, they cannot be looked upon as pathological products. As they are enucleated cells they are destined sooner or later to die out. They must, however, as such or at the time of their formation, play an important part in the economy of *Lingula.* It is certain that the blood corpuscles, leucocytes and spindle bodies have their respective offices. Among several hypotheses which suggest themselves in regard to the function the of spindle

bodies the following seems to me the most probable. As the cœlomic epithelium of other animals often perform the excretory function, so the cells forming the dendritic organs and the epithelial ridges accumulate waste products found dissolved in the cœlomic fluid. The blood corpuscles now force their way into these regions in order to take up the waste substance accumulated there. At the same time they are turned into the spindle bodies and set free into the body cavity again, being in the end collected in the peduncular cavity; in short, *the spindle bodies function only at their formation as the eliminators of waste products.*

III. OTOCYSTS.

Morse ('78) is the only investigator who describes the occurrence of the otocysts in the adult *Lingula.* Unfortunately his paper has not been published in full, so that no details are forth-coming. The only thing I can learn is that "their [otocysts'] position and general appearance recall the auditory capsule figured by *Claparède* in certain tuticolous Annelids" (p. 157).

On the other hand, in his recent work Blochmann ('00) pronounced thus positively upon the absence of the otocysts: "Nun kann ich zunächst mit voller Sicherheit behaupten, dass bei erwachsenen *Lingula* Otocysten nicht vorhanden sind" (p. 124). He ('98) regarded the vesicles found in the larvæ of *Discinisca* and referred to as the otocysts by Fritz Müller ('60, '61) as the funnels of the nephridium. Moreover he claimed to have found a duct leading from this organ to the exterior.

In the free-swimming larvæ of *Lingula* Brooks ('78) rightly observed the otocysts, but he was not able to study the young *Lingula,* and was very cautious in his statements about the fate of these vesicles.

For the purpose of determining how long the otocysts which are present in the larvæ of the 5–15 p. c. stage persist, I searched after the structures in many individuals at the identical place where they are situated in the larvæ, and in all cases I could discover there a pair of sharply outlined vesicles which can be identified as the otocysts. The structure I was able to find in our *Lingula* is probably one and the same organ that MORSE discovered twenty three years ago, since the vesicles are very conspicuous and there are no other organs which can be mistaken for the otocysts.

The otocysts are found even in the oldest individuals I have examined (45 mm. in shell length). They are a pair of vesicles of so conspicuous a size that an experienced observer can easily see them with the naked eye. In young *Lingula* (4.5–10 mm. in shell length) with translucent shells, we can readily detect with strong reflected light a pair of otocysts under the dorsal valve, the otoliths in constant dancing motion and the blood corpuscles running about the otocysts. In older individuals the shell increases in thickness and prevents the transmission of light: in such cases we are able to detect the vesicles only in fixed specimens. To have the best surface aspect of the organ the material should be fixed with chromo-acetic or some other mixture with chromic acid, since in a specimen thus treated the connection between the valve and animal becomes loose, so that the shell can easily be peeled off from the soft part without danger of injuring the latter.

With this general remark I shall enter upon a detailed description of the otocysts. In a dorsal view of the fixed specimen whose dorsal valve is taken off we can at once make out the otocysts imbedded in the supporting substance, upon the

gastoparietal band, a little posterior to the median half of the *occlusor anterior*, and near the bottom of the lateral indentation of the body cavity. Fig. 20 (Pl. II.) shows the position of the organ. Just distal to this vesicle the pallial sinus comes into communication with the body cavity.

Generally speaking the organ is lenticular, but there are not wanting variations: in a dorsal view it is sometimes nearly triangular with rounded angles, while in other cases it is almost circular. As a rule it is broader than it is long. Its walls are not of equal thickness, but are thickest on the median and lateral walls, and thinnest on the dorsal and ventral sides. There is also a great variation in size: the following is an average of the measurements of the organ in various sections.

Length	... ...	145 μ
Breadth	... ...	160 μ
Height	... ...	40 μ
Thickness of the walls	thickest portion ...	35 μ
	thinnest „ ...	8 μ

In tracing a series of longitudinal sections (Pl. II., Figs. 21, 22) as soon as the valve at the entrance of the pallial sinus comes to an end, and in the same section in which the nephridium is about to open to the exterior on the ventral side the otocyst becomes visible. Following the sections toward the median plane we soon find that the otocyst is a closed sac, as is seen on a surface view, and that it bears not a little resemblance in structure to the same named organ occurring in *Arenicola grubii* studied by E. Ehlers (Taf. XIII., Fig. 37). It is situated just anterior to the attachment of the gastroparietal band to the dorsal body wall, so that it has a lamella of the supporting substance at the

posterior end in common with the above band. The greater part of the ventral face, the entire anterior face and a small part of the dorsal face of the otocyst is free and coated with a layer of longitudinal muscle which attains a considerable thickness at the anterior end. The otocyst itself is composed of an external layer of the supporting substance and an inner sac formed by a thick epithelium. The supporting layer is of different thickness at different places: it is thickest at the posterior edge and on the median and lateral sides, while in other parts it remains thin. Interior to the supporting layer there is a very thick epithelial sac, composed of tall columnar cells with nuclei near their bases and placed at several different levels. The nuclei are of a spindle shape, and have a great affinity for stains. The epithelial cells are highest along the edge formed by the meeting of the two surfaces of the lens-shape. The cells are throughout of the same structure and no other elements occur among them. At the tip of these cells facing toward the cavity there is no indication of the cuticula formation, but there fine rod-like granules are seen, which with a high degree of certainty are to be regarded as the basal pieces of cilia, indicating that the inner cavity of the otocyst was covered with cilia, although in fixed specimens no cilia could be detected. In the cavity there are found some thirty spherical otoliths, varying greatly in diameter, the largest measuring 3–4μ. They are compact, highly refractive and take a somewhat deep hæmatoxylin stain which proves that they are organic in composition and hence not introduced from without, but secreted from the walls. Along the margins of the lenticular vesicle there is left a ring-shaped space filled with granular substance and in one region a few muscle fibres. The former is no doubt nervous in nature, but I must confess that but little is known

about the origin and termination of the nerve fibres among the epithelium.

From the above description no one would doubt the presence of the otocysts in the adult *Lingula*, at least in the Japanese form, and my account is confirmatory of MORSE's discovery.

Physiologically the organ just described would probably subserve a static function, as in similar organs found in other animals. The terms "otocyst" "otoliths" are employed here simply in the morphological sense.

IV. HEART.

Into the macroscopical feature of the heart I shall not enter, as it has already been fully described by HANCOCK ('59) and BLOCHMANN ('00). In sagittal sections of the young *Lingula* (Pl. II., Fig. 23, *h*.) the finer structure of the organ is best seen. In the region which we designate as heart, the blood vessel increases slightly in calibre. The constrictions of the vessel are not constant, varying according to the state of contraction at the time of killing. The vessel is here composed of a tube of columnar or cubical cells and the inner muscular layer. The epithelial cells vary considerably in thickness in different places. As a rule the cell has an enlarged tip, and an attenuated base. The nuclei placed in the middle of the cells are rather compact and stain intensely. Interior to the epithelial layer a thin coating of muscle fibres is found. The fibres do not appear to run longitudinally, but to take a somewhat screw-like course. The movement of the fluid therein contained is due to the contraction of the muscle. At the transverse dorsal ridge of the stomach, upon which the heart is situated, the layer of

the supporting substance attains a considerable thickness projecting for a short distance posteriorly (Pl. II., Fig. 24). To this protuberance small bundle of muscle secures its attachment. The muscle has a sheath of cell layer as in other muscles. It runs ventral to the heart vessel, and posteriorly gradually attenuating into a fine string, is finally inserted on the dorsal wall of the stomach. The muscle serves probably to draw the posterior part of the stomach nearer the region of the gastroparietal band, facilitating the movement of food particles in the alimentary canal, and indirectly of the lymph fluid in in the heart tube.

V. OVARY AND TESTIS.

On the sexual organs and elements of *Lingula* BLOCHMANN did not touch at any place in his recent paper ('00). It may, therefore, not be superfluous to devote a few pages to this point. As for finer studies on ovogenesis and spermatogenesis I shall put them off for some future time.

Lingula is an animal of distinct sexes as is the case with other Brachiopods. MORSE was the first to emphasize this fact, for he was able to study *Lingula* in life (73 *b*, p. 38). Thirteen years later BEYER ('86), advanced another view, maintaining that *Lingula* is hermaphrodite. This conception originates from HANCOCK's statements. As both the sexual elements are very much alike in appearance, the last named author did not distinguish the testis from the ovary; or, if not, the specimens he studied were all female. At any rate he did not notice the true testis in his monograph ('59) and regarded the dendritic proliferating zone of the spindle bodies on the walls of the body cavity as the testis. BEYER found the same structure as HANCOCK's supposed

testis in the epithelial ridge of the pallial sinus and mistook the spindle bodies for spermatophores. Moreover he extended his view, regarding the round compact bodies also found in the epithelial ridge as young ova. As I have explained at great length in Section II, these two enigmatical bodies are not sexual elements at all.

In *Lingula* the formation of sexual elements is restricted entirely to the ileo-parietal band, and the sexual organs in the pallial sinus found in other Brachiopods are entirely wanting. Both the ova and spermatozoa are nothing else than modified epithelial cells on either side of the ileo-parietal band, as has already been described by many writers on the Testicardines.

The sexes of *Lingula* are after some experience easily distinguished from without. Seen through the translucent shell the females appear somewhat dark brown, while the males lighter brown or even whitish. The distinction can best be seen in individuals of a medium size, for in older animals the shell increases in thickness and obscures these features.

a. Ovary.

As to the structure and development of the ovary : in free-swimming larvæ with eight pairs of cirri there are distinctly seen, though few in number, primary germ-cells,[1] some which are distinguished from other epithelial cells by their larger size, granular cytoplasm, and vesicular nuclei.

In young *Lingula*, 4.5 mm. in shell length the sex is already differentiated ; the undifferentiated condition, therefore, must be sought for in individuals younger than this. In Pl. II., Fig. 26

1. *Vide* N. YATSU. On the Development of *Lingula anatina*. This volume, Art. 4, p. 60.

the ileo-parietal band and a portion of the nephridium from a transverse section through a young *Lingula* (5.5 mm. in shell length) is shown. Here we see at once that the ileo-parietal band is nothing but a folding of the visceral layer of the mesoblast, which covers the nephridia as well as the alimentary canal. Moreover in the figure we can distinguish two kinds of cells which constitute the ileo-parietal band: the one with vesicular nuclei, and the other with compact nuclei. The former are those which have already acquired the character of ova, while the latter kind of cells are those which still remain as ordinary epithelial cells. The supporting substance between the two layers of the ileo-parietal band is not as yet formed.

In young *Lingula* of 9–14 mm. shell length the layers of the ileo-parietal band bearing the young ova are subjected to a high degree of folding, so that in sections they give a dendritic appearance.

In the female which has almost attained maturity, the ovary appears as a conspicuous mass of cells of a darker brown than the liver, filling up the main part of the body cavity, and protruding even into the "Erker." The ova (Pl. II., Fig. 27) are prism-shaped, pentagonal or hexagonal in section, and slightly rounded out on their free surfaces. They measure 60 μ in diameter and 90 μ in height. Their cytoplasm (Pl. II., Fig. 28) contains minute yolk granules uniformly distributed. In larger ovarian ova vacuoles are found scattered throughout the peripheral portion. The vacuoles apparently result from the dissolving away of the yolk granules.

The nucleus, enclosed by a definite membrane, lies always near the free surface: a portion which must bear some relation to the metabolic function of the ovum (Pl. II., Fig. 27). The

nucleus is vesicular and stains less intensely than cytoplasm, as if it were quite devoid of chromatic substance.

In young ova the nucleolus is single but in older ovarian ova there appear accessory nucleoli (*a. nl.*) which are usually found closely apposed to the principal nucleolus (*p. nl.*) (Pl. II., Figs. 28, 29 *a*, *c*). But sometimes they detach themselves from the principal one (Pl. II., Fig. 29 *b*). The accessory nucleolus is not always spherical, in some cases assuming an irregular outline (Pl. II., Fig. 29 *b*). The two kinds of nucleoli can easily be differentiated by gentian violet, the principal one staining darker than the accessory, as is shown in the figures (Pl. II., Figs. 28, 29). Consequently it seems that the accessory nucleoli are less dense than the principal. In the Testicardines the presence of two kinds of nucleoli is clearly figured by VAN BEMMELEN ('83), but he says nothing about it. VOGT and JUNG ('88) mention that often two nucleoli are present. The supporting substance (Stützsubstanz) of the ileo-parietal band is found as a thin membrane between two layers of ova (Pl. II., Figs. 27, 28). This membrane BEYER ('86) neither mentions nor figures. At some places the membrane increases in thickness and gives a vacuolated appearance (Pl. II., Fig. 30).

Even in the region where ova are developed cells are of course not all converted into them. The follicle of the ovum is formed at the expense of interstitial cells, which become extremely flattened, so that their presence is perceived only by their compact and spindle-shaped nuclei (Pl. II., Fig. 28). Of the cells which have started to become ova, some lying pressed between two adjacent ova are arrested in their early development and remain small. That these degenerating ova take a hæmatoxylin stain more readily than others, shows that they are at the beginning of yolk-formation.

Yellowish brown pigment granules are found scattered here and there along the supporting substance of the ileo-parietal band and among the ova. They are compact and polygonal granules of various sizes (Pl. II., Figs. 27, 30). These granules must have been formed by a process of pigment degeneration of the young ova (early arrested in their development) and even of the ripe ova. In large specimens of *Lingula* collected at the end of the summer or in the fall, the ovary is found very much reduced in size as dirty brown masses with black spots. On cutting such an ovary into sections we meet with diverse stages of karyolysis and plasmolysis of the ova; the latter at first provided with vesicular nuclei come to increase in consistency and break up into granules. But I could not determine whether it is only the ova left behind after the ripe ova had been deposited that are subjected to degeneration or whether losing the opportunity of discharging, the entire ovary, with both ripe and unripe ova, turns into pigment granules. Must probably both these things take place. LANKESTER ('73) found in *Terebratula vitrea* the yellow matter among the ovarian ova and this he considered as the envelope of escaped ova (p. 93). JOUBIN ('86) states that on keeping *Crania* the eggs atrophied and turned into brown bands (p. 255).

b. Testis.

In male individuals the spermatozoa are formed only in the region corresponding to that in which the ova are produced in the female. About the supporting substance of the ileo-parietal band is a tolerably thick layer of cells with vesicular nuclei; this layer consists of the spermatogonia and spermatocytes. Ex-

terior to this there is another thicker layer of compact nuclei, being the clusters of the heads of spermatozoa. The outer surface of the latter layer has a ciliated appearance owing to the presence of the tails. Spindle bodies force their way into the narrow spaces of the testes, probably bearing some important physiological relations to sperm cells. The above two layers of testis are noted by VOGT and JUNG ('88) in their Lehrbuch (p. 724).

The spermatozoon has a head, slightly pointed at the anterior end and rounded posteriorly (2 μ), a very short middle piece (1 μ) and a comparatively long tail (40 μ) (Pl. II., Fig. 25).

At what age individuals discharge their sexual elements I could not determine. It is certain that *Lingula* of about 30 mm. in shell length discharge eggs, as I have observed the act. As to the male, the spermatozoa are met with even in so young an individual as one 7 mm. in length. Whether spermatozoa which are so precociously developed are retained in the body for a long time or are soon discharged, is quite unknown.

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Abbreviations used in Plates.

a. nl.....accessory nucleolus.
ant. dr. lv. .....anterior dorsal lobe of liver.
bl. cp. .....blood corpuscle.
cl. m. .....circular muscle of the stomach.
cp.....compact body.
d. o. .....dendritic organ.
dr. sh. .....dorsal shell.
ep. rd. .....epithelial ridge.
foll. .....follicle cell.
gl. cll. .....gland cell of mantle.
gm. cll.....germ cell.
gst. pr. bd. .....gastroparietal band.
h. .....heart.
il. pr. bd.....ileo-parietal band.
leu.....leucocyte.
lat.....M. lateralis.
lv. .....liver lobule.
mg. l. .....marginal lacuna.
m. pr. .....parietal muscle.
n. .....nucleus.
nph. .....nephridium.
obl. int.....M. obliquus internus.
occ. ant. .....M. occlusor anterior.
ot. .....otocyst.
otl.....otoliths.
p. nl.....principal nucleolus.
pll. sn. .....pallial sinus.
pig.....pigment.
r. g. .....refractive granu'e.
spd. .....spindle body.
spl. .....supporting layer.
stm. .....stomach.
vt. sh. .....ventral shell.

PLATE I.

Plate I.

Fig. 1.—Blood corpuscles taken from a living specimen of the adult *Lingula*. × 310.

Fig. 2.—Blood corpuscle treated with acidulated methyl green. × 800.

Fig. 3 *a*, *b*.—Two leucocytes taken from a living specimen. × 380.

Fig. 4.—Star-shaped leucocyte, from a hæmatoxylin-erythrosin preparation. Blood corpuscle is also drawn for comparison of size. × 729 (immers).

Fig. 5.—Transverse section through the epithelial ridge. × 729 (immers).

Fig. 6.—Leucocyte embracing a spindle body; a compact body by it. × 729 (immers).

Fig. 7 *a*.—Spindle body with two bundles. × 380.
b.—Spindle body with three bundles. × 800.
c.—Spindle body with a constriction. × 380.
d.—Spindle body with a bundle bent within the membrane. × 380.
e.—Fragment of spindle body. × 380.
f.—Spindle body with fibres running in various directions. × 380.
g.—Spindle body in the dendritic organ with very loose fibres in the cell membrane. × 729.

Fig. 8 *a*.—, *b*.—, *c*.—} Three spindle bodies treated with acidulated methyl green. × 490.

Fig. 9.—Transverse section through the mantle perpendicular to the pallial sinus. Here two epithelial ridges are seen as the section passes the point of bifurcation of the pallial sinus. × 80.

Fig. 10.—Transverse section through the mantle perpendicular to the epithelial ridge. Hæmatoxylin-orange G-erythrosin preparation. × 490.

Fig. 11.—Portion of a section of the mantle. × 420.

Fig. 12.—Dendritic organs (*d. o.*) on the ventral body wall exposed. Sublimate preparation. × 2.

Fig. 13.—Transverse section through a dendritic organ of the adult. × 500.

Fig. 14.—Transverse section through the young *Lingula* of 5.5 mm. shell length, showing only the dendritic organ. × 729 (immers).

Fig. 15.—Blood corpuscle in a stage of transformation into the compact body. The nucleus is pushed aside and the cytoplasm becomes granular. × 729 (immers).

Fig. 16.—Transverse section through the epithelial ridge of the adult. A compact body is imbedded in the epithelium. Three blood corpuscles are shown for comparison. × 729 (immers).

Fig. 17.—Two compact bodies of different sizes; both are much smaller than usual compact bodies. The splitting can here be seen. × 729 (immers).

Fig. 18.—Compact body from the dendritic organ, half of which has been metamorphosed into fibres. × 729 (immers).

Fig. 19.—Spindle body with the remnant of a compact body from a section through the dendritic organ. × 729 (immers).

Plate II.

Fig. 20.—Left anterior portion of a young *Lingula* of 5 mm. shell length, showing the otocyst. Unstained clarified preparation. × 80.

Fig. 21.—Sagittal section through a young *Lingula* of 6.5 mm. shell length. Acetic-sublimate preparation. × 80. *lv.* liver lobule.

Fig. 22.—Otocyst of the same section as that drawn in Fig. 21. × 500. *otl.* otoliths.

Fig. 23.—Portion of sagittal section through a young *Lingula* of 6.5 mm. shell length, showing the heart. × 380.

Fig. 24.—Portion of sagittal section through a young *Lingula* of 7.5 mm. shell length, showing the heart and the muscle running below it. × 729 (immers).

Fig. 25.—Spermatozoa, from living material. × 500.

Fig. 26.—Portion of transverse section through young *Lingula* of 5.5 mm. shell length, showing the young ova in the ileo-parietal band. A portion of the nephridium is also represented. × 729 (immers).

Fig. 27.—Section through the ovary of the adult. × 729 (immers).

Fig. 28.—Full grown ovarian ovum from the above section. × 490.

Fig. 29.—Three nuclei of ovarian ova, each with a principal nucleolus and accessory nucleoli.

Fig. 30.—Section through the ovary of an old individual killed late in the autumn. × 490.

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Art. 2.—Nitrilosulphates. By E. DIVERS and T. HAGA, Science College, Imperial University, Tōkyō.

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TOKYO, JAPAN.

VOL. XVII., ARTICLE 6.

On Some Fossils from the Islands of Formosa and Riu-Kiu (=Loo Choo).

By

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Geological Department, British Museum,

and

Richard Holland,
Hon. Treasurer, Zoologists' Association, London.

With Plates I—IV.

(Communicated by Prof. Kotō).

CONTENTS.

I. INTRODUCTION.

The following report on some fossils from Formosa and the Riū-Kiū Islands has been drawn up from material collected by Mr. S. Yoshiwara, and entrusted to us for examination and description by Professor Dr. Kotō of the Science College, Imperial University, Tōkyō, Japan. Three groups of organisms are represented in the collection, *viz.*, Bryozoa, Foraminifera and plants, the different members of which exhibit certain characters enabling us to recognize particular geological horizons.

The oldest rock identified is an Orbitoidal-limestone which occurs in both the Pacific areas referred to; an example from Riū-Kiū (Iriomoté Island) exhibiting an abundance of organisms of an exceedingly dwarfed character (see Pl. I, fig. 4). We have already called attention to the presence of this limestone at Formosa,[1] in which we recognized *Orbitoides* (*Lepidocyclina*) *Verbeeki* associated with *Lithothamnium* (*Rosenbergi*=) *ramosissimum*, and other microzoa, and which we regarded as of Miocene age.

On the present occasion we have determined similar and additional fossils in this material, all of which appear to confirm our first views as to its geological horizon; moreover the presence of *Lithothamnium ramosissimum* so abundantly represented in the "Leithakalk" of the Vienna Basin, would strongly suggest that these Pacific limestones might be referred to the "Tortonian" stage of the Miocene epoch, that is, according to the European standard of geological nomenclature.

Of presumably much later date than the Orbitoidal rock first referred to, the collection contains examples of a raised

1. Newton and Holland: *Journ. Geol. Soc. Tōkyō*, 1900, Vol. VII, pp. 1–4.

coral-reef formation which enters into the structure of the Riū-Kiū Islands. A certain modern facies possessed by this material has inclined us to regard it as of Pleistocene age. It is in fact very similar lithologically to some of the reef limestones of Christmas Island (Indian Ocean) which immediately succeed the Miocene beds of that area and which have been ably described by Dr. ANDREWS and his co-adjutors in "The Christmas Island Report" of 1900.

A slight variation in colour and lithological character is observed in our specimens of the Riū-Kiū reef-limestones, and they differ sometimes with regard to the prevailing Foraminifera contained in them. Thus the rock from Unten on the west coast of Okinawa is crowded with *Operculina complanata* and *Pulvinulina repanda*. On the other hand in the rock from Motubu near Unten the *Operculina* is absent though the *Pulvinulina* is plentiful. In addition to these organisms, there occurs a modern type of *Lithothamnium*.

The age of the raised reef-limestones of the Pacific Islands has always been a troublesome problem to the geologist, insuperable difficulties becoming manifest when he attempts to divide up these deposits into the various horizons of the Tertiary and Quaternary systems.

Such a result can only be attained by an examination of carefully collected material from the different terraces of elevation, so that each assemblage or colony of organisms would be available for study and comparison. The present samples are by no means sufficient to allow us, with any certainty to make a definite statement on this subject and we prefer, tentatively, to regard our specimens from the Riū-Kiū Group as favouring the view that they belong to some part of the Post-Pliocene series.

This collection contains a third material consisting of a loose, sand earth of dark colour, which was obtained from Itoman, Southern Okinawa, where it is said to be "overderlaid discordantly by raised coral-reefs." Whether this represents detrital matter brought down from the interior or a mere surface accumulation such as a beach deposit, the fact remains that it is largely composed of foraminiferal tests belonging to forms found in the surrounding seas.

So far as the literature is concerned very little is apparently known of the palæontoloy of either Formosa or the Riū-Kiū Islands.

One of the earliest references on this subject is by Mr. ARTHUR CORNER who[1] recorded the occurrence of "*Monstis Hawai*" in Formosa, on the top of a high cliff of fossiliferous limestone at a place called the Dragon's Head, which he thought indicated the "Permian period of Palæozoic times."

According to H. B. GUPPY[2] a modern limestone formation at Ape's Hill, Takaw, S.W. Formosa, has yielded *Scutella*, *Cyclolites*, *Ostrea*, *Pecten*, etc., although none of them properly determinable and therefore of little importance for horizonal purposes. KLEINWÄCHTER[3] follows with a coloured geological map of southern Formosa, and alludes to "*Lithostrotia*" occurring in the Mountain limestone of that area, besides recording "*Nummus lævigata*," as an additional fossil to GUPPY's list of specimens found in the limestone of Ape's Hill. In the "Geological Remarks" of this same memoir mention is made of the

1. *Proc. Roy. Geogr. Soc.* (London) 1875. Vol. XIX, p. 515. *Monotis Hawai* was originally described by Meek and Hayden from the Permian rocks of north-eastern Kansas (*Trans. Albany Institute* 1858. Vol. 4).

2. *Journ. North China Branch Roy. Asiatic Soc.* 1882, Vol. 16 (*n. s.*), pp. 13–16.

3. Same Journal, 1884, Vol. 18 (*n. s.*), pp. 37–53, with a geological map of South Formosa.

reef-limestones of southern Formosa, occurring at various altitudes above the sea level forming a compact rock "built up by generations of Zoophytes."

Several fossils [*Pecten*, *Ostrea*, *Lutraria ?*, *Cardium ?*, *Echinodiscus* (*Amphiope*) *bioculatus* Agassiz, *E. bisperforatus*, and ? Crustacean fragment] found in association with the coal-beds by Mr. David Tyzack, at Kelung, North Formosa, were determined as of Miocene age by Professor G. A. Lebour[1] in 1885.

Prof. Kotō,[2] next, briefly refers to the raised coral reef-limestones of Formosa and Riū-Kiū, and also recognizes in the latter-named Group a three-fold zonal structure, synonomous with that observed by Prof. E. Suess in the Lesser Antilles: (1) the volcanic belt; (2) the mountainous islands; (3) the exterior belt, which comprises the Miocene and Quaternary formations.

Further, as previously mentioned, the presence of an Orbitoidal-limestone in the northern part of Formosa was made known by ourselves, rather more than twelve months since, after an examination of some microscopical sections of fossiliferous rocks forwarded to us by Professor Kotō. From the character of the organisms detected in those sections we were inclined to regard the limestone as of Miocene age.

In concluding these preliminary observations we desire to express our best acknowledgments to Mr. H. W. Burrows, F.G.S., for the interest he has taken in our work and the help he has afforded us more especially in the preparation of many of those beautiful photographs which adorn our plates. We wish to thank, also, Professor Dr. Kotō for the privilege of being allowed to work out this material, our report upon which, we hope, may

1. *Trans. North England Instit. Min. Engineers Newcastle*, 1885. Vol. 34, pp. 77–81, etc.
2. Journ. Coll. Sci. Imp. Univ. Tokyo, 1899. Vol. XI, Part 2, pp. 90, 98.

form an acceptable contribution to the palæontological history of these Islands.

II. DESCRIPTION OF THE FOSSILS.

A. Bryozoa.

Cellepora formosensis, sp. nov.

(Plate II, figs. 2, 4, 5 and 6; Plate III, fig. 1; Plate IV.)

In some rough "Notes on Microscopic Sections of Limestones" from Formosa which we furnished to Dr. Kotō of Japan in 1900 and which (although we had not prepared them for publication) Dr. Kotō kindly published in the *Journ. Geol. Soc. Tokyo*, June 1900, Vol. VII, we referred several times to the occurence of a large "chambered organism." The material which Dr. Kotō has now sent to us includes the Formosa fossil which is shown of the natural size in Plate II, figs. 4, 5, and 6. This fossil is very dense and highly crystalline; it is entirely free from matrix and had been cut in two directions before it reached our hands. On polished surfaces and in thin sections it still furnishes details of its microscopic structure. Figure 6 of Plate II gives the vertical view of the specimen, but the cutting process has robbed it of some of its height; fig. 4 gives the basal view and there the loss sustained by cutting is shown by plaster filling; fig. 5 gives the actual view of the basal section of the upper segment shewn in fig. 6; Plate IV is a reproduction of an enlarged photograph of the central portion of fig. 5; fig. 2 of Plate II presents a microscopic section of the fossil viewed by transmitted light; and fig. 1 of Plate III gives

another portion of the same section as seen by reflected light with a black back-ground. The fossil is identical with the "chambered organism" referred to in the notes mentioned above and proves to be an undescribed species of Bryozoa of the genus *Cellepora* which we designate, from the place of its origin, *Cellepora formosensis.*

It is notoriously difficult to distinguish accurately for purposes of diagnosing species, the "characters" of fossil specimens of such Bryozoa as the Celleporæ. This is so even in the case of specimens from beds such as those of the English Coralline Crag where the fossils have suffered no very great change in their calcareous parts during the process of fossilization. It is more especially difficult where, in cases like the present, the organisms have become thoroughly mineralised and all the chambers have become filled up with crystalline calcite.

By cutting such a specimen however in two directions at right angles to each other and viewing the polished surfaces as solid objects by reflected light it is possible to see some of the characteristic features of the individual cells. This is made possible by the fact that the polished crystalline calcite reveals the microscopic structure for a short distance below the surface and so the apertures and other features of some of the earlier formed cells, which have become imbedded but not altogether obscured by the subsequent growth of overlying chambers, are brought to light. In a word the presence of clear crystalline calcite in the chambers of the fossil, *to the exclusion of an opaque substance,* furnishes an approach to the opportunity for examination by section which is given by the comparatively unaltered Celleporæ from the English Crags. Failing a cut and polished surface of a crystalline specimen it has been found serviceable to examine as an opaque object an ordinary microscopic section if it be not cut too thin.

Figure 1 of Plate III shows such a section cut from our Formosan specimen and viewed by reflected light and with a black background. Fig. 2 of the same Plate, placed beside it for comparison, is a micro-photograph of the same magnification from the cut surface of a solid segment of a specimen of *Cellepora tubigera* BUSK, from the English Coralline Crag of Broom Hill, Suffolk. It will be observed that in this latter specimen, in several places *at the bottom of the cells cut through*, there can be plainly seen the orbicular orifice of the cell below with its sinus in front; and in other places can be seen the vestiges of the avicularia. Not so clearly, but nevertheless far more plainly than in the same slide viewed by transmitted light (compare Plate II, fig. 2, and Plate III, fig. 1) the main zoœcial characters are shown in the section from the Formosan specimen (Plate III, fig. 1, and Plate IV). After a careful examination of several sections as well as the cut surfaces of our specimen we are able to give the "characters" of the species as follows:—

Zoarium: Massive; surface mammillate. The base of the specimen under description was free. The whole was supported by growing around some branched organism which became imbedded along the long axis of the fossil, and whose place is now occupied by the cores of stalagmitic material shown in section in the middle portions of Plate IV and of fig. 5 of Plate II. The mammillate character of the surface is indicated not only by the present exterior aspect of the fossil, which might conceivably be due to erosion, but also by the lines of growth shown in the horizontal section, Plate II, fig. 5. Dimensions of specimens:—Height=75 millim. Base=75×80 millim.

Zoœcia: Urceolate; contiguous; walls thin; aperture not more than one half the diameter of the cell; orbicular, with a sinus in front.

Avicularia: Numerous; irregularly distributed over the zoarium, arising sometimes in front and sometimes at the sides of the apertures.

Cellepora formosensis is undoubtedly allied to the *Cellepora pumicosa* Linn. of our present seas; to the *C. tubigera* of the English Crag, which is by some authors looked upon as identical with *C. pumicosa;* and to *C. mammillata* Busk. Generally, *C. formosensis* differs from these in the relatively larger dimensions of the zoœcia and the more delicate walls, and in the arrangement of the avicularia.

Occurrence: The figured specimen was obtained by Mr. Yoshiwara from the limestones of Sha-kō-kō near Roku-ryo, North of Kee-lung harbour, North Formosa. We found the same species in the limestones from Rei-suiko, 10 miles S.W. of Tai-hoku, the chief town of Formosa; also in the limestones from Shin-ko-gai, 10 miles due S. of Tai-hoku. We have now found it in the Orbitoidal limestone from Sonai, Iriomoté Island, Riū-Kiū (=Loo Choo Islands of European maps). All these limestones appear to be of Miocene age.

Cellepora sp. (Pl. III, fig. 7.)

Our slides contain a few specimens of another species of *Cellepora* with smaller cells but we have not had sufficient material to enable us to work out the species.

One of the specimens is shown in fig. 7 of Plate III, associated with *Orbitoides* (*Lepidocyclina*) *angularis*.

Occurrence: In the Orbitoidal limestone from Sonai, Iriomoté Island, Riū-Kiū.

B. Foraminifera.

Orbitoides.

The genus *Orbitoides* is represented in the limestone from Iriomoté Island by innumerable specimens—the rock being crowded with them throughout (see Plate I, fig. 4)—but there appear to be but two, or at the most three, species present. The striking feature about them is their small size. The dimensions of the specimens are remarkably uniform and there is no trace in our slides of large examples. All the specimens belong to the *Lepidocycline* group which is characterised by the possession of lozenge-shaped or spatuliform chambers in the median plane.

Orbitoides (Lepidocyclina) angularis, sp. nov.

(Pl. I, figs. 1 and 6, Pl. III, fig. 7.)

Characters: The species is dimorphic—that is to say, some individuals have the initial chamber large, while in others it is very small, practically invisible. They are distinguished here as Form A and Form B respectively.

Form A.—Shell discoidal; central area of disc somewhat flattened and slightly tuberculate; from edge of this flattened area the shell rapidly decreases in thickness; margin of disc thin, slightly swollen and rounded at the extreme edge. Chambers above and below the median plane irregular in shape but somewhat regularly disposed. External dimensions of shell 3 mm. in

width by 1 mm. in thickness; inside dimensions of the two central chambers taken together about .21 mm. in width by .14 mm. in depth; chambers of the median plane very minute; long axis of lozenge about .02 mm.

Form B.—Similar to the Form A in all respects save that the initial chamber is too small to be measured.

Occurrence: Very common in the limestone from Sonai, Iriomoté Island, Yayeyama Group. The Form A in our slides is more common than the Form B.

Orbitoides (Lepidocyclina) sumatrensis, Brady.

(Pl. I, fig. 7.)

O. sumatrensis, Brady: *Geol. Mag.*, 1875, p. 536, Pl. XIV, fig. 3; and *Jaarb, Mijn. Ned. Ooste-Indië*, 1878, Vol. VII, Pt. II, Pl. II, fig. 3.

O. (*Lepidocyclina*) *sumatrensis*, Newton and Holland: *Ann. Mag. Nat. Hist.*, 1899. Ser. VII, Vol. III, p. 259, Pl. X, figs. 7–12.

O. (*Lepidocyclina*) *Sumatrensis*, Jones and Chapman: *Monograph of Christmas Island*, 1900, p. 244, Pl. XX, fig. 6.

To this species we refer the form shown in fig. 7 on Plate I. It differs from the Sumatran and Bornean specimens only in its smaller dimensions which are 1.5 mm. by .85 mm.

Occurrence: Met with rarely in the Iriomoté limestone. The Sumatran specimens were collected by Dr. Verbeek from the marl-rock of Nias Island, off the West coast of Sumatra.

Those from Borneo were obtained from the Gomanton Hill limestone and from pebbles taken from the bed of the River Malinam. The examples recorded by Messrs. JONES and CHAPMAN came from the Tertiary limestones of Christmas Island.

Orbitoides (Lepidocyclina) Verbeeki, NEWTON and HOLLAND.

O. papyracea, BRADY: *Geol. Mag.*, 1875. Pl. XIV, fig. 1, p. 535 (*non* Boubée).

Lepidocyclina species g. and k., VERBEEK et FENNEMA: *Descr. géol. de Java et Madoura*, 1896. Vol. I, Pl. XI, figs. 173–175, 177–180; Vol. II, p. 1178.

O. (*Lepidocyclina*) *Verbeeki*, NEWTON and HOLLAND: *Ann. Mag. Nat. Hist.*, 1899. Ser. VII, Vol. III, p. 257, Pl. IX, figs. 7–11; Pl. X, fig. 1.

O. (*Lepidocyclina*) *Verbeeki*, JONES and CHAPMAN: *Monograph of Christmas Island*, 1900, p. 245.

One or two very small forms, which are probably to be referred to this species, occur in the Iriomoté limestone. The Sumatran specimens were collected by Dr. VERBEEK from limestones on the W. coast of that Island and from the adjacent Island of Nias. The Bornean specimens were obtained from pebbles found in the bed of the River Malinam. The examples recorded by Messrs. JONES and CHAPMAN came from the Tertiary limestones of Christmas Island. We have ourselves noted it from the Formosan limestones (Journ. Geol. Soc. Tōkyō, June 1900, Vol. VII).

Operculina complanata, (DEFRANCE).
(Plate I, figs. 3 and 5, Plate III, fig. 3.)

Lenticulites complanata, DEFRANCE: *Dict. Sci. Nat.*, 1822. Vol. XXV, p. 453.
Operculina complanata, ORBIGNY: *Ann. Sci. Nat.*, 1826. Vol. VII, p. 281, Pl. XIV, figs. 7–10.

The *Operculinæ* from the Riū-Kiū material are numerous and very interesting. In some cases the rock specimens described as from "raised coral reefs" are very largely composed of them and the reef at Kamé-zu on the south coast of Tokuno-shima has yielded the gigantic specimen shown on Plate I. Figs. 3 and 5, a specimen which in its unbroken condition, must have been one of the largest Operculines yet known. Fig. 5 gives the exact present size of this noteworthy specimen and the dotted line indicates what its dimensions must have been before the later chambers had been for the most part broken away. Some part of these later chambers have been destroyed since the specimen came into our possession. In order to determine the fossil at all we were obliged to grind away each surface so as to render the interior chambering visible. The irregularity and brittle character of the test made it impossible to proceed as far as could have been desired with the grinding process but enough has been done to allow of a photograph being taken which shows clearly the Operculine chambers.

We have considered it best on the whole to refer these examples to the species *complanata* because they undoubtedly belong to the group of which *O. complanata* (DEFRANCE) may be taken as the type. At the same time we think there has

been a tendency, particularly among English authors, to make this species include too great a variety of forms. There is for instance a very considerable and constant difference between the modern *Operculina complanata* as figured in the Challenger Report (Vol. IX, Pl. CXII, figs. 3, 4, 5, and 8) and the *O. complanata* from the Burdigalian (=Langhian) of Bordeaux. The whole genus needs careful revision. This, however, is not the occasion for any attempt in that direction.

Occurrence: *Operculina complanata* in recent seas is essentially a shallow water form and is met with only in tropical and sub-tropical latitudes. As a fossil it is recorded from the chalk of Maestricht and Minnesota, from the Eocene of Central Europe and India, from the Miocene of Italy and of Muddy Creek (Victoria) and in great profusion in the Burdigalian (=Langhian) of the Bordeaux area. The Riū-Kiū specimens are from the "raised coral reefs" of Tokuno-shima and Okino-yerabu; and from a "10 foot thick bed in raised coral reef" from Unten, west coast of the Island of Okinawa.

Operculina complanata, (Defrance) var **granulosa,** Leymerie.
(Pl. III, figs. 4 and 5.)

Operculina granulosa, Leymerie: *Mem. Soc. géol. France*, 1846. Ser. II, Vol. I, p. 359, Pl. XIII, fig. 12 *a*, *b*.
O. complanata, var. *granulosa*, H. B. Brady: *Chall. Report*, 1884. Vol. IX, p. 743, Pl. CXII, figs. 6, 7, 9 and 10.

This species is exceedingly numerous in the dark earthy looking material from Itoman, S. Okinawa, which is described as "overlaid discordantly by the raised coral reefs." Besides the

Operculinæ it has yielded numerous other species of Foraminifera as will be seen from the appended "Tabular Statement of Determinations."

Occurrence: Itoman, Southern Okinawa, Riū-Kiū Islands.

Carpenteria, sp. (Pl. II, fig. 3: Pl. III, fig. 6.)

The genus *Carpenteria* has played an important part in building up the Orbitoidal-limestone of Iriomoté Island. Fragments are very numerous in the sections and possibly two or more species are present. We have however not had sufficient material at our disposal to satisfy us in referring our examples to definite "species." The specimen shown in Pl. II, fig. 3 appears to bear a strong resemblance to the *Carpenteria capitata* described by Messrs. Jones and Chapman from the Tertiary limestones of Christmas Island (Monograph of Christmas Island, p. 246, Pl. XX, fig. 7). The specimen figured on Plate III, fig. 6, does not at first sight look like a *Carpenteria*, but from numerous specimens in our slides connecting it with undoubted examples of the genus there can be no question as to its affinities.

Occurrence: Iriomoté limestone, Riū-Kiū Islands.

Linderina, sp.? (Pl. I, fig. 2).

We are in some doubt as to the proper affinities of the organism represented in the figure quoted above. It certainly bears a very striking resemblance to the horizontal section of *Linderina brugesi* Schlumberger, as figured by M. Schlumberger (Bull. Soc. géol. France, 1893, Sér. III, Vol. XXI, Pl. III, fig. 9); and in the Orbitoidal limestones of Gomauton Hill,

Borneo, we found numerous undoubted specimens of the genus (Ann. Mag. Nat. Hist., 1899, Sér. VII, Vol. III, p. 262, Pl. X, fig. 6). The most careful search through our slides cut from the Orbitoidal limestone of Iriomoté Island, however, yields no specimen which will throw further light on the organism here represented and we can only assign it provisionally to the genus *Linderina*.

Occurrence : Iriomoté limestone, Riū-Kiū Island.

Amphistegina vulgaris Orbigny. (Pl. II, fig. 1.)

Amphistegina vulgaris, d'Orbigny : 1823, Modèles, Liv. 2e No. 40, *Ann. Soc. Nat.*, 1826. Vol. VII, p. 305, No. 8.
Amphistegina lessonii, d'Orbigny : 1823, Modèles, Liv. 4, No. 98, *Ann. Sci. Nat.*, 1826. Vol. VII, p. 304, No. 3.
Amphistegina lessonii, Brady : 1884, *Chall. Report*, pp. 740, 741, Pl. CXI, figs. 1–7.

Associated with the *Orbitoides*, *Carpenteria* and *Lithothamnium* which make up the great mass of the Iriomoté Island limestone, are a few other Foraminifera among which *Amphistegina vulgaris* is the most conspicuous. The specimens are small in common with most of the other organisms in the limestone, but they are fairly numerous.

Occurrence : *Amphistegina vulgaris* (including *lessonii*) is in recent seas mostly confined to tropical and subtropical latitudes and as a rule is found in shallow water. It has been recorded as a fossil from the Eocene of France and Bavaria. It is very characteristic of the Miocene deposits generally and has been

found in the Pliocene of many localities. Our examples are from the Iriomoté limestone, Riū-Kiū Islands.

Pulvinulina repanda, (F. and M.)

Nautilus repandus, Fichtel and Moll: 1798, *Test. Micr.,* p. 35, Pl. III, figs. *a–d.*

Pulvinulina repanda, H. B. Brady: *Chall. Report,* 1884, Pl. CIV, fig. 18, p. 684.

Pulvinulina repanda, Jones and Chapman: *Report Christmas Island,* 1900, p. 228, Pl. XX, Fig. 1.

This well known form occurs plentifully in the "raised Coral-reefs" at Tokuno-shima, Unten and Motubu. At Tokuno-shima it is found in association with *Operculina complanata.* In the other localities mentioned the *Operculinæ* are absent.

C. Plantæ.

Lithothamnium ramosissimum, Reuss.
(Plate I, fig. 8.)

Nullipora ramosissima, Reuss: *Nat. Abhandl. Haidinger,* 1848. Vol. II, Part I, Pl. III, figs. 10, 11, p. 29.

Nullipora ramosissima, Unger: *Denkschr. K. Akad. Wiss.* (Wien), 1857. Vol. XIV, Pl. V, figs. 18–22, pp. 23, 38.

Lithothamnium ramosissimum, Gümbel: *Abhandl. K. bayerischen Akad. Wiss. München,* 1871. Vol. XI, Part I, p. 34, Pl. I, fig. 1.

Cumulipora Rosenbergi, MARTIN : *Samml. Geol. Reichs-Mus. Leiden*, 1881. Vol. I, Part I, pp. 12–14, 64, Pl. III, fig. 7.

Lithothamnium Rosenbergi, MARTIN : *Samml. Geol. Reichs-Mus. Leiden*, 1881. Vol. I, Part II, pp. 70, 79 ; and *ibid.*, 1882. Vol. I, Part III, pp. 153, 155.

Lithothamnium ramosissimum, ROTHPLETZ : *Zeitsch. Deutsch. Geol. Ges.*, 1891. Vol. XLIII, p. 320.

Lithothamnium ramosissimum, K. NISHIWADA : *Journ. College Sci. Imp. Univ. Tokyo*, 1894. Vol. VII, Part III, p. 233, Pl. XXIX, figs. 1–3.

Lithothamnium (*Cumulipora*) *Rosenbergi*, NEWTON and HOLLAND : *Journ. Geol. Soc. Tokyo*, 1900. Vol. VII, No. 81, p. 1.

Lithothamnium Rosenbergi, S. YOSHIWARA : *Journ. Geol. Soc. Tokyo*, 1900. Vol. VII, No. 81, p. 22.

Besides the organisms already referred to as occurring in the Orbitoidal-limestones of the Riū-Kiū Islands we have to add that of the well-known Nullipore.

Lithothamnium ramosissimum. Our illustration (Pl. I, fig. 8) exhibits a vertical section (× 70) of one of the branches made up of the innumerable rectangular cells and arranged in the usual concentric layers. Among fossil Calcareous Algæ this species is of considerable importance as a reef-building organism. At Leitha near Vienna it enters largely into the structure of the so-called "Leithakalke," a limestone belonging to the Middle Miocene and which according to Prof. DE LAPPARENT[1] may be included in the Tortonian division of that formation. It is of

1. Traité de Géologie, 1900, Ed. 4, p. 1547.

frequent occurrence in the limestones of many of the islands lying off the eastern coast of the Asiatic Continent, Mr. NISHIWADA having first recognized it in the rocks of Japan, although Dr. MARTIN at a much earlier period had reported its presence in the Pacific area, at Timor, New Guinea, Amboina, etc., but under the name of *L. Roseubergi*, a form which we now consider structurally equivalent to the *ramosissimum* of REUSS. The difficulty of defining the species of *Lithothamnium* found in a fossil state, has already been alluded to by Solms-Laubach[1] and others so that until the whole subject has been more systematically treated than heretofore, we have thought it advisable to place MARTIN's name in synonomy. Under MARTIN's species, also, we ventured to call attention last year to the appearance of this organism in the limestones of northern Formosa. The species is of interest from a stratigraphical point of view, because it is so far known only from rocks of the Miocene Period and probably indicates the middle portion known in Europe as the "Tortonian" stage.

Occurrence: Species of Miocene age have been recorded from Europe, Timor, New Guinea, Formosa, Japan, etc.; we have now identified it in the Orbitoidal-limestone of Sonai, Iriomoté Island, Riū-Kiū.

---

1. Fossil Botany by Solms-Laubach; English translation by H. E. F. GARNSEY, 1891 p. 45.

III. TABULAR STATEMENT OF DETERMINATIONS.

Genera and Species.	Orbitoidal Limestones.		"Raised Coal Reefs."		Material "overlaid discordantly by Raised Coral Reefs."
	Formosa.	Irionoté Island.	Tokunoshima & Uten.	Motubu near Uten.	Itoman, S. Okinawa.
A. Bryozoa.					
Cellepora formosensis, sp. nov....	×	×			
„ *species*...		×			
B. Foraminifera.					
Amphistegina vulgaris, ORB.		×			×
Biloculina bulloides, ORB....					×
„ *depressa*, ORB....					×
Bolivina costata, ORB.					×
„ *robusta*, BRADY....					×
Bulimina aculeata, ORB.					×
„ *elegans*, ORB.					×
Carpenteria sp.		×			
Cristellaria calcar, (LINN.)					×
„ *crepidula*, (F. and M.)...					×
„ *fragaria*, (GÜMBEL)					×
„ *tenuis*, (BORNEMANN)					×
Globigerina bulloides, ORB.					×
Linderina, sp.		×			
Marginulina glabra, ORB.					×
Miliolina oblonga, (MONTAG.)					×
„ *pulchella*, (ORB.)...					×
„ *seminulum*, (LINN.)					×
„ *tricarinata*, (ORB.)					×
„ sp.		×			
Nodosaria (*Dentalina*) *communis*, ORB.					×
„ *pyrula*, ORB.					×
„ *radicula*, LINN.					×
„ (*Dentalina*) *vertebralis*, (BATSCH)					×
Nonionina umbilicatula, (MONTAG.)...					×
Operculina complanata, (DEFRANCE)		×			
„ „ var. *granulosa*, LEYMERIE.					×
Orbitoides (*Lepidocyclina*) *angularis*, sp. nov. ...		×			
„ „ *sumatrensis*, BRADY. ...		×			
„ „ *Verbeeki*, NEWT. & HOLL.		×			
Planorbulina mediterranensis, ORB....					×
Polymorphina lactea, W. and J.					×
„ *communis*, ORB....					×
Polystomella craticulata, (F. and M.)					×
Pulvinulina repanda, (F. and M.)...			×	×	×
Rotalia Beccarii, (LINN.)...					×
„ *papillosa*, BRADY.					×
Sagrina raphanus, P. and J....					×
Spheroidina dehiscens, P. and J.					×
Textilaria gramen, ORB.					×
„ *quadrilatera*, SCHWAGER					×
Truncatulina precincta, (KARRER)...					×
„ *ungeriana*, (ORB.)					×
„ *Wuellerstorfi*, (SCHWAGER)...					×
Uvigerina pygmæa, ORB....					×
C. Plantæ.					
Lithothamnium ramosissimum, REUSS	×	×			
„ sp.			×	×	

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NOTE.—With the exception of specimen represented in Plate III, fig. 2, which is in the collection of Mr. H. W. BURROWS, the whole of the material described in this paper has been returned to Professor KOTÔ of Tokyo, Japan.

PLATE I.

V. EXPLANATION OF THE PLATES.

Plate I.

Orbitoides (*Lepidocyclina*) *angularis*, sp. nov., Form B. From Sonai, Iriometé Island, Yayeyama Group, Riū-Kiū.

FIG. 1.—Horizontal section ... ... × 40.

FIG. 6.—Vertical section ... ... × 35.

? *Linderina*, sp. From same locality as above.

FIG. 2.—Horizontal section ... ... × 60.

Operculina complanata, (DEFRANCE). Found in an elevated coral-reef at Kamézu, south coast of Tokuno-shima between Okinawa and Oshima, Riū-Kiū.

FIG. 3.—Side view ... ... × 2.

FIG. 5.— Do. ... ...nat. size.

Orbitoidal Limestone of Sonai, Iriomoté Island, Yayeyama Group, Riū-Kiū.

FIG. 4.—Section showing the small and crowded character of the organisms ... ... × 7.

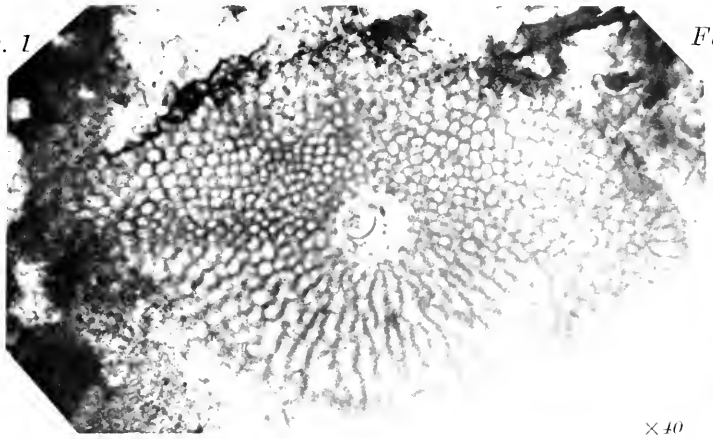
Orbitoides (*Lepidocyclina*) *sumatrensis*, BRADY. Form B. From the Orbitoidal limestone as above.

FIG. 7.—Vertical section ... ... × 60.

Lithothamnium ramosissimum, (REUSS). From same limestone and locality as before.

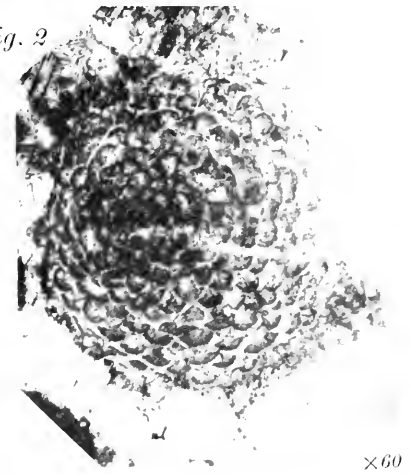
FIG. 8.—Vertical section of a branch, showing the concentrically arranged layers of rectangular cells.

Fig. 1



×40

Fig. 2

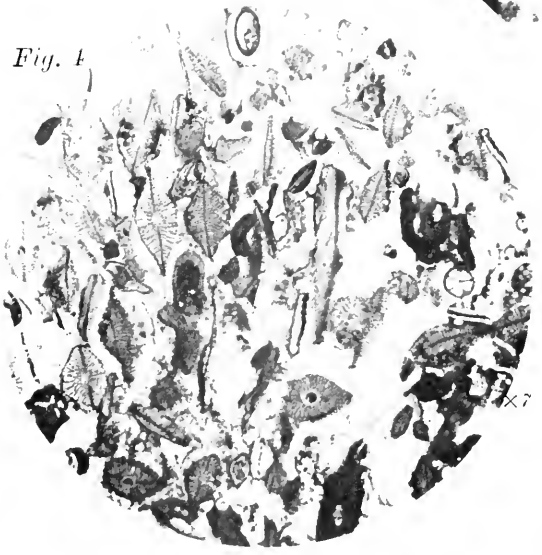


×60

Fig. 3.

×2

Fig. 4

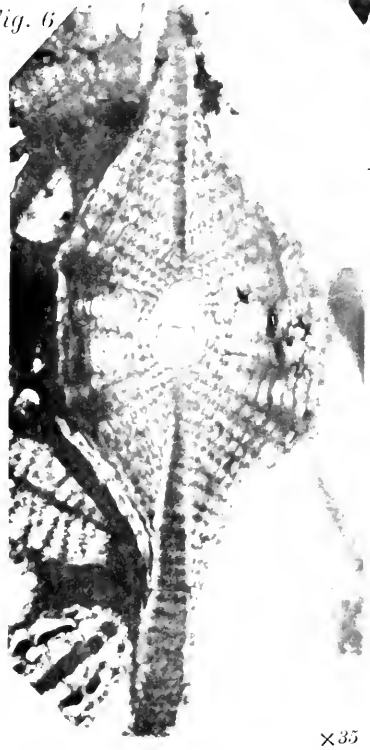


×7

Fig. 5

Nat. size.

Fig. 6



×35

Fig. 7

×60

Fig. 8

×70

H. W. Burrows,
P. Highley, } *Photo.*

TOKIO PRINTING CO.

ORGANISMS (FORAMINIFERA, &c.)
of the RIÛ-KIÛ LIMESTONES.

Plate II.

Amphistegina vulgaris, Orbigny. From the same Orbitoidal-limestone as before.

Fig. 1.—Oblique section ... ... × 60.

Cellepora formosensis, sp. nov. From the limestone of Sha-kō-kō, south east of Tō-shi-yen, Northern Formosa.

Fig. 2.—Section of specimen viewed by transmitted light ... × 20.

Fig. 4.—Basal view ... ...nat. size.

Fig. 5.—Horizontal section ... ...nat. size.

Fig. 6.—Side view ... ...nat. size.

Carpenteria sp. From the Orbitoidal limestone of Iriomoté Island, previously mentioned.

Fig. 3.—Vertical section ... ... × 20.

Fig. 1

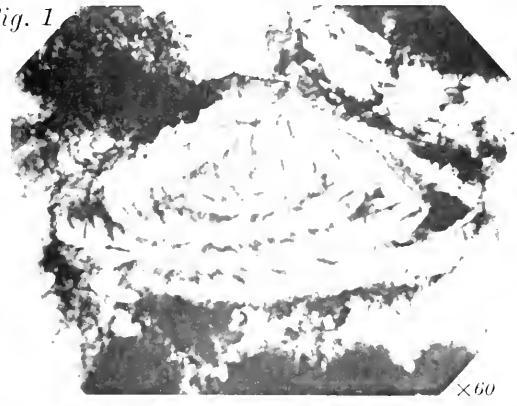


Fig. 2

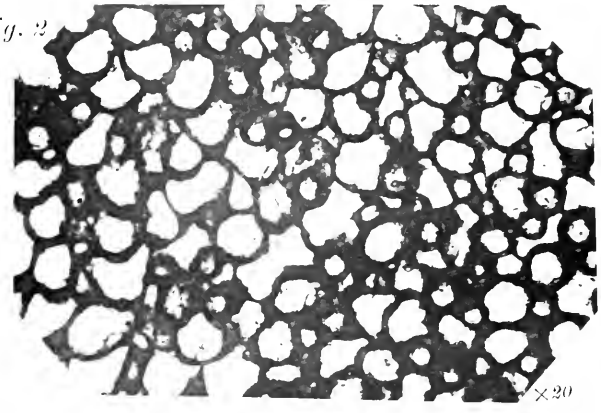


Fig. 3

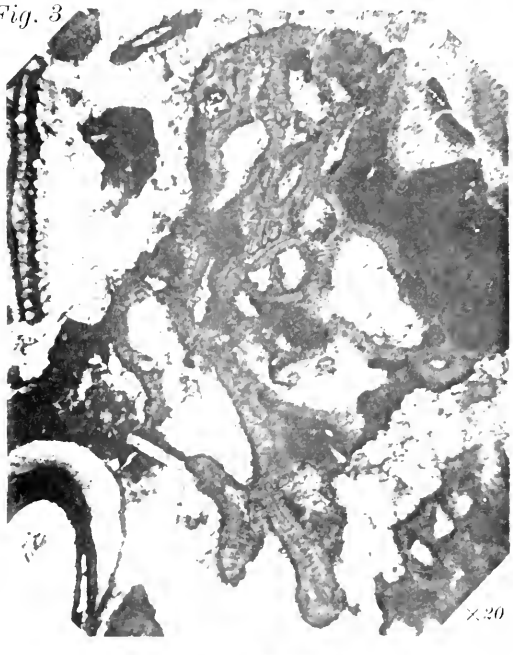


Fig. 4

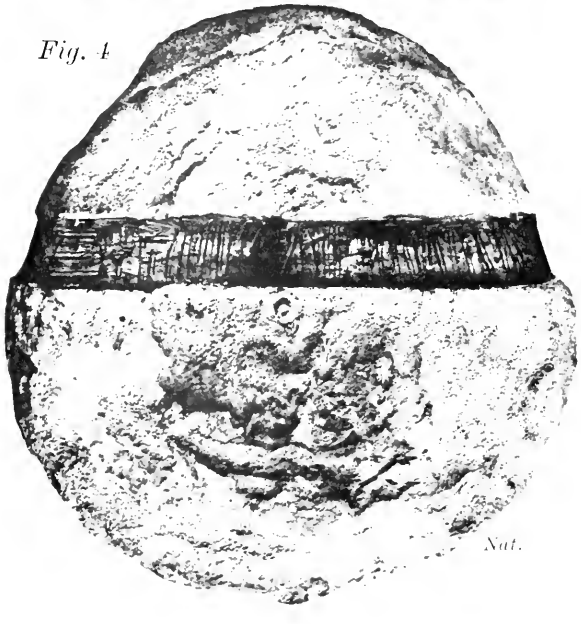


Fig. 5

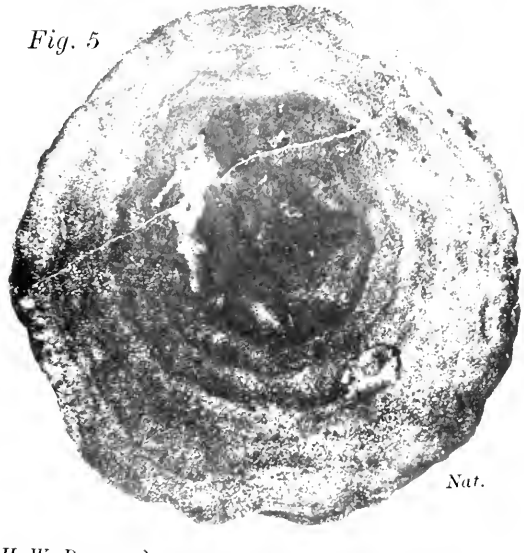
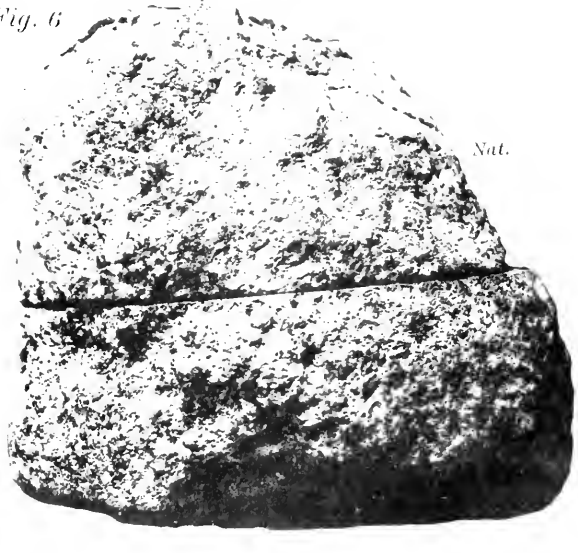


Fig. 6



H. W. Burrows, H. R. Holder, } Photo.

BRYOZOA AND FORAMINIFERA

from the LIMESTONES of FORMOSA and RIÛ-KIÛ.

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PLATE III.

Plate III.

Cellepora formosensis, sp. nov.

FIG. 1.—Section of specimen from Formosa figured on Plate II, viewed by reflected light.

Cellepora tubigera, BUSK. From the English Crag deposits—for comparison.

FIG. 2.—Section of a solid segment viewed by reflected light. × 20.

Operculina complanata, (DEFRANCE). Occurring in a 10 feet thick bed in raised coral reef; from Unten. West coast of the Island of Okinawa, Riū-Kiū.

Fig. 3.—Vertical sections... ... ... × 20.

Operculina complanata var. *granulosa*, LEYMERIE. Isolated form, from a loose sandy material occurring at Itoman, Southern Okinawa, Riū-Kiū.

FIG. 4.—Horizontal section ... ... × 15.

FIG. 5.—External view ... ... × 15.

Carpenteria sp. From the Iriomoté Island, Orbitoidal limestone, previously alluded to.

FIG. 6.—Vertical section... ... ... × 35.

Cellepora sp. Associated with *Orbitoides* (*Lepidocyclina*) *angularis*. Found in the Iriomoté Island Orbitoidal Limestone previously mentioned.

FIG. 7.—Section viewed by transmitted light ... ... × 20

Fig. 1

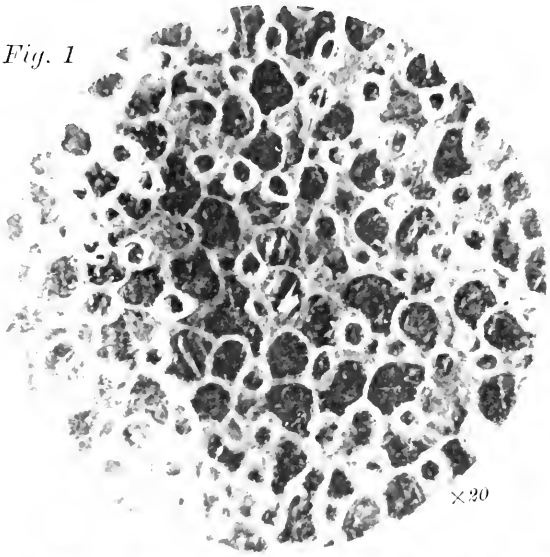


Fig. 2

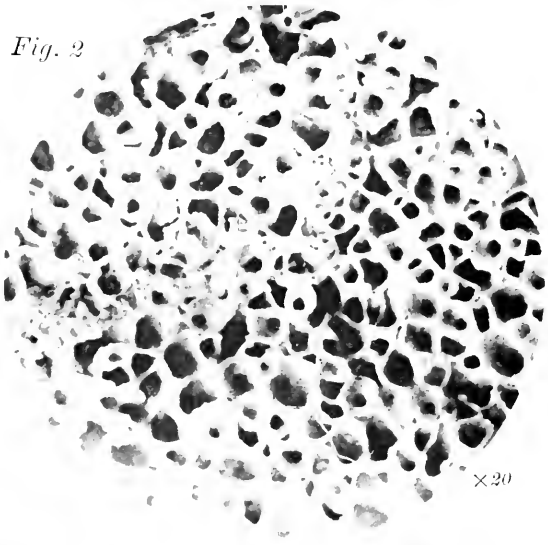


Fig. 3

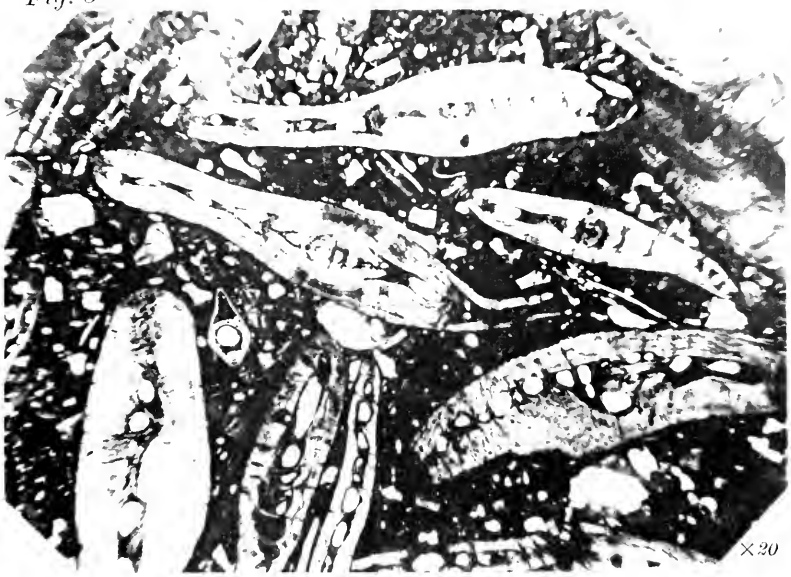


Fig. 4

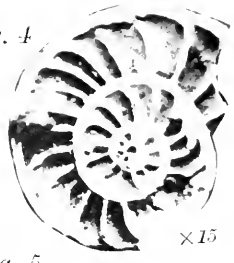


Fig. 5

Fig. 6

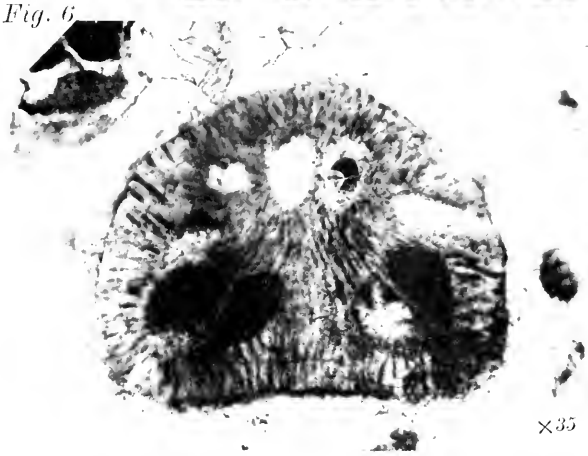
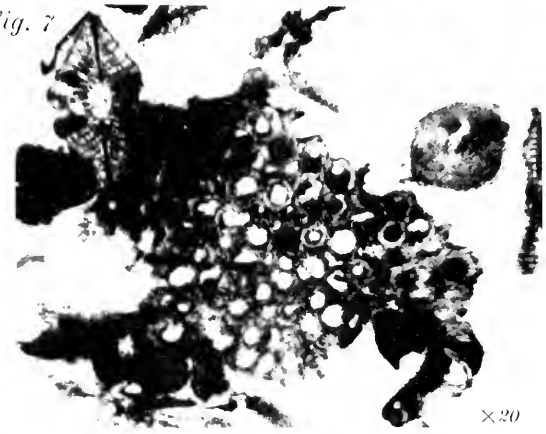


Fig. 7



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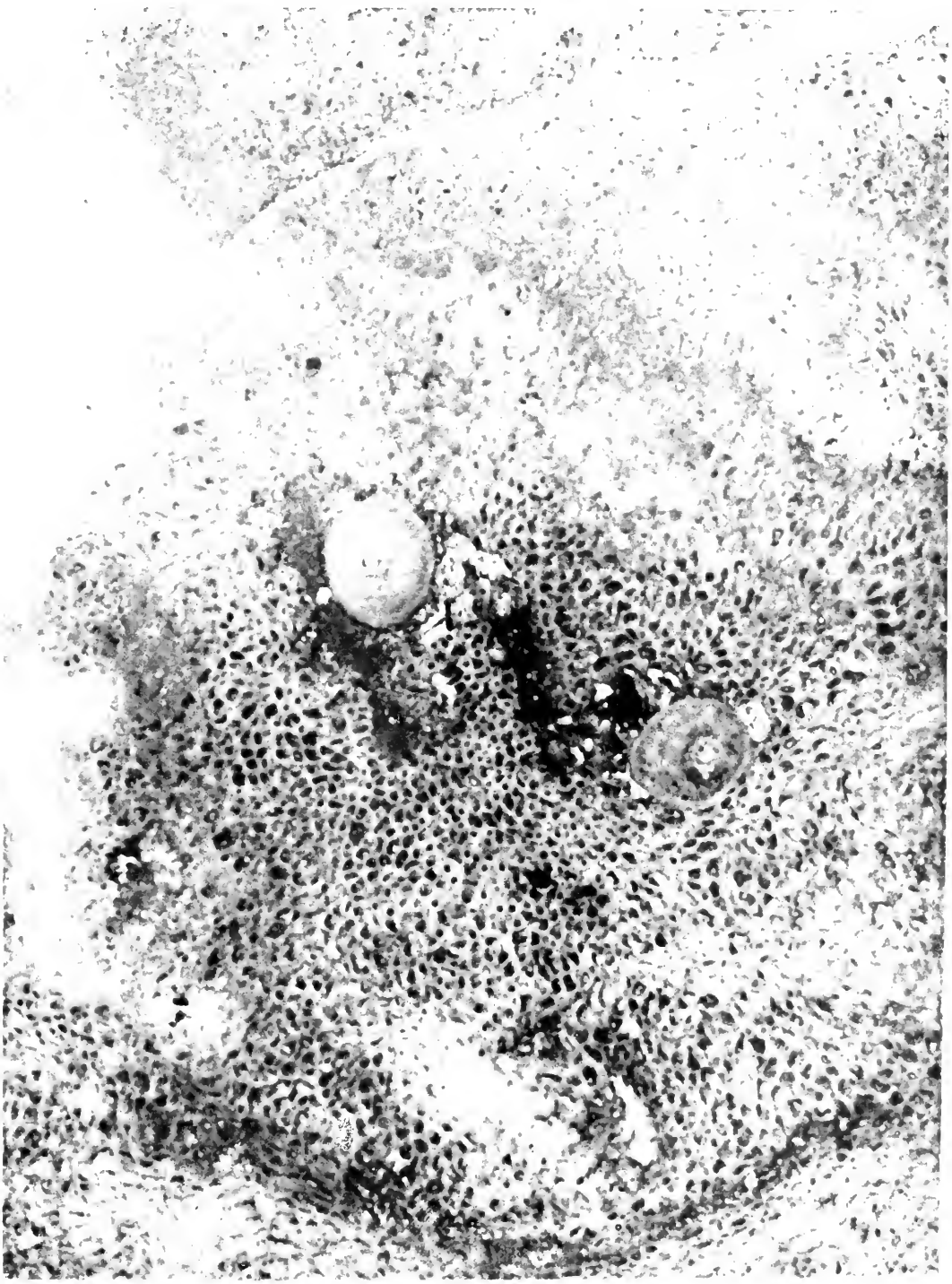
BRYOZOA AND FORAMINIFERA
from the LIMESTONES of FORMOSA and RIÛ-KIÛ.

Plate IV.

Cellepora formosensis, sp. nov. Central portion of specimen represented by Fig. 5 of Plate II, showing stalagmitic cores round which the organism has developed ... ... × 7



H. R. Holder, Photo.

CELLEPORA FORMOSENSIS, sp. nov.

Central part, magnified 7 diameters.

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JOURNAL OF THE COLLEGE OF SCIENCE, IMPERIAL UNIVERSITY,
TOKYO, JAPAN.

VOL. XVII, ARTICLE 7.

Some New Scyphomedusæ of Japan.

By

Kamakichi Kishinouye,

Imperial Fisheries Bureau, Tokyo.

With two plates.

Having been engaged for several years in collecting and studying the Scyphomedusæ of the waters around Japan, no less than twenty-two species of the group have thus far become known to me from the region mentioned. The majority of them are new to science, many showing noteworthy peculiarities in their structure. Some of them have already been described by me in the "Zoological Magazine" (Tokyo). In the present paper I propose to deal with a few forms that have come under my observation and which differ remarkably from the related forms hitherto known.

STAUROMEDUSÆ.

Stenoscyphidæ, nov. fam.

Stauromedusæ with simple, undivided umbrella margin, without adradial lobes; the eight principal tentacles are transformed

to adhesive anchors. Secondary tentacles are clustered in eight adradial groups. Coronal muscle ring-shaped. Adhesive peduncle at the aboral end of the umbrella.

This new family is proposed for a medusa which stands between *Tesseridæ* and *Lucernaridæ* and which thus serves to connect these two families. More precisely speaking, the medusa is closely allied to the subfamily *Depastridæ* on the one hand and to the subfamily *Haliclystidæ* on the other; at the same time it can not well be placed under either of these.

Stenoscyphus. nov. gen.

Stenoscyphidæ with four-chambered peduncle and eight separate genital glands.

Stenoscyphus inabai.

(Figs. 1, 2.)

Depastrum inabai, KISHINOUYE, 1893. In: Zool. Mag. Tokyo, Vol. V, pp. 416–418, with woodcuts. (In Japanese).

The umbrella or the calyx is very narrow and elongate, generally widening somewhat abruptly at the oral end, so that it presents the shape of a long prism or rather of a funnel with a long and wide tube. It is more or less quadrate in cross-section. The length (or height) is from two to three times the breadth at the umbrella margin. The latter is not divided into lobes or arms.

The peduncle is more or less quadrate like the umbrella and possesses four interradial grooves (fig. 2). Its length is

from one half to one-fifth that of the umbrella. The adhesive surface at the aboral end is wide. The cavity of the peduncle or the basal stomach is divided into four chambers by the union of the interradial tæniola. In young specimens of a length under 9 mm., the peduncle is not divided into separate chambers. The interradial tæniola begin to unite near the pyloric portion.

The exumbrella is generally smooth. It is furnished here and there with small groups of nematocysts. It is very thin and consists of three layers: the ectoderm, the gelatinous layer and the endoderm.

The whole surface of the subumbrella is beset with very large, spherical groups of nematocysts, each group opening by a small pore at the end of a little neck. This neck projects only a little above the surface of the subumbrella.

The coronal or marginal muscle is ring-shaped; it is not divided, but well developed. In preserved specimens, this muscle is generally contracted so that the animal is rather narrow at the oral end.

The interradial, longitudinal muscles are divided into two heads at the oral end. Each head of the muscle is attached to the root of the adradial bunch of tentacles. These muscles are well developed and run through the whole length of the animal, from the base of the peduncle to the umbrella margin.

The eight principal tentacles are transformed into the adhesive marginal bodies, generally known as anchors. They are large, round and sessile. They are about half as long as the diameter of the peduncle.

The secondary tentacles, the "Succursal Tentakeln" of HAECKEL, are grouped into eight adradial bunches, in each of which are to be counted about twenty-five tentacles in four or five

longitudinal, and five or six transverse, rows. These tentacles are short, capped, and are found in the subumbrella. They nearly disappear from sight, when the coronal muscle is contracted. They are highly adhesive. In a very small specimen, only 2 mm. in length, the secondary tentacles were six in number, arranged in two longitudinal rows; in another, 4.5 mm. long, from nine to ten tentacles were present in three rows, and in still another of 7 mm. length, eleven tentacles in three rows. Generally new tentacles are added outside the group of older ones.

The central stomach cavity is very long and columnar. It is four-lobed in transverse section. The manubrium or the buccal stomach is short and nearly as long as broad. It presents a cross-like shape in transverse section. The gastral filaments are long, simple and numerous, being arranged in two longitudinal rows, which run very close to each other on the internal axial wall of each infundibulum. They are found in the central stomach only.

The coronal umbrella cavity is conical and occupies from one-third to one-fourth of the length of the calyx; its wall is entirely covered with groups or glands of nematocysts. The four interradial infundibular deepenings reach the aboral end of the calyx. They are tolerably uniform in diameter throughout their whole length. Two longitudinal, ectodermal thickenings run along the abaxial side of these deepenings and are connected at the aboral end. These thickenings consists of very high ectoderm cells, rich in nematocysts.

The genital glands, eight in number, are band-like. They run side by side in twos along the interradial muscles. Each gland consists of numerous sacs, of which there are in old specimens about fourty, arranged approximately in two rows. In young specimens the sacs are less in number and are arranged

in a single row. New genital sacs are added at the distal end of the genital gland.

The color is generally dark brown flecked with white. The adhesive anchors and the base of the peduncle are light red. The genital glands and the tip of the secondary tentacles present a dark brown color. The manubrium is yellowish, while the subumbrella is pale green.

The size of a full-grown animal measures 25 mm. in length, excluding the peduncle.

A specimen of this interesting medusa was first discovered by Mr. MASAMARU INABA in 1893 at Kata-ura in the Province of Kii. It was kindly given me for examination, together with the sketches and notes made by the discoverer. On account of the peculiar shape and movements of the animal Mr. INABA at first took it for a worm. Some years since the same medusa was found near the Marine Station at Misaki during winter months.

The animal hangs down from the frond of *Sargassum* on which it is attached. It can detach itself of its own accord, but is devoid of swimming power. Being remarkably contractile in both umbrella and peduncle, the body-length is reduced by almost one half when contracted. As the body has adhesive apparatus at both its extremities, it can effect a locomotion very much like that of a leech.

Lucernaridæ.

Schizodiscus, nov. gen.

Lucernaridæ without mesogonial pouches and without adhesive anchors. Umbrella deeply divided. Peduncle four-chambered,

without muscular fibres in the tæniola. Structure of the genital gland not simple.

Schizodiscus nagatensis.

(Figs. 3–6.)

Lucernaria nagatensis, OKA, 1897. In: Zool. Mag. Tokyo, Vol. IX, pp. 1–4, pl. I; Annot. Zool. Jap. Vol. I, pp. 141–145, with woodcuts.

The umbrella is of an extraordinary form. It is not goblet-shaped, but quite flat and deeply divided. The animal, when fully expanded, is about five times as broad as long. The eight adradial lobes or arms are somewhat recurvate. They are united in pairs and the four perradial arches are about twice as deep and wide as the four interradial arches.

The peduncle is short, being subequal in length to the umbrella, and more or less conical in shape. It is four-chambered; but in young specimens it is one-chambered, the tæniola being separate. These begin to unite near the pyloric portion. In the peduncle we find no muscle, as we do in the case of *Craterolophus tethys*. The adhesive surface of the peduncle is more or less quadrate and has many small furrows beside four large interradial furrows (fig. 5).

The exumbrella is quite smooth in the calyx, but shows a slight rugosity in the peduncle owing to the presence of many small groups of nematocysts. In an old specimen, I have found that the surface of the peduncle is divided into numerous small areas by a network of grooves. The surface looks as if paved. The exumbrella consists of three layers: a layer of columnar ectoderm cells, a firm gelatinous layer and a layer of glandular

endoderm cells. The gelatinous layer is thickest in the pyloric portion.

The subumbrella is broader than the exumbrella. Its surface is wavy for the greater part, but rugose near the umbrella margin as well as in the perradial regions. The rugose portion near the umbrella margin is often turned over towards the aboral side. The rugosity is caused by large groups or glands of nematocysts lying underneath. The subumbrella also consists of the three layers. Its ectoderm cells are higher than those of the exumbrella and many of them are in possession of nematocysts. The gelatinous layer is exceedingly thin.

The umbrella cavity is hardly distinguishable. The very small interradial concavities found in the central disc of the body may be compared to the infundibular deepenings of other Stauromedusæ.

The interradial, longitudinal muscles are well developed. They run between the adradial bunch of tentacles and the pylorus, without extending into the peduncle.

The coronal or marginal muscle is cut into eight separate pieces, each of which is shaped either like the letter U or V. The perradial pieces are much longer and broader than the interradial pieces.

The eight adradial lobes are united in pairs, giving to the animal the form of a Greek cross. The distal end of each adradial lobe is pointed, bent at a right angle to the remaining part and turned towards the oral side. Each united pair of the adradial lobes may be longitudinally folded, as it were, on the oral side. Moreover it may be bent towards the mouth. It is longer than the diameter of the central undivided portion of the umbrella.

Primary tentacles are not present; in place of them we see a dark pigment for each.

The secondary tentacles are short, capped and situated on the abaxial side of the bent extremity of each adradial lobe. They are adhesive. The tentacular tract is somewhat triangular, having two long sides and a short base. The tentacles, about five in number and situated at the base of the triangular tract, have each a thick, flattened disc on the abaxial side of its stalk.

The central stomach cavity is very short, about equal in length to the buccal stomach. The latter is likewise short and presents a cruciate form in the transverse section.

The gastral filaments (fig. 6) are rather few in number but well developed, branched and crowded together in eight short phacelli at the axial end of the genital glands. Apart from the phacelli I have found a few, large and simple filaments at the pylorus.

The genital glands are represented by eight long and broad bands, each of which consists of numerous large, laterally oblong sacs and extends from the tip of the adradial lobes nearly to the margin of the pylorus, where the longitudinal muscle terminates.

The color is variable, simulating that of the environment. It is sometimes dark brown, sometimes dark green. The nematocysts-groups are generally colorless.

The diameter of the umbrella in a full-grown individual is about 30 mm.; total height of the body, about 7 mm.

A single specimen of this medusa was discovered by Mr. Oka in April, 1896, at Kogushi in the Province of Nagato. He proposed to name it *Lucernaria nagatensis*, should the species prove new to science. The somewhat popular description given by him of the medusa as also his rather diagrammatic figures

are not entirely satisfactory. Some years since, this medusa was found also at Misaki in winter.

DISCOMEDUSÆ.

SEMOSTOMÆ.

Pelagidæ.

Kuragea, nov. gen.

Pelagidæ with fifty-six tentacles and sixty-four marginal lobes.

Kuragea depressa, nov. sp.

(Fig. 7.)

The umbrella is flat, discoidal, about three times as broad as high. The sixty-four marginal lobes are oblong and of unequal size. The ocular lobes and the lobes by the sides of the adradial tentacles are larger than any other, while those next to the ocular lobes are the smallest. The sixteen ocular pouches are long and oval in form, their distal ends being about half as broad as those of the tentacular pouches. The oral arms are broad and rich in folds. The tentacles are unequal in thickness, those at the sides of the ocular lobes being slender. The total length of both the oral arms and the tentacles can not be determined, as they are but incompletely preserved in the single type-specimen examined.

The exumbrella shows a sixteen-rayed, stellate marking. On

* *Kuragé*, the Japanese name for medusæ.

its oral side is to be seen the peculiar figure formed by the combination of several grooves, which is of such general occurrence in medusæ of the Pelagidæ and the Cyaneidæ. Centrally there is a sixteen sided polygon, the diameter of which is about half that of the central gastric cavity. From each angle of the polygon a groove radiates toward the umbrella margin. These radial grooves alternate with the partition walls between the radial pouches.

The genital glands are four in number. Each gland is much folded and bent like the Greek letter ω.

The gastral filaments are many and long.

Diameter of the umbrella 85 mm.; its height 30 mm. The unique specimen, on which this new species is based, was obtained at Misaki in 1900.

RHIZOSTOMÆ.

Cepheidæ.

Rhizostomæ with cup-shaped umbrella which consists of a central dome, generally covered with warts or furnished with radial furrows, of a circular groove and a broad brim. Radial muscle-fibres conspicuous. Subgenital cavities generally four in number, but sometimes coalesced into one. Subgenital ostia to the cavity are very small. Network of the canal system narrow. Radial canals numerous. No circular canal. Oral arms generally one-winged.

It was necessary to make some alterations in the family character in order to receive the new forms of medusa next to be described. Notwithstanding that these show many novel points

in their structure, I believe it but proper to range them under the Cepheidæ.

Microstylus, nov. gen.

Cepheidæ with a four-lobed subgenital cavity and very small appendages among oral frills. Oral arms one-winged.

Microstylus setouchianus.

(Figs. 8–10.)

Cotylorhiza setouchiana, Kishinouye, 1899, in litteris Mus. Zool. Univ. Tokyoensis.

Umbrella hood-shaped. Central dome covered with fifty or more warts; separated from the broad brim by a circular groove; its central portion is generally thin, especially in young specimens. The dome surface with numerous minute brown dots, arranged in radial lines, converging towards the apex of each wart. The umbrella is very thick, but becomes suddenly thin in the brim (fig. 9).

The umbrella margin is divided into from fifty to sixty lobes, *i. e.*, each octant of it is furnished with from six to eight oblong velar lobes between every two smaller ocular lobes.

The straight, uniformly distributed radial muscles seen in the subumbrella gradually become fainter peripherad, finally to disappear altogether before reaching the brim (fig. 10).

The oral disc is a little depressed and octagonal in shape. From it hang down eight oral arms. On each interradial abaxial side of the disc there is a small round opening communicating with the subgenital cavity (figs. 8, 9). The sides of the disc are

covered with numerous brown dots, of which those on the perradial sides differ in form and arrangement from those on the interradial sides.

The subgenital cavity is flat; though not divided into four separate cavities, it is four-lobed, being separated by four perradial septa. The length of each of these septa is about one fourth that of the broadest part of the cavity (fig. 9). In continuation of these septa there are narrow gelatinous thickenings in the gastrogenital membrane or the roof of the subgenital cavity. Naturally these gelatinous thickenings form a cross. This cross is a little curved and is not much separated from the upper surface of the oral disc, while the remaining portion of the gastrogenital membrane is very thin and loose.

The canal system in general resembles that of *Netrosoma typhodendrium* L. SCHULTZE. Of the interocular radial canals there are twenty-four. The eight ocular canals are larger than other radial canals and run straight to the umbrella margin (fig. 10). So do likewise the eight adradial canals, but these are not so distinct. The sixteen remaining canals are branched just outside the oral disc and together with other canals form a complicated network. The meshes are mostly polygonal near the central portion of the subumbrella, but become rectangular near the periphery. Near the margin of each marginal lobe, the network ends with only one mesh, which sends a very short, blind canal towards the umbrella margin. For about one-third its length at the axial portion, the ocular canal is free from side branches. There is no circular canal. The eight brachial canals originate from depressions on each side of the perradial septa of the subgenital cavity. These adradial depressions in the central stomach cavity correspond to the pillar canals of other monodemniate

Rhizostomæ. The brachial canal gives off a horizontal branch towards the axis of the body. Such horizontal canals reunite and form a very short crucial canal at the centre of the oral disc (fig. 9).

The oral arms are one-winged and curved abaxially at the lower half (figs. 8, 9). They are a little longer than the umbrella radius. They are divided into two at the distal end and give off many branchlets in a feather-like manner. The upper arm is nearly equal in length to the lower arm. Among sucking frills, there are many small, short appendages. Those at the junction of the oral suture are longer, triangular in cross-section, and have prickly appearance.

The umbrella diameter measures 10–20 cm.

The central dome of the umbrella and the sides of the oral disc have many dark brown dots. Genital glands pinkish; oral frills brown. A specimen which I have obtained at Senzaki, on the coast of the Japan Sea, had a very beautiful light blue color.

This medusa is known thus far from Setouchi-umi (Inland Sea) as well as from Misaki and Senzaki. It is found in August and September and is known among fishermen by the name of *Ebi-kuragé* (shrimp medusa), since a commensal shrimp is often present under its umbrella.

This medusa is closely related to *Cephea*, *Cotylorhiza* and *Netrosoma*.

Perirhiza, nov. gen.

Cepheidæ with a deeply divided subgenital cavity, small subgenital ostia and long, numerous appendages among oral frills.

Gelatinous wall of the oral disc entirely destitute of gastrovascular canals. Oral arms three-winged.

Perirhiza nematophora, nov. sp.

(Figs. 11–13.)

The umbrella is hood-shaped. Its central dome is very high and covered with many large and numerous small conical warts, about thirty in number. Many of the warts are bent or crooked near the apex. Larger warts are situate in the centre of the umbrella, while smaller ones are chiefly found near the foot of the dome and sometimes also scattered between or upon the larger warts. The umbrella is thickest at the groove separating the central dome from the peripheral hanging portion.

The umbrella margin is divided into from eighty to ninety lobes, *i. e.*, there are eight or nine oblong velar lobes between every two ocular lobes, which are very small and much receded from the general umbrella margin. The velar lobes in each octant are united by a thin membrane as is the case in some species of the genus *Cephea* (fig. 13).

In the subumbrella we find thickly crowded and well developed muscular ridges running radially. At the axial part of the brim the radial muscles disappear, while instead circular muscles present themselves (fig. 13).

The oral disc, octagonal in outline, is not distinct, as the subgenital ostia are very small and compressed and the oral pillars, short, broad and inconspicuous. Its oral side is strongly vaulted upwards; the gelatinous wall forming it is destitute of the gastrovascular system, which is a remarkable fact. The central portion of the oral side of the disc is free of the sucking frills.

Those on the axial side of each two oral arms unite in perradial planes and form four perradial wings of sucking frills. The wings are very short (fig. 12). At each junction of the oral suture ("Pfeiler-gabel" of HAECKEL) there hangs down an appendage of very large size.

The subgenital cavity lies between the umbrella and the oral disc. It is divided into four large lobes, separated from one another by as many long perradial septa. The length of each septum is about three-eighths the greatest breadth of the cavity. We do not find crucial gelatinous thickenings continuous to these septa. The gastrovascular membrane is not loose. There are four interradial gelatinous ridges which run directly from the lower lip of the subgenital ostia. The latter are very small and of crescent shape.

The oral arms are short, thick and stout. They belong to a primitive type of the three-winged arms and show many characteristic features. They are shorter than the umbrella radius and do not project out of the umbrella margin. The upper arms are nearly coalesced and form a large part of the octagonal oral disc. The upper arms project from both sides of the oral disc, axially as well as abaxially. The lower arms are also very short, being of nearly the same length as the upper arms. The axial wing of the oral arms is very well developed, giving out a profuse quantity of branches which completely cover the axial portion of the oral arm. The abaxial wings are wide; they are developed upwards. Appendages of the oral arms are very long, numerous and circular in cross-section. There are from six to eight of them on each abaxial wing; much more numerous are they on the axial wing. They are canalled and many of them are longer than the umbrella diameter. Specially long and large appendages

are found at the junction of the oral suture and near the lower end of the oral arms.

The gastrovascular system is remarkable in many points. It forms a very fine network in the subumbrella (fig. 13). The eight ocular canals run straight and are easily distinguishable from others, though there exists no special difference in calibre. Between each two ocular canals we find five or six canals which soon divide into numerous smaller ones. Radial muscular ridges receive many small blind branches from the network of the canal system. The circular canal is wanting. Each velar lobe receives a very minute network of the canal system. The central stomach cavity is nearly circular, not cruciate. No canal is contained in the oral disc, but in its lower surface a canal is seen in each of the adradial ridges or keels of the oral crisps (fig. 12).

The umbrella in the preserved state measures 14 cm. in diameter.

The animal is colorless, with the exception of the margins of the velar lobes which are brown.

The medusa is known from Misaki. It is found in the colder months of the year.

Tokyo, 25 Jan., 1902.

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PLATE I.

Plate I.

Fig. 1.—*Stenoscyphus inabai*. 3× natural size.
Fig. 2.—Peduncle of *Stenoscyphus inabai*, showing its adhesive surface.
Fig. 3.—*Schizodiscus nagatensis*. 2× natural size. Oral view.
Fig. 4.—*Schizodiscus nagatensis*. 2× natural size. Aboral view. The peduncle is bent aside.
Fig. 5.—Peduncle of *Schizodiscus nagatensis*. Adhesive surface.
Fig. 6.—Gastral filament of *Schizodiscus nagatensis*.
Fig. 7.—*Kurogea depressa*. Natural size.
Fig. 8.—*Microstylus setouchianus*. $\frac{1}{2}$ natural size.
Fig. 9.—Longitudinal section of *Microstylus setouchianus*. The left half represents a section through a perradial plane, and the right half that through an interradial.

Fig. 2.

Fig. 7.

Fig. 1.

Fig. 5.

Fig. 6.

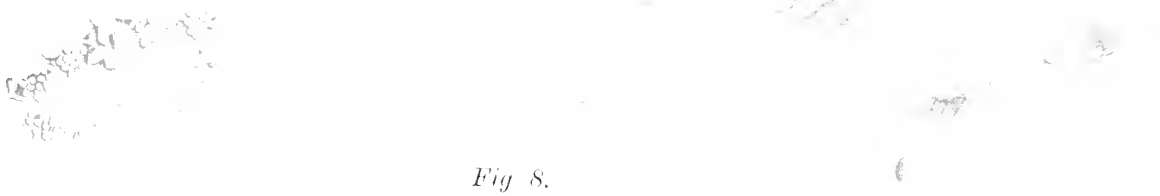

Fig 8.

Fig. 3.

Fig. 9.

Fig. 4.

1—2, Stenoscyphus inabai. *3—6, Schizodiscus nagatensis.*
7, Kuragea depressa. *8—9, Microstylus setouchianus.*

PLATE II.

Plate II.

Fig. 10.—Portion of the umbrella of *Microstylus setouchianus*, showing the canal system and the radial muscle.

Fig. 11.—*Perirhiza nematophora*. $\frac{1}{2}$ natural size.

Fig. 12.—Longitudinal section of *Perirhiza nematophora*. The left half represents a section through a perradial plane, and the right half that through an interradial.

Fig. 13.—Portion of the umbrella of *Perirhiza nematophora*, showing the canal system and the muscles.

Fig. 11.

Fig. 10.

Fig. 13.

Fig. 12.

10, Microstylus setouchianus. 11–13, Perirhiza nematophora.

JOURNAL OF THE COLLEGE OF SCIENCE, IMPERIAL UNIVERSITY
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VOL. XVII., ARTICLE 8.

Preparation of Sulphamide from Ammonium Amidosulphite.

By

Edward Divers, and **Masataka Ogawa.**

Sulphamide occurs among the products of the spontaneous decomposition of ammonium amidosulphite. That this appeared to be the case was mentioned in the paper describing this salt (this Journal, 1900, **18**, 187). It had then been isolated not only in too small a quantity to admit of its purification and full analysis, but in a way that rendered its identity almost doubtful. The decomposed amidosulphite had been extracted with 95 per cent. alcohol, the residue from the evaporated voluminous solution extracted with undried ether, and the again very voluminous solution evaporated. Half a gram of crystalline residue from about 150 c. c. of the ether solution was thus obtained, answering the tests for sulphamide, but melting much above 81°, tasting not bitter, and yielding a little too much sulphur on analysis. Then, too, we had failed to get silver sulphamide from the aqueous solution of the decomposed amidosulphite, owing, as we afterwards found, to our having used ammonia in excess. All these points differed, or seemed to differ, from TRAUBE's description, and caused us to hesitate in pronouncing the substance to be sulphamide. Since then, we have obtained it in larger quantity and pure, and

thus become certain that sulphamide is a little soluble in absolute alcohol and even very slightly so in dry ether, that it melts at 91°, and that its silver derivative is insoluble in ammonia alone, but soluble in ammonia in presence of the ammonium nitrate which its mother-liquor always contains. The publication of HANTZSCH and HOLL'S important contribution to the knowledge of sulphimide and sulphamide (*Ber.*, 1901, **34,** 30), in which TRAUBE'S account of sulphamide (*Ber.*, 1893, **26,** 609) is amended, affords welcome confirmation, so far as it goes, of the correctness of our own observations.

Hitherto, as well is known, sulphamide has only been got from sulphuryl chloride and ammonia, a mode of preparing it which HANTZSCH and HOLL have shown to be most laborious and unprofitable, and the difficulty of getting it in this way has quite recently induced Ephraim to try to obtain it from sulphuryl chloride by means of urethane, but without success (*Ber.*, 1902, **35,** 776). Sulphuryl chloride is stated to give only 1–2 per cent. of pure sulphamide, whilst ammonium amidosulphite, by a process not unduly troublesome, yields 10 per cent. of its weight, and probably much more by skill and care. In order to prepare the ammonium amidosulphite and decompose it afterwards, ammonia in excess and sulphur dioxide are led into a closed flask, fitted with a thermometer and an exit-tube dipping in mercury. To absorb the heat caused by the combination of the gases, the flask is held in a bath of brine and crushed ice, which is more effective when the flask contains some ether and is kept in motion, because then the salt does not stick to the walls of the flask as a waxy, badly-conducting coating. The rate of flow of the gases is to be regulated by the operator's ability to prevent the temperature in the flask from rising much above 10°. The inside

of the apparatus, the gases, and the ether are all to be dried before use.

When as much amidosulphite has been formed as may be wanted or be convenient to prepare, the cooling mixture in the bath is replaced by water, and a slow stream of dry hydrogen passed through the flask, whilst the temperature of the water is slowly raised to about 70° and then kept at that point for five or six hours, or so long as ammonia continues to come off in any quantity. During this operation, the ether, if used, also evaporates. The sulphamide is all formed at temperatures not higher than 30°–35°, and a higher temperature is here employed only for the purpose of destroying as much as possible of the thionic compounds which are formed along with the sulphamide and would, at a later stage, consume much silver nitrate and undesirably produce much ammonium nitrate, if present. The employment of a higher temperature than 70°, to destroy all the thionic compounds, is not possible, because then the sulphamide itself would be decomposed.

When the flask has cooled down, enough ice-cold water is poured in to dissolve all its contents other than a considerable quantity of sulphur, left by the destroyed compounds. To the yellow, unfiltered solution, which has been poured into an open vessel, barium hydroxide is added in quantity a little more than sufficient for the salts it precipitates, among which are sulphate, imidosulphite, and thiosulphate. In order to lessen the dilution of the solution of sulphamide, the barium hydroxide is used in mixed solution and crystals, as obtained by rapidly cooling a hot, concentrated solution. The precipitate is to be filtered off, though it is not very easy to get a bright filtrate, and, even when this is accomplished, the filtrate soon becomes turbid again, owing to

further production of sulphate by the decomposing salts present in it. This does not matter, however, and to the turbid filtrate silver nitrate is added, just so long as it continues to give a precipitate. The barium hydroxide will have liberated much ammonia, but a good deal of this will have evaporated during the time taken up in filtration, especially if the precipitation has been carried out in an open vessel. What remains of it interferes only temporarily with the silver precipitation and does not usually need external neutralisation. For, so much acid is formed as the result of a very rapid decomposition of the precipitated silver salts (in which they change from white to black), as to be more than enough to neutralise the ammonia remaining in the solution, and also to dissolve up any silver sulphamide that may have been thrown down at first. When the mother-liquor has become thoroughly acid or, exceptionally, has been made so by adding nitric acid, and still holds silver in solution, it is filtered from the black precipitate and just neutralised with ammonia. Any slight precipitate then formed is also filtered off and rejected; it contains no trisulphimide. The filtrate holds little else than sulphamide and ammonium amidosulphate, and if evaporated over sulphuric acid, would yield both these substances in characteristic crystals. But to isolate the sulphamide, it is to be precipitated from this solution by silver nitrate and ammonia, that is, by Traube's method. The silver sulphamide, thus obtained, is almost pure, there being no such acid matter present as is met with when the sulphamide has been prepared from sulphuryl chloride. In that case, a viscid silver salt accompanies the silver sulphamide, and, according to Hantzsch and Holl, can only be removed from it by a process entailing the destruction of much of the sulphamide. Even in the present case, however, the amido-

sulphate, left with the sulphamide, may cause a little difficulty, unless care be taken.

On referring to the memoir on amidosulphuric acid in the Journal of the College of Science for 1896 (**9**, 239–241, "amidosulphonic acid"), it will be found there stated that when an alkali is added in suitable quantity to a solution of mixed silver nitrate and potassium amidosulphate, a bright yellow, amorphous salt is precipitated, which is very probably $AgHN.SO_3.K$, and is soluble in and ultimately decomposed by excess of alkali. It is now found that, in precipitating silver sulphamide in presence of ammonium amidosulphate, as in the present case, a very small quantity of a bright-yellow substance, probably ammonium argent-amidosulphate, is apt to accompany the silver sulphamide, and that, in order to circumvent this liability and, at the same time, to avoid loss of the silver sulphamide through its solubility in ammonia in presence of ammonium nitrate, precipitation should be carried out in the following way. Having added more silver nitrate, dilute ammonia is dropped in, slowly and with stirring, until the solution is slightly alkaline. The precipitate is quickly filtered off and washed free from mother-liquor. The solution is again treated with silver nitrate and ammonia, as before, in order to see whether any more sulphamide is thrown down. This being quickly filtered off, if it be desired to obtain a sight of the yellow compound, a few drops of ammonia may be added, and dilute silver nitrate very slowly dropped in, when it will be produced.

The silver sulphamide, perhaps a little yellow after all, is to be dissolved in dilute nitric acid, ammonia added to slight alkalinity, as before, and then two or three drops of silver nitrate. After a repetition of this treatment, the precipitate is stirred up

with exactly enough dilute hydrochloric acid to decompose it, just as TRAUBE directs. The filtrate from the silver chloride, which must not be acid, gives the sulphamide in good crystals, when it is evaporated in the desiccator. It is to be recrystallised, and, since it is exceedingly soluble, the mother-liquors must be worked up, if a good yield is wanted.

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Studies on the Hexactinellida.

CONTRIBUTION II.

(The genera *Corbitella* and *Heterotella*).

By

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With one plate.

While in Paris in the early part of this year, I was enabled, through the courtesy of Professor EDMOND PERRIER and the friendly assistance of Dr. CH. GRAVIER, to examine, amongst other things, the old types of *Corbitella speciosa* (QUOY GAIMARD) and of *Heterotella corbicula* (BOWERBANK). For the perplexing history of our knowledge of these little known forms, the reader is referred to a paper by F. E. SCHULZE ('00).

Another Euplectellid, *Eudictyum elegans* MARSHALL, has also long stood in need of re-examination as being closely allied to, if not identical with, one or the other of the above mentioned forms. On the occasion of a visit last year to Amsterdam, an opportunity was given me of examining the type of MARSHALL; and subsequently, Professor MAX WEBER has kindly supplied me with a sample of its tissues to make studies on.

I now propose to give an account of my observations on the above mentioned Euplectellids. The following may be mentioned at once as consequences to be derived from the results I obtained on the systematic: GRAY's genera *Corbitella* and *Heterotella* should be kept up as distinct; the former to comprise not only QUOY and GAIMARD's *speciosa* but also MARSHALL's *Eudictyum elegans* as well as F. E. SCHULZE's *Tegeria pulchra*, and the latter to stand represented by the single original species *corbicula* of BOWERBANK.

Corbitella speciosa (QUOY et GAIMARD).

(Plate, figs. 1–12.)

Aleyoncellum speciosum. QUOY and GAIMARD, '33, p. 302; Pl. XXVI, fig. 3.—MILNE EDWARDS, '36, p. 586.—FILHOL, '85, p. 284; Pl. VI, fig. 1. (*Not* BOWERBANK, '67, pp. 353–358.—*Nor* BOWERBANK, '69, p. 344; Pl. XXIV, figs. 8–11).

Aleyonellum speciosum. OWEN, '49, p. 205, (*not Aleyonellum gelatinosum*).

Euplectella speciosa in part. GRAY, '66, p. 487.

Aleyoncellum sp. BOWERBANK, '67. pp. 358–359 (*not* pp. 353–358).

Corbitella speciosa. GRAY, '67, p. 530; Pl. XXVIII, fig. 1.—GRAY, '72, p. 457.—SCHULZE, '00, p. 156.

Habrodictyon speciosum. W. THOMSON, '68, p. 131; Pl. IV. fig. 2 (*not* fig. 2 *a*).—CARTER, '73; pp. 361, 367.

Habrodictyon speciosum in part. MARSHALL, '76, p. 129.

Habrodictyum speciosum in part. SCHULZE, '86, p. 42.—SCHULZE, '87, p. 99.

The type, preserved in the Muséum d'Histoire Naturelle, Jardin des Plantes, is to be considered as the only specimen known of this species. It is known that the specimen was given to QUOY and GAIMARD by M. MERKUS, the governor of the Moluccas, and in accord therewith the label attached to it bears the record: "des Moluques: Mm. QUOY et GAIMARD. Exp^{on} D'Urville. 1829." W. THOMSON's ('68, p. 132) statement that the specimen is labelled to quite another effect is clearly an error. The labelling quoted by him belongs, as was suggested by GRAY ('68, p. 173), to a different sponge (specimen A of *Heterotella corbicula*, which shall be treated further on).

The figures of the specimen, prepared after a photograph and given by both GRAY ('67) and W. THOMSON ('68), are excellent and well fitted to give a correct idea of its general appearance. QUOY and GAIMARD's figure of the same, as well as that to be seen in FILHOL's ('85) work, are not entirely satisfactory.

Besides the original describers, BOWERBANK ('67, pp. 358–359) and W. THOMSON (*l. c.*) have both given us accounts of the specimen from independent observations. Singularly enough, BOWERBANK at the time was somehow not cognizant of the fact that he had to deal with QUOY and GAIMARD's type of "*Alcyoncellum speciosum*"; for, while calling by that old name the Philippine *Euplectella aspergillum* OWEN, the very type of QUOY and GAIMARD was laid down by him as representing a different species of the same genus.

Although I have but little to add to the existing descriptions of the general appearance of the specimen, I may be allowed to give here my own notes in full.

The specimen may be said to consist of little besides the

skeletal framework, in which all the component parenchymalia are rigidly fused together by synapticular formations from the base to the sieve-plate inclusive. The loose parts have been almost entirely lost. In this denuded state the specimen is tubular and phallus-like, gradually expanding from the base to the somewhat flatly rounded upper end. Total length 210 mm. Cross-section of the body, which is irregularly circular, measures in diameter : 64–62½ mm. near the upper end, 45–42 mm. at the middle and 36–33 mm. near the lower end.

The closed basal end is obliquely truncated. Compact basidictyonal incrustation is to be seen at this end, and also in an isolated irregular patch close to, but on one side of, this end. One point in the description given by QUOY and GAIMARD is in disagreement with the facts. According to them the body should be *open* at the extremity opposite to that which is dilated and rounded, although they have made mention of certain indications that the sponge must have been fixed by one of its ends. In their figure also the basal end presents the appearance of being open, which is certainly a misrepresentation, probably of the engraver. Probably the authors were to a degree misled by this appearance of their figure.

The upper end of the body is closed by an arching over, as it were, of the lateral wall. This passes over gradually and without essential change in texture into the terminal part which should represent the sieve-plate. The absence of a demarcation of any sort between the sieve-plate and the lateral wall has been repeatedly emphasized by writers ; but, in my opinion, it is by no means to be excluded that in the original unmacerated state of the specimen there was present a marginal ridge consisting of a loose tissue such as might easily have been lost.

The entire latticework-like wall is thin except at the region of basal incrustations. The meshes which should have each contained an osculum (both parietal and cribellar, *cfr.* IJIMA, '01, p. 39) are rather wide. The beams, filigree-like in structure, are never more than $\frac{1}{4}$ mm. thick. They combine to form a latticework of an irregular kind (*vide* fig. in either GRAY '67, or THOMSON '68). Many of the stronger beams are seen to pursue a flexuous course, on the whole longitudinally directed; some others run, at least for a short distance, in a direction more inclined to the transverse or the oblique. However, irregular deviations in the course as well as in the branching and anastomosing are of such frequent occurrence that it is scarcely possible to distinguish separate systems of beams such as are seen in *Euplectella*. So far as the skeletal tube goes, there exists no indication of parietal ledges. Although I have no observation on the megascleric elements of the beams, it seems to me more than probable that both the comitalia and principalia parenchymalia are, at least mainly, diactins.

Of the interstitial loose tissue, I have fortunately found some vestiges still left, scantily attached here and there to the skeletal framework. They had to be searched for by means of a lens. By careful manipulation with pins or a pair of pincers, I succeeded in securing the tissue in a quantity sufficient to make of it a number of microscopical preparations, an examination of which revealed some points of great systematic importance to the genus and the species.

W. THOMSON ('68, p. 131) communicated some information respecting the loose spicules of this species, but it was so little that not much use could be made of it for the systematic. And moreover, soon after I had gone into the study of the Euplec-

tellids in the Paris museum, I had to conclude that one of the spicules mentioned and the single one figured by him (*l. c.*, fig. 2 *a*)—a spicule which chiefly weighed with him in regarding this species as a distinct one—does not belong to the species at all! I recognize in that spicule the small spiny hexactin (*vide* Pl., figs. 20–23) which is characteristic to, and not uncommonly found in, *Heterotella corbicula* and which is easily distinguishable from a similar spicule of *Corbitella speciosa*. The said spicule was held by W. THOMSON as probably identical with BOWERBANK's "bifurcate rectangulated hexradiate spicule" (BOWERB. '58, Pl. XXV, fig. 38; also '64, Pl. VIII, fig. 188). I think W. THOMSON was quite correct in this identification; for, BOWERBANK's above named spicule was taken, not from *C. speciosa*, but from "*Aleyoncellum corbicula*" obtained in 80 fathoms off the Island of Bourbon (*vide* BOWERB., '67, p. 358). As before mentioned, W. THOMSON by an unfortunate confounding of labels ascribed to the only specimen of *C. speciosa* the labelling "*Alcyoncellum corbicula* VAL. Tiré par 80 brasses de profondeur dans la rade de St. Denis de Bourbon" etc. Probably this labelling and the spicule in question belonged together, but neither of them to the *C. speciosa*. This tends also to explain that W. THOMSON has entirely overlooked the spiny microxyhexactin and the discohexasters soon to be described from *C. speciosa*.

To return to my own observations on the loose tissue spiculation, the parenchymalia (accessoria) consist mainly of the well-known, long, slender diactins, with which are associated a not inconsiderable quantity of hexactins. The diactins may be as thick as 20 μ, but the majority are much thinner and of a filamentous appearance (down to 7 μ in thickness near the center).

The spicular center is ordinarily simply annulated, occasionally supplied with cruciately disposed knobs; ends of rays being rough-surfaced as usual. Occasionally slender tauactins came under observation. The hexactins are of various sizes. The larger ones may be said to be of a medium size and strength. Often one axis is considerably longer than the two others, the elongated axis not seldom forming bundles in association with diactin elements.

Of common occurrence are similarly rayed pentactins, in which the unpaired ray exceeds all the others in length (about 100 μ long; thickness near the center 7 μ or less). Many, if not all, of these are probably to be looked upon as the gastralia (Pl., fig. 4).

At places dagger-like hexactins of varying sizes are met with in the preparations. Hilt-ray 50–200 μ long, 4–10 μ thick near the center; guard-rays about as long as, or somewhat longer than, the hilt-ray in the same spicule; length of blade-ray usually several times that of the hilt-ray. A large spicule of this category may reach 1 mm. in total length. Some at least of such hexactins are undoubtedly the dermalia. So, for instance, the rather small hexactins (Pl., fig. 2) in which the short hilt-ray of about 50 μ length is quite or nearly smooth with rounded termination; and also the tolerably large ones (Pl., fig. 1) in which the hilt-ray (about 200 μ long, 10 μ thick near the central node and gradually tapering outwards to a point) bears in close apposition graphioconal rhaphides, besides being distinguished from the other rays on account of the more extensive roughness of its surface. The roughness of the dermal hilt-ray is however never very pronounced.

The larger hexactins of the parenchyme seem to diminish

gradually in size down to such small and slender-rayed oxyhexactins as measure only 160 μ or under in axial length and about 3 μ in breadth of ray near the center. These may be called microxyhexactins. They occur in scattered distribution, though at places several may be found side by side. There are to be distinguished two kinds of the microxyhexactin, *viz.*, the smooth (Pl., fig. 3) and the spiny (Pl., fig. 9).

Of these the spiny microxyhexactin is more numerous and certainly more highly characterized. The rays are not infrequently of unequal length in the same spicule; they are either nearly straight or slightly bent. Except in the basal part, each of them is supplied with a varying number of rather slender spines (up to 7 μ in length). These are irregularly distributed, and indefinite as to their direction, being sometimes recurved, sometimes obliquely outwardly directed and at other times projecting nearly vertically. Their number on a ray may be quite small, sometimes even only two or three. In certain cases the few spines present were quite obsolete, a fact which seemed to indicate a gradational transition between the spiny and the smooth microxyhexactins. Axial length of the spiny microxyhexactins generally 120–130 μ, exceptionally 280 μ. Similarly spinose microxyhexactins occur also in *C. elegans*, *C. pulchra*, *Regadrella okinoseana* and *Dictyaulus elegans*, and further in a peculiarly modified form in *Heterotella corbicula*.

Of a somewhat doubtful nature are the small, thick-rayed spicules of stunted appearance, which are met with in some numbers in certain parts of my preparations (Pl., figs. 5–8). They are usually pentactins but occasionally a hexactin, a tauactin or a compass-needle-like diactin. The stout-looking rays are smooth, nearly uniformly broad throughout, and with rounded

terminations. They may be so short as to measure only 40 μ in length with a breadth of 10 μ; but the size and the proportions are subject to much variation, some of the spicules wellnigh approaching the ordinary pentactin gastralia or the hexactin parenchymalia in these respects. The general appearance of the spicules strongly reminded me of the oscularia of certain *Euplectella*. Apparently the same kind of spicules was made known by MARSHALL ('75, p. 212, Pl. XVI, figs. 66 *e–h*) from *Corbitella elegans* and was held by him to be the oscularia. I doubt the correctness of this interpretation. So far as is known to me, the occurrence of well differentiated oscularia is confined to the genus *Euplectella;* and moreover, I have found in *Regadrella okinoseana* spicules similar to the kind in question scattered among the parenchymalia (IJ., '01; Pl. VIII, figs. 27, 28, 34).

The hexasters consist of the floricome, the discohexaster and the graphiocome.

The floricome was found in some numbers in more or less fragmentary states. It measures 72–83 μ in diameter. The principals are slender and bear each a perianth of 7 or 8 terminals of the usual character. The claws, of which there are three or four to each terminal plate, are very small. In many cases of the rosette, only the basal parts of the terminal perianth remained, the missing parts having been broken off.

Comparatively large and strong-rayed discohexasters (Pl., figs. 10 and 11) occur in great abundance. Diameter 100–130 μ, occasionally reaching up to 145 μ (on an average 120 μ). Each exceedingly short or almost obsolete principal bears 2 or 3 diverging terminals, which, thickening somewhat towards the outer end, finally terminate in a convex, marginally pronged, conspicuous disc, about 16 μ in diameter as measured from tip to

tip of two oppositely standing prongs. The prongs are strong, recurved; in number 5–8, usually 6, to each disc. Now and then the rosette under consideration takes the form of a discohemihexaster (fig. 11), in which one or more of the principals have only one terminal in a straight line while the remaining principals have two in the usual disposition. In a few cases I have observed a uniterminal ray crooked near the base in much the same way as is known to sometimes occur in oxyhemihexasters of certain Rossellids. Purely hexactinose form of the discohexaster in question (=F. E. SCHULZE's Derivat-Hexactin or Discohexactin, which terms I think had better be avoided as liable to lead to a misconception) is also met with, though only very rarely. Such a hexactinose discohexaster exactly corresponds in shape with the same of *Corbitella elegans* (Pl., fig. 13) but is smaller by nearly one-half.

Possibly a second form of discohexaster, differing considerably from the one above described, is to be ascribed to the species. I say this on the strength of the single case I have discovered of the very small, incompletely preserved discohexaster, which I have shown in Pl., fig. 12. It measured only 40 μ in diameter. From each short principal there arise divergingly slender, rough-surfaced terminals, 3 or 4 in number. The terminal disc is composed of about 6 minute claws forming an irregular umbel; at any rate the claws are not uniformly recurved and seem to spring at variable angles from the end-point of the terminal.

The graphiocome has never been observed intact. Nevertheless, its presence in the species is not to be doubted from the occurrence of fine rhaphides (about 150 μ long), either isolated or grouped into bundles, and which, as I have shown in the last Contribution, take their origin as the terminals of graphiocomes.

Corbitella elegans (MARSHALL).

(Plate, figs. 13–15.)

Eudictyon elegans. MARSHALL, '75, p. 211; Pl. XVI, figs. 66 *a–l.*—MARSHALL, '76, p. 129.

Eudictyum elegans. SCHULZE, '86, p. 43.—SCHULZE, '87, pp. 103–104.—SCHULZE, '00, pp. 164–165.

The possibility of the little known *Eudictyum elegans* MARSHALL being generically and even specifically identical with *Corbitella speciosa* has been assumed by both MARSHALL and F. E. SCHULZE. The result of my examination of MARSHALL's type, preserved in the Museum of the Zoölogical Garden in Amsterdam, tends to show that while the species should decidedly be ranged under the same genus as *C. speciosa*, the extension of the identity to the point of species is scarcely justifiable. At least so long as the differences which I shall point out in the sequel remain not bridged over by intermediate transitions, I consider it expedient to keep up MARSHALL's species under the name of *Corbitella elegans.*

The locality of the single specimen on which the species is based has never before been made known. On the label attached to it, it stands thus: "Coll. van der Hucht. Molukken." The specimen thus comes from the same islands as *C. speciosa*, a fact which made me at first presume its identity with the species just mentioned, as had been suggested by MARSHALL and SCHULZE. As known through the original describer the two specimens here alluded to closely resemble each other in general appearance and, I may add, in spiculation too, except in certain points to which attention will soon be called.

The type of *C. elegans* is a complete specimen ; only it is strongly macerated on the external surface, so that the cuff and parietal ledges, if these were originally at all present, must have entirely fallen away. A considerable quantity of the loose tissue however still exists in connection with the skeletal lattice-work, while the internal surface of the wall seems to be tolerably well preserved. Nothing unusual in the quantity of the loose tissue on this surface attracted my attention ; it is of much the same character and appearance as I know it to be in *Regadrella* or in *Euplectella*. The statement of MARSHALL ('75) that the "flake-tissue" lies on the inner side of the skeletal lattice-work applies to the actual condition only in a relative sense that that tissue has been lost from the outside by abrasion.

The clavate tubular body measures 232 mm. in height. Inferiorly it gradually narrows towards the solid basal knob, close to which extremity the width is 34 mm. The irregularly rounded upper extremity is 101 mm. wide, as measured in one way. The wall at this end shows a considerable—evidently an abnormal—outbulging towards one side, so much so that the body appears as if bent at the top. For the measurements given above I am indebted to the kindness of Professor MAX WEBER.

Of the main parenchymal bundles or the skeletal beams, not a few pursue a more or less distinctly longitudinal course ; the rest run more or less obliquely, freely intersecting and anastomosing with one another. Thus the skeletal lattice-work is of an irregular character. A rather extensive area at the upper end is to be considered as representing the sieve-plate. The beams and the meshes at that part look much like those in the sieve-plate of either *Regadrella okinoseana* or *Euplectella marshalli*. However, as was mentioned by MARSHALL, they pass over

gradually and without any abrupt change in character into those of the lateral parietes.

According to MARSHALL the sponge consists of unfused spicules, which statement, as he himself gave it to be understood, is based on but a hasty observation with the naked eye. To me it at once seemed apparent that all the main skeletal beams—those of the sieve-plate included—consisted of spicules soldered together in the usual manner. The truth of this observation was later confirmed by microscopic examination of a beam fragment as well as by a note received from Professor MAX WEBER, who, at my request, kindly determined the fused state of elements in the sieve-plate beams. Spicular fusion existing in this structure, there can be no question as to the same condition obtaining in the skeletal lattice-work of the lateral wall; for, it is a recognized fact that the soldering together of spicules begins at the base and proceeds continuously towards the apex of the sponge-body.

Of the loose tissue half a dozen preparations stand at my disposal for study. So far as are represented in these, the supporting parenchymalia are mainly diactins (length 4 mm. and under; breadth near the center 6–45 μ) and much less frequently hexactins with slender rays and of variable dimensions under a medium size. There also occur at intervals the same slender microxyhexactins, in both the smooth and the peculiarly spiny variety, that were found in *Corbitella speciosa*. Axial length of microxyhexactins 330–150 μ; breadth of ray near the center 3 or 4 μ. Scattered here and there are the small, plump-looking, 2–6 rayed spicules which had been described and figured by MARSHALL ('75, figs. 66 *e–h*) and to which I have already alluded under *Corbitella speciosa* (p. 8). Axial length 57 μ and over; thickness

of ray 6–16 μ. Occasionally I have found this peculiar spicule adhering to the shaft of the dermalia.

In details of character all the spicules thus far noticed agree with the corresponding elements in *Corbitella speciosa*; so that, what I have said concerning them may be considered to hold good here also.

Sword-shaped hexactins of variable size, which by the manner of their arrangement distinctly prove themselves to be the dermalia, are plentifully represented in my preparations (Pl., figs. 14 and 15). They have mostly strong rays, measuring 20 μ or more—up to 27 μ—in breadth near the central node, while in other and weakly developed cases the breadth may not exceed 8 μ. The total length of the spicule may reach nearly 1 mm. The hilt-ray, up to 200 μ in length, narrows but slightly towards the rounded outer end; it is nearly or quite smooth throughout. In certain cases I have seen that ray reduced to a knob; and in some others it was the blade-ray that was so unusually shortened as to differ not much in length from the paratangential rays. Whether the much thicker rays of average dermalia can be looked upon as one of the distinguishing characters of the species in contrast with *Corbitella speciosa*, will require testing with more specimens.

The gastralia are not represented in my preparations.

As regards the hexasters, the occurrence of graphiocomes is certain, since the rhaphides (145 μ long) are not uncommonly met with, either scattered or grouped into bundles.

The floricome measures 98–114 μ in diameter. It is therefore considerably larger than in *Corbitella speciosa* (72–83 μ). Each slender principal bears a perianth of 6–8 terminals. Terminal plate with 5 or 6, moderately large claws.

A difference much more remarkable than that in the size

of the floricomes exists between this and the foregoing species in the nature of the discohexaster. Whereas in *C. speciosa* this kind of rosette occurs predominantly in the ordinary hexaster form and only exceptionally in the modified hexactinose form, the relative proportion of these two forms as they occur in *C. elegans* is just the reverse. A further point of important difference lies in the fact that the hexactinose discohexaster of the latter species is usually nearly twice or more than twice as large as any discohexaster in the former (Pl., fig. 13).

The hexactinose discohexaster of *C. elegans* was figured and remarked upon by MARSHALL. In all my preparations it occurs in very great abundance and in most places in crowded disposition. Axial length 220–264 μ. The six rays arranged as in a regular hexactin are usually straight or nearly so. Near the central node about 7½ μ thick, they narrow somewhat outwardly but to thicken again near their junction with the convex terminal disc. Breadth at the middle about 6 μ. The terminal disc measures up to 19 μ in diameter. Its recurved marginal prongs are strong; in number 5–8, usually 6, to each disc. Altogether the disc is shaped exactly like that of the discohexaster of *C. speciosa*.

In the figure of this characteristic spicule given by MARSHALL ('75, fig. 66 *b*) one important point requires a correction; *viz.*, the axial filament is represented by him as reaching right up to the terminal disc. This is by no means the fact. Examination of the spicule in glycerine reveals that the axial cross is confined to the central node, in a manner known to me to be the case in all hexactinose rosettes derived from hexasters (LJ., '97, pp. 44–45). I do not know how to account for another figure of MARSHALL's (*l. c.*, fig. 66 *d*), in which a spicular ray with axial filament is shown as being forked into two disc-bearing

branches, unless it be a faulty representation of a discohexaster principal bearing two terminals.

As already alluded to, the same discohexaster in the ordinary form, in which each principal is supplied with 2 or 3 terminals, is however not wanting; but such forms occur every rarely indeed. In fact I have met with only two such cases, measuring respectively 122 μ and 165 μ in diameter. They were thus much smaller than the hexactinose variety, and this fact seemed to bring the relationship between *C. elegans* and *speciosa* very close indeed.

Finally, the peculiar spicule mentioned and figured by MARSHALL (*l. c.*, figs. 66 *i* and *k*), which shows some resemblance to the central portion of a graphiocome but is much too large for it, has not been discovered by me. Probably he was right in regarding that spicule as of extrinsic origin.

Corbitella pulchra (F. E. SCH.).

Tegeria pulchra. Narr. Chall. Cruise, '85, fig. 158.—F. E. SCHULZE, '86, p. 41.—F. E. SCHULZE, '87, p. 94; Pl. VII, Pl. VIII and Pl. XI, figs. 1–3.—F. E. SCHULZE, '95, pp. 35, 49.

The genus *Tegeria* was instituted by F. E. SCHULZE for the single species, *T. pulchra*, known in a unique specimen that was obtained by the "Challenger" near the Fiji Island from a depth of 1115 meters. From the detailed descriptions given by that investigator, it clearly follows that that species can not be held generically separate from either *C. speciosa* or *C. elegans*. The name *Tegeria* should be put down as a synonym of GRAY's older name *Corbitella*.

Especially close seems to be the resemblance in spiculation between *C. pulchra* and *C. speciosa.* Under general agreement in shape and structure, the two species have in common not only the smooth and the spiny microxyhexactins, the graphiocome and the floricome, but also similarly characterized discohexasters in both the ordinary and the hexactinose form (=F. E. SCHULZE's Discohexactin).

So far as our knowledge goes, the following points in the structure and spiculation of *C. pulchra* seem to be noteworthy as offering probably useful data for its differential diagnosis:

1. The body, whose length (200 mm.) does not fall much short of that of the known specimens of *C. speciosa* and *elegans*, is in shape ventricose, not clavate.

2. The upper end of the body, instead of being covered by a sieve-plate, is overarched by a wreath of projecting rays belonging to the principalia marginalia. This condition has been assumed by F. E. SCHULZE ('95, p. 35) as possibly due to a partial loss of a sieve-plate, such as is found in *Dictyaulus elegans* F. E. SCH. Now while that possibility can not be wholly set aside, it seems to me equally possible that we have here to do with the same perfectly natural phenomenon as the coronal wreath of *Regadrella komeyamai* IJ. ('01, p. 253).

3. Assuming there was originally a sieve-plate, this could not have passed over (to judge from SCHULZE's figure) so insensibly into the lattice-work of the lateral wall as in either *C. speciosa* or *elegans.* Moreover there exists a marginal ridge which seems to be tolerably persistent.

4. The parenchymal bundles, while irregularly crossing one another in the greater part of the body, are arranged in regular longitudinal and transverse systems near the upper end.

5. The synapticular fusion of spicules does not extend to the upper end of the body.

6. The strongest parenchymal principalia are predominantly stauractins; more seldom triactins and diactins.

7. The dermalia, which vary much in size, should have all the rays running out to a fine point.

8. The floricome, according to my computation from the figure in the Challenger Report, should measure about 100 μ in diameter, which size is about the same as in *C. elegans* but considerably larger than in *C. speciosa*.

9. Of the two forms of the discohexaster the ordinary seems to be by far the commoner as in *C. speciosa*. Both forms should be of about the same size. For the hexactinose form, F. E. SCHULZE has given 170 μ for axial length. This indicates the size of the discohexaster to be larger than in *C. speciosa*, and considerably smaller than in *C. elegans* so far as the hexactinose form is concerned.

Heterotella corbicula (BOWERBANK)

(Plate, figs. 16–23.)

Euplectella sp. BOWERBANK, '58, Pl. XXV, figs. 37 and 38 (*probably also* figs. 35 and 36 and Pl. XXVI, fig. 5).

Alcyoncellum corbicula. VALENCIENNES *in literis*, Mus. d'hist. nat. Paris.—BOWERBANK, '62, pp. 1103, 1104.—BOWERBANK, '64, p. 176.—BOWERBANK, '67, p. 358.

Alcyoncellum sp. BOWERBANK, '64, Pl. VII, fig. 187 (*probably also* figs. 185 and 186) and Pl. VIII, fig. 188 (? *also* fig. 195).

Heterotella corbicula. Gray, '67, p. 531; Pl. xxviii, fig. 2. —Gray, '72, p. 457.

Habrodictyon corbicula. W. Thomson, '68, p. 129; Pl. iv, fig. 1.—Carter, '73, pp. 361, 367.

Habrodictyon speciosum in part. Marshall, '76, p. 129.

Habrodictyum speciosum in part. Schulze, '86, p. 42.—Schulze, '87, p. 99.

Corbitella corbicula. Schulze, '00, p. 156.

In the zoological museum of the Jardin des Plantes there exist three specimens bearing the name "*Alcyoncellum corbicula.*" All come from the Island of Bourbon. In my opinion they represent a distinct species for which a distinct genus should be allotted.

Two of the specimens are evidently those that were remarked upon by Bowerbank ('67, pp. 358, 359); all the three seem to have been examined by W. Thomson ('68, pp. 129–131). For the sake of reference I will call them Specimens A, B and C.

Specimen A. This is the specimen which is labelled "*Alcyoncellum corbicula* Val. Tiré par 80 brasses de profondeur dans la rade St. Denis de Bourbon par Mr. Leschenault, 1819." Of all the three specimens it is the one that earliest became known, in that Bowerbank, as early as 1858, had figured some spicules from it and in 1867 had given a short account of it, claiming its distinctness as a species from all other "*Alcyoncellum*" known to him.

The specimen is the torn upper end of a tubular Euplectellid sponge, consisting of a partly damaged, roundish and flatly convex sieve-plate, 50–58 mm. in diameter, and of the adjoining part of

the lateral wall to an extent of about 55 mm. or less in length. A short distance from the top, the diameter of the body must have measured nearly 70 mm. The lateral wall slightly closes in towards the sieve-plate margin, somewhat as in *Regadrella phœnix* (Ij., '01, p. 267). The entire length of the original individual can of course not be ascertained. That the base was firmly fixed to a hard substratum may be concluded from the state of that part in Specimen B.

The sieve-plate closely resembles that of *Euplectella* or of *Regadrella*. The meshes are oval, ovoid, triangular or quadrangular with rounded corners, some of the largest measuring 3½ mm. across. The beams are laterally compressed and measure up to 1 mm. or thereabout in breadth as seen from above. Many of them are perceptible as more or less radially directed, especially in the peripheral part of the sieve-plate.

The ring-like edge of the sieve-plate is well marked. Here all around was probably originally present a low continuous ridge, which is at places still preserved with fine palisade-like marginalia projecting out to a length of nearly 2 mm.

The lateral wall, much lacerated on the outside, is rather thin. Its general appearance is very much like that of the same part in *Euplectella imperialis* or in *E. marshalli*, in a similarly macerated state of preservation. Externally are seen a number of parechymal bundles running for the greater part obliquely. Beneath these is the more strongly developed system of longitudinal bundles. As has been correctly noted by Bowerbank ('67, p. 358), these bundles for the most part terminate when they reach the marginal ring; the rest cross it and pass continuously into the sieve-plate beams. Internal to all is the system of wavy transverse bundles, which together with the longitudinal forms a

tolerably regularly checkered lattice-work. The meshes of the lattice-work contain each a parietal osculum. This is circular, measures up to 2 mm. in diameter and is separated from nearest neighbors by a space of 2–3 mm. Nowhere in the specimen is the ankylosis of spicules observable.

The spiculation of this specimen will be described in detail, after we have dealt with the two other specimens.

Specimen B. This bears the labelling "*Alcyoncellum corbicula*. Bourbon. M. Sachet. 1857."* It is the complete specimen of which an account was first given by Bowerbank in '67, p. 359, who held it as specifically different from all other specimens bearing the same name in the Paris museum. A very good figure of the same specimen, prepared after a photograph, was shortly afterwards given by Gray ('67, fig. 2) and also by W. Thomson ('68, fig. 1) together with a description based on original observation. As this specimen, besides being entire, differs somewhat in general appearance from Specimen A, a special description will not be out of place.

The sponge is tubular, shaped somewhat like a glass-tumbler, abrupty truncated above and somewhat tapering downwards. In the upper part it is roundish in cross-section; inferiorly it becomes four-cornered, finally to become compressed at the base. At this end the wall is broken through but is furnished in parts with a few knob-like points of attachment to a hard substratum. Total length of body 105 mm. Greatest breadth, close to the upper end, 92–96 mm.

* The wording on the label as given by W. Thomson ('68, p. 131) should run somewhat differently. Either the labelling has since been changed or W. Thomson did not quote correctly, perhaps by a slip of memory.

The nearly horizontal sieve-plate at the upper end is irregularly circular, measuring 50–60 mm. in diameter. In the middle it is broken; against the lateral wall it is bounded by a low, compact-looking marginal ridge. Meshes of the plate shaped as in Specimen A, but on the whole somewhat larger, measuring up to 4 or 5 mm. across. Beams laterally compressed, up to $\frac{3}{4}$ mm. in width as seen from above. The nodes may however be $1\frac{1}{2}$ mm. broad. The stronger beams show a somewhat radial arrangement.

The lateral wall is thick, measuring at places 5 mm. or over in thickness. Pit-like or shallower depressions give quite an uneven appearance to the external surface. The bottom of each such depression is occupied by a round parietal osculum, which may measure 2 mm. in diameter. The oscula are arranged irregularly, not in regular rows. The spaces between the oscula are made up of loosely connected fine fibers or ill-defined bundles of fibers, which pursue an indefinitely directed and irregularly flexuous course, intersecting at low angles and anastomosing in all directions. Only in the uppermost portion of the wall one perceives that many of the bundles show a tendency to a more or less longitudinal direction. On the whole the wall, as seen from the exterior, appears irregularly latticed and woolly.

On the internal surface is seen a distinct system of rather widely separated, compact-looking, wavy and intersecting bundles which are in general transversely directed.

The entire sponge is quite soft. The external surface has been subjected to much laceration, but there still remains in the parenchyme a large quantity of dried-up soft parts and of microsclerae. Evidently ankylosis between the spicules nowhere exists except in parts of the basal knobs in direct contact with the substratum.

The sponge differs from Specimen A in its much thicker wall and in the more irregular disposition of its skeletal bundles. Striking as is the difference at first sight, I consider it as due to individual circumstances. Not improbably we have here to do with a specimen fully developed in all parts but stunted in general growth. I am inclined to think that the checker-like arrangement of the main parenchymal bundles in Specimen A, as also in the corresponding portion of *Corbitella pulchra*, is related to the relatively young state of the part concerned. After that part has reached a certain stage of development, an irregularity in the arrangement of the bundles may set in, as is factually seen in the lower and therefore the older portion of *Corbitella pulchra*. This is all the more conceivable in *Heterotella corbicula*, since here the parenchymalia are totally wanting in large stauractins, whose paratangentially disposed rays might permanently determine the vertical intersection of the bundles. Moreover, if in Specimen A the external oblique bundles should undergo a further quantitative development, a great approach in its appearance towards that of Specimen B would be the result.

As regards spiculation the two specimens are practically identical even to details, so far as my observations go. For this reason I consider it superfluous to enter specially into its description.

Specimen C. The labelling of this runs: "*Aleyoncellum corbicula* Val. de Bourbon. Donné par M. Sachet 1857." It is the torn upper end of the body, much mutilated and preserved pressed between two plates of glass. In the middle is the irregularly oval sieve-plate, 54 mm. and 68 mm. in diameters. Around this is attached a small adjoining portion of the lateral wall,

much rent and flattened out. As the specimen is sealed up between the glasses I have not undertaken a microscopic investigation of its spicules; but in general structure it so closely resembles Specimen A that I have little doubt of the two being specifically identical.

Spiculation. The following description of the spiculation is principally based on my observations on Specimen A. It may however be considered to hold good also for Specimen B, unless otherwise mentioned. Of each specimen I have four microscopic preparations to make studies on.

The regularly checker-like arrangement of the main skeletal bundles in Specimen A made me at first assume the presence of large stauractions among their elements. But in this I was mistaken. For the principalia of the said bundles as well as of the parenchyme in general, I have found only diactins. These are long and bow-like or bent in the middle in an elbow-like manner. The center is even-surfaced. The larger principalia in the main bundles may be 15 mm. long and 150 μ thick at the center.

The accessoria parenchymalia, occuring partly as comitalia and partly in loose arrangement, are predominantly long and filamentous diactins of the usual character. Length up to 5 mm. or more. Breadth near the middle down to about 4 μ. Center four-knobbed, less frequently simply annulated. Occasionally the four knobs are prolonged into regular rays, thus converting the spicule into a hexactin in which one of the axes is more or less elongated in excess over the others. Quite or nearly regular hexactins of a medium or smaller size and with slender pointed rays, are also not wanting among the accessoria parenchymalia.

Such hexactins, 200–300 μ in axial length and 3–6 μ in breadth of rays near the center, were at places not uncommon. When very small (under 100 μ in axial length), as they were sometimes found to be, they may deserve to be called smooth microxy-hexactins. But these are quite rare.

The dermalia are mostly sword-shaped hexactins (Pl., figs. 17 and 18) of variable size and strength. While a small one may measure only half a millimeter or thereabout in length, a large one may be over one millimeter long. In thickness of rays the variation ranges from 9 μ up to 30 μ, as measured close to the center. The comparatively short guard-rays and the prolonged blade-ray have rough ends, ultimately terminating in conical points. The short hilt-ray, generally under 100 μ in length, tapers somewhat outwards and ends rounded; it is either smooth all over or sparingly beset with low microtubercles. Not uncommonly the hilt-ray is reduced to a mere knob (Pl., fig. 19) or has even entirely disappeared; the spicule is then a pentactin with the unpaired ray much prolonged. It can nevertheless be recognized as a dermalia on account of its arrangement in association with other unmistakeable hexactin-dermalia. Moreover, different stages in the reduction of the hilt-ray are plentifully represented in the varied length of that ray in different dermalia.

Certain pentactins with much more slender rays, which I have occasionally met with in isolated positions, gave me the impression that I had before me elements of the gastral skeleton.

The marginalia (Pl., fig. 16) are hexactins in which the distally directed ray is specially developed and the longest. They again are quite variable in size and strength; they are, I believe, connected with the dermalia by all sorts of intermediate sizes and forms. Tolerably well developed marginalia have the distal ray

considerably over one millimeter in length and 40 μ thick near the base but slightly thicker farther outwards. The outer end of the ray is tapering, but the extreme tip is found invariably broken off. Except at the basal portion and also to a certain extent at the outer end, the ray is furnished with low, conical and irregularly distributed tubercles, which are however never very numerous. The apices of the tubercles, not always pointed but often rounded, are directed either laterally or obliquely outwards.—A spicule exactly like the marginalia here described was also discovered in the preparations of Specimen B, which, by macroscopic observation, showed no trace of a marginal palisade.

The sieve-plate beams are composed mainly of the parenchymal diactins, which are here somewhat shorter than in the lateral wall. Certain hexactins situated in the midst of these diactins are certainly to be considered as likewise parenchymal; but other hexactins and pentactins of regular shape and occuring superficially in some numbers undoubtedly represent the dermalia —and perhaps also the gastralia—in this region of the body. The said hexactins and pentactins have an axial length of 260 μ on an average; the tapering rays vary in thickness at the base from 4 μ to 15 μ. Among these spicules I have sometimes observed, more frequently in Specimen B than in Specimen A, abnormalities in the form of distorted rays, of tubercular formations or of amalgamated parts of other spicules. The irregular looking spicule given by W. Thomson ('68) in his fig. 1 *a* undoubtedly comes under this head.

From both Specimens A and B I have a parietal oseulum cut out and made into preparations. In these I do not find any specially differentiated oscularia.

I now come to consider the peculiar spiny microhexactin

(Pl., figs. 20–23), which constitutes the most characteristic kind of spicules in this genus and species. In my opinion it is directly derived and not far removed from the spiny microxyhexactin that we have seen in *Corbitella speciosa*, *C. elegans*, etc., and at the same time it may represent to a degree a stage in the transformation of a spiny oxyhexactin into an oxyhexaster. The spicule is the one that has been figured by BOWERBANK ('58, Pl. XXV, fig. 38; '64, Pl. VIII, fig. 188) under the name of "bifurcated rectangulated hexradiate spicule." Apparently the identical spicule has also been figured by W. THOMSON (*l. c.*, fig. 2 *a*) and alleged by him to belong to *Corbitella speciosa*.

The spicule in question occurs in the parenchyma of both Specimens A and B, not very abundantly but at intervals in rather scattered distribution. Its size is somewhat variable: axial length 64–100 μ in Specimen A; 76–136 μ in Specimen B. In the center is a small spherical node, whence arise the fine, gradually tapering rays, not more than 2 μ thick at base. Some of the six rays may be perfectly simple, running out to a fine point, as in a smooth microxyhexactin (see Pl., figs. 22 and 23). But more generally the rays are armed with tolerably long, slender and branch-like spines, which are however never numerous. The usual number is 1–3, at most 4, to a ray. The spines arise at quite indefinite points in the space from about the middle to the outer end of the ray, and are generally directed obliquely outwards, though cases of a retroverted or of a vertically outstanding spine were sometimes met with. After taking origin they are either nearly straight or curved one way or the other, always running out to an exceedingly fine point.

When a spine or spines spring out laterally from a ray that keeps up a tolerably straight course to the end, as is occasionally

the case, the entire ray is in all essential points comparable to that of a spiny microxyhexactin (Pl., fig. 9), such as we have seen in *Corbitella speciosa* or *C. elegans*. Sometimes a ray is seen to end rather abruptly, apparently without being broken off at the point, and to send out a spine close to that end at varying angles. This leads over to cases in which a ray appears simply bent at a certain distance from the outer end, and also to those in which a ray, though nearly straight throughout, shows a sudden diminishing in caliber at a similar position. I have therefore been led to the belief that in certain cases—but not in all—the terminal portion of a ray, much as it may took like a direct elongation of the ray itself, is to be considered as a spine of secondary nature and not as a part of the ray proper. This would be much the same as the relation between a terminal and a principal in a hexactinose hexaster.

Further it very frequently happens that towards their outer end the rays appear more as if split into two or three, symmetrically or asymmetrically divergent branches, rather than as bearing one or two spines at the spot (see figs.). Nevertheless, a close observation may sometimes reveal the fact that one of the branches, owing to a slight difference in caliber or in the manner of origin, may not improperly be interpreted as a part of the ray proper and the rest as secondary appendages or spines. But at other times all the fine branches are so exactly similar in appearance that it is impossible to attempt the distinction. I am greatly inclined to believe that in many, if not all, such cases what appear as branches are really all spines borne at the extreme end of a ray, similarly to the terminals in an oxyhexaster are. A demonstration of the extent of the axial filament in the rays should make the matter clear. However, by examining the spicules

in glycerine and under a high power of the microscope, I could indeed trace the filament for some distance from the central node into the rays, though nowhere with as much distinctness as in a larger spicule, and soon it always became quite indistinguishable, probably on account of the fineness of the rays themselves. So that, no use could be made of the observation in clearing up the question.

The hexaster present in the species is only of two kinds: *viz.*, the floricome and the graphiocome. Notwithstanding a thorough search made in my preparations, I have failed to discover a third hexaster form. Whether this negative character is due to a loss by the species or is to be regarded as primary, is of course difficult to say. Possibly the absence is to a certain degree compensated by the presence of the interesting spiny microhexactin above described.

The floricome in Specimen A was mostly represented by fragments; only in two cases was it found nearly intact, measuring in diameter 94 μ and 100 μ respectively. In Specimen B several could be measured, ranging 82–92 μ in diameter. The floricome is of a delicate appearance; principals slender; terminal claws of moderate strength.

The graphiocome as such was never found; but the occurrence here and there of unmistakeable rhaphides sufficiently attests its presence in the species.

In conclusion, a few words about the Euplectellid subfamily to which the forms treated of in this Contribution belong, to be followed by summarized statements of the characters of these.

The subfamily in question is the Tægerinæ of F. E. SCHULZE. Granting that this is to be kept up as separate from the Euplectellinæ, there seems to be a necessity for renaming it, since *Tegeria* has sunk to the rank of a synonym of *Corbitella*. I therefore propose to call the subfamily by the name of Corbitellinæ.

The definition of the subfamily ***Corbitellinæ*** would be somewhat as follows :

Euplectellidæ of saccular or tubular body, always firmly attached by the base to solid substratum. Superior end of body mostly with a sieve-plate. Lateral wall with parietal oscula which are devoid of specially differentiated oscularia. Skeletal lattice-work usually irregular; fused or unfused. Principalia parenchymalia as a rule diactins, sometimes stauractins. Accessoria parenchymalia slender-rayed diactins, hexactins, etc. Spiny microhexactins present in many. Hexasters generally include floricome and graphiocome, in addition to which there usually occur one or more other hexaster varieties.

The genera and species sufficiently known to be referable to this subfamily are :

1. *Corbitella speciosa* (Q. and G.). Moluccas.
2. *C. elegans* (MARSH.). Moluccas.
3. *C. pulchra* (F. E. SCH.). Fiji Is.
4. *Heterotella corbicula* (BOWERB.). Bourbon Is.
5. *Regadrella phœnix* O. SCHM. Atlantic, E. Pacific.
6. *R. okinoseana* IJ. Sagami Sea, Indian Ocean.
7. *R. komeyamai* IJ. Sagami Sea.

8. *Walteria flemmingi* F. E. Sch. N. of Kermadec.
9. *W. leuckarti* Ij. Sagami Sea.
10. *Dictyaulus elegans* F. E. Sch. Indian Ocean.
11. *Dictyocalyx gracilis* F. E. Sch. S. Pacific.

Diagnoses of the Corbitellinæ described in this Contribution.

Genus **Corbitella** Gray.

Corbitellinæ with sieve-plate; saccular, thin-walled, phallus-like. Lateral wall with numerous, round and irregularly arranged parietal oscula. Spiny microxyhexactin present. Besides the floricome and the graphiocome, there occurs a tolerably strongly developed form of discohexaster in which each terminal ends with a convex disc with strong, recurved, marginal prongs.

Corbitella speciosa (Q. and G.).—*Corbitella* with body broadest at the arched upper end. Skeletal beams, including those of the sieve-plate, fused together. Principalia parenchymalia probably diactins; accessoria diactins and hexactins. Discohexaster in the ordinary hexaster form or in the hemihexaster form; rarely hexactinose; diameter 100–130 μ, occasionally up to 145 μ. Diameter of floricome 72–83 μ.

Corbitella elegans (Marsh.).—Quite like *C. speciosa* except in the following points: Discohexaster predominantly in the hexactinose form; diameter 220–264 μ. Diameter of floricome 98–114 μ.

Corbitella pulchra (F. E. Sch.).—*Corbitella* with ventricose body; skeletal beams in the uppermost part of the wall arranged

longitudinally and transversely (always?). Sieve-plate present (?). Skeletal beams unfused in the upper part of the body. The strongest principalia parenchymalia are mostly stauractins. Discohexaster predominantly in the ordinary hexaster form; sometimes hexactinose; diameter about 170 μ. Floricome about 100 μ in diameter.

Genus **Heterotella** Gray.

With a single species.

Heterotella corbicula (Bowerb.).—Corbitellinæ of saccular shape, the lateral wall slightly closing in towards the margin of a flatly convex sieve-plate. With numerous, round, irregularly arranged parietal oscula. Skeletal beams unfused. Principalia parenchymalia diactins; accessoria diactins and hexactins. Microhexactins present, in which the rays are sparingly supplied with long, slender spines; such a ray sometimes appearing like a long oxyhexaster-principal with 1–3 terminals. Floricome and graphiocome only present; no discohexaster.

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PLATE.

Illustrating: I. IJIMA, Studies on the Hexactinellida. Contribution II.

The Genera *Corbitella* and *Heterotella*.

Explanation of Plate

Figs. 1–12, *Corbitella speciosa* (Q. and G.).

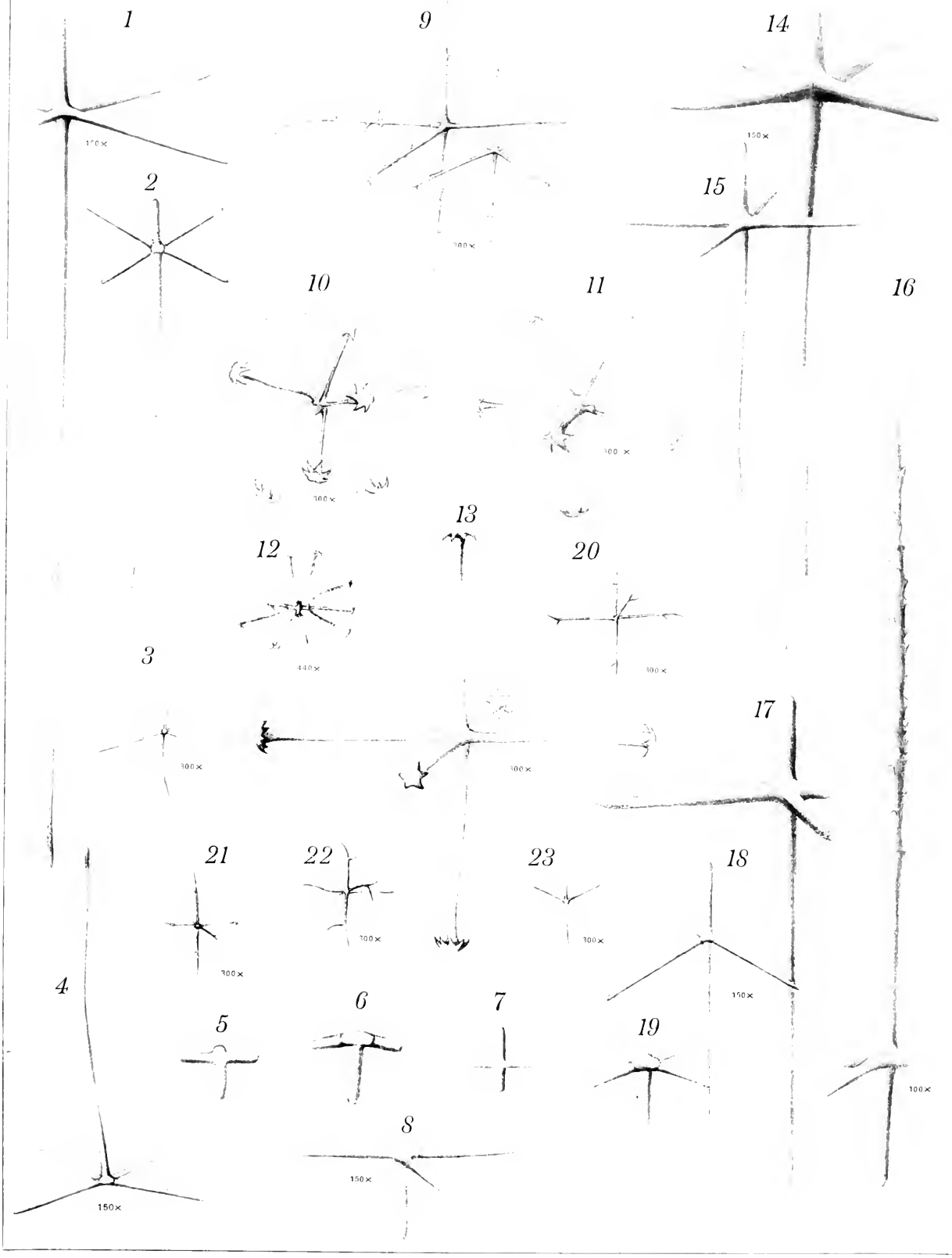
Figs. 1–2. A large and a small dermalia. 150×.
Fig. 3. A smooth microxyhexactin from the parenchyma. 300×.
Fig. 4. A gastralia. 150×.
Figs. 5–8. Small, plump-looking spicules of various shapes and dimensions, from the parenchyma. 150×.
Fig. 9. Two spiny microxyhexactins from the parenchyma. 300×.
Fig. 10. A discohexaster with 2 or 3 terminals to each principal. 300×.
Fig. 11. A discohemihexaster. 300×.
Fig. 12. A small discohexaster found only once. 440×.

Figs. 13–15, *Corbitella elegans* (Marsh.).

Fig. 13. A hexactinose discohexaster. 300×.
Figs. 14, 15. Dermalia. 150×.

Figs. 16–23, *Heterotella corbicula* (Bowerb.).
All from Specimen A.

Fig. 16. A marginalia. 100×.
Figs. 17–19. Dermalia. 150×.
Figs. 20–23. Spiny microhexactins. 300×.



1–12, CORBITELLA SPECIOSA. 13–15, CORBITELLA ELEGANS. 16–23, HETEROTELLA CORBICULA.

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Studies on the Parasitism of *Buckleya Quadriala*. B. et H., a Santalaceous Parasite, and on the Structure of its Haustorium.

By

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With one plate.

I. INTRODUCTION.

The family Santalaceæ includes, according to HIERONYMUS,[1] twenty-six genera, some of which are already known as hemiparasites.[2] But investigations on this family are far from being complete and especially those species known as hemiparasites can scarcely be said to have been adequately treated, if we except some widely distributed species belonging to the genus *Thesium*. The inadequacy of our knowledge of this family has led HIERONYMUS to remark most properly that probably other genera of the Santalaceæ should be counted among the category of the hemiparasites.[3]

1). HIERONYMUS, Santalaceæ. ENGLER und PRANTL, Die natürlichen Pflanzenfamilien, III. 1, 1889, p. 202.

2). These are *Henslowia*, *Phacellaria*, *Osyris*, *Santalum*, *Comandra*, *Thesium*, *Arjona*, and *Quinchamalium*.

3). HIERONYMUS, loc. cit. p. 203.

It must therefore be admitted that studies on the ecology and physiology of these plants, interesting and important as they are, are still wanting, whereas about the habits of hemiparasites belonging to other families we have a more or less clear knowledge, owing to the numerous investigations of KOCH,[1] PEIRCE,[2] HEINRICHER,[3] WETTSTEIN,[4] etc.[5]; for instance, the gradation of accommodation from autophytic towards parasitic nature was found to prevail among several kinds of plants in a certain family. So far as the Santalaceæ are concerned, the results obtained up to the present time are, as already indicated, very meagre, and leave much to be desired, especially on the mutual relations between the host and the parasite.

With the view of throwing some light upon this subject, I took up *Buckleya Quadriala* for study, as this plant is easily accessible to us. It will be proper to give first an account of its parasitic nature and then to proceed to the structure of its haustorium.

1). KOCH, Ueber die directe Ausnutzung vegetabilischer Reste, etc. Ber. d. deutsch. bot. Gesellsch. Bd. V, 1887, p. 350; ——, Zur Entwickelungsgeschichte der Rhinanthaceen. I. Rhinanthus minor Ehrh. Jahrb. f. wiss. Bot. Bd. XX, 1889, p. 1 and II. Euphrasia officinalis L. Jahrb. f. wiss. Bot. Bd. XXII, 1891, p. 1.

2). PEIRCE, On the Structure of the Haustoria of some Phanerogamic Parasites. Ann. of Bot. Vol. VII, 1893, p. 291.

3). HEINRICHER, Die grünen Halbschmarotzer. I. Odontites, Euphrasia und Orthantha. Jahrb. f. wiss. Bot. Bd. XXXI, 1898, p. 77; II. Euphrasia, Alectrolophus und Odontites. Jahrb. f. wiss. Bot. Bd. XXXII, 1898, p. 389; III. Bartschia und Tozzia, etc. Jahrb. f. wiss. Bot. Bd. XXXVI. 1901. p. 665; IV. Nachträge zu Euphrasia, Odontites und Alectrolophus. Jahrb. f. wiss. Bot. Bd. XXXVII. 1902, p. 264; ——, Zur Entwickelungsgeschichte einiger grüner Halbschmarotzer, (Vorl. Mittheil.). Ber. d. deutsch. Bot. Gesellsch. Bd. XVII, 1899, p. (244); ——, Auf dem Wege vom Halbparasitismus zum absoluten Parasitismus. Sond. Abdruck aus Ber. d. naturwiss.-medicin. Vereins in Innsbruck. XXV, 1899/1900.

4). WETTSTEIN, Monographie der Gattung Euphrasia. Leipzig 1896.

5). BONNIER, G., Sur l'assimilation des plantes parasites à chlorophylle. Comptes rendus. Bd. 113, 1891; ——, Recherches physiologiques sur les plantes vertes parasites. Bull. d. l. soc. bot. d. France et d. l. Belgique 93, p. 77; CANNON, W. A., The Anatomy of Phoradendron villosum Nutt. Bull. of the Torrey Bot. Club. vol. 28, 1901, p. 374; VOLKART, A., Untersuchungen über den Parasitismus der Pedicularis-Arten. (Inaug.-Diss.). Zürich 1899; SPERLICH, A., Beiträge zur Kenntniss der Inhaltsstoffe in den Saugorganen der grünen Rhinanthaceen. Beihefte z. Bot. CentBl. Bd. XI, 1902, p. 437.

So far as my knowledge goes, there is no literature specially concerned in the study of *Buckleya*. G. A. CHATIN, in his elaborate work, "Anatomie comparée des végétaux (Plantes parasites)," has given a study of *Buckleya distichophylla* among others, but he has given nothing about the structure of its root-system.[1] A comparative anatomy of the Santalaceae was then made by M. BEHM,[2] who has studied all known species of *Buckleya*, *viz.*, *B. distichophylla*, *Quadriala* and *umbellulata*; but his purpose was to find out some anatomical characters for identifying sterile specimens, so that he confined himself merely to an investigation of the structure of leaves and stems, leaving the root-system entirely out of account. It seems to me that the genus *Buckleya* has till now been considered as an autophyte; for example, it was included by CHATIN, in his above cited work, among "plantes non parasites."[3]

There is, however, good reason to think that he did not examine the root, because even a most superficial examination of the root-system should be quite sufficient to convince one of its parasitic nature.

As to the structure of the haustorium in the Santalaceae the works of PITRA,[4] SOLMS-LAUBACH[5] and SCOTT[6] are especially instructive. PITRA studied the anatomy of the haustorium of *Thesium ramosum*; SOLMS confirmed, in *T. pratense*, the results of PITRA's

1). 2nd edition 1892, p. 372 and Pl. LXXI.

2). BEHM, Beiträge zur anatomischen Charakteristik der Santalaceen. Bot. Centlbl., Bd. LXII, 1895, p. 65.

3). CHATIN, loc. cit. p. 372.

4). PITRA, Ueber die Anheftungsweise einiger Phanerogamen Parasiten an ihre Nährpflanzen. Reprinted from Bot. Ztg. Bd. XIX. 1861, p. 66.

5). H. GRAF z. SOLMS-LAUBACH, Ueber den Bau und die Entwicklung der Ernährungsorgane parasitischer Phanerogamen. Jahrb. f. wiss. Bot. Bd. VI, 1867–68, p. 509.

6). J. SCOTT, Untersuchungen über einige indische Loranthusarten und über den Parasitismus von Santalum album. Bot. Ztg. Bd. XXXII, 1874, p. 129, (Uebersetzt von SOLMS-LAUBACH).

investigation. SOLMS also studied the structure of the haustoria of *Osyris alba*, but he did not examine, it seems to me, a sufficient number of them. SCOTT, who was the first discoverer of the parasitic nature of *Santalum album*, made a thorough investigation of the external morphology of its haustorium, while on the other hand, our knowledge on the internal structure is still far from being complete.[1]

II. PARASITISM OF BUCKLEYA.

Buckleya Quadriala is a diœceous shrub widely distributed in the central part of Japan. It has lanceolate or oval, opposite leaves and very inconspicuous greenish flowers on the tip of the shoot. Old stems and branches are furnished with a grayish soft corky layer, which may be stripped off in irregular thin sheets. It is well known to common people on account of its wide occurence and especially of its edible fruit, which is crowned with four narrow leafy bracts, thus closely resembling our shuttle-cock. The seeds are oval and enclose fatty substance around the small cylindrical embryo. As the plant is generally periodically cut down, so a very old stem was inaccessible to me, but at the age of nearly 40 years it measures about 3 meters in height and 9 centimeters in diameter. Formerly it was thought that this shrub could not be successfully transplanted ; and the fact was well known to gardeners that, though its seeds easily give rise to seedlings, yet these soon cease to grow and sooner or later perish. The reason of this fact seems, however, to have remained unknown. Recently the interesting fact of the parasitic nature

1. SOLMS studied the structure of the haustorium of *Santalum album* on a specimen sent to him by SCOTT. See the foot note of SCOTT's paper (loc. cit. p. 148).

of this plant was first announced by Professor SHIRAI in his book on plant diseases written in Japanese.[1] This discovery led me to make somewhat detailed studies of this plant. First of all, I intended to determine its hosts by means of a close examination of its roots, while I was obtaining numerous haustoria for anatomical study. The plants thus determined as hosts till now are as follows:—*Cryptomeria japonica*, *Abies firma*, *Chamaecyparis obtusa*, *Quercus glandulifera*, *Carpinus japonica*, *C. yedoensis*, *Fagus japonica*, *Rhododendron sinense*, *Alnus firma*, *Fraxinus longicuspis*, *Lespedeza Buergeri*, *Ilex macropoda* and *Stachyurus praecox*.

Besides I was struck with the remarkable phenomenon that the plant seems to be able to select its host, for in a certain region where several kinds of conifers grow side by side, I have always failed to find any *Buckleya* where pine-trees grow, while on the other hand it is found abundantly and in a flourishing condition where other conifers as such *Cryptomeria*, *Abies*, or *Chamaecyparis* stand. Even where foliage trees and *Abies* grow mingled together, *Buckleya* is found most abundantly near the latter and in such cases a close examination always shows that *Buckleya* is parasitic on the *Abies*. My culture experiments with the seed of *Buckleya* showed that all the plants used were capable of being hosts, as, for instance, all the seedlings of *Buckleya* developed haustoria as soon as their young roots came in contact with those of other plants such as *Quercus glauca*, *Podocarpus sinensis*, *Torreya nucifera*, etc., and even with those of *Pinus Thunbergii*, though this fact seems to be contradictory to what had been observed in nature.

The development of the parasite in the cases of various hosts,

1). SHIRAI, Plant Diseases 1891 (In Japanese).

however, seems to display a certain unmistakable inequality of degrees, noticeable already at the end of the first growing period: the most vigorous development is undoubtedly attained by seedlings feeding on *Abies* and *Cryptomeria*. As the cultivation is yet at its beginning, it is not possible to decide conclusively whether all these hosts are really able to maintain *Buckleya* for any great length of time. However, from the mode of development of the parasite, both in nature and in my experiments, it seems possible to conclude that *Buckleya* prefers *Abies* and *Cryptomeria* as its host.

III. DESCRIPTION OF THE HAUSTORIUM.

If we examine a young *Buckleya*, one year old, we see that the whole system of the root from the hypocotyl to the fine rootlets is provided with numerous small haustoria firmly adhering to the fine rootlets of the host (Fig. 1), but in the older parasite the axial main part of the root is free from haustoria, while they are present on the lateral roots and mostly on the distal portions. The form and size of the haustorium vary within wide limits. The form seems to depend mainly upon the size of the host and especially upon the stage of development of the haustorium itself. The young haustorium is generally roundish especially when it attacks slender roots; but that fully grown is elliptical in surface view, the major axis running across the long axis of the host-root (Fig. 3), and dome-shaped in side view (Fig. 5. *c*.). Its external form may partly depend upon its internal structure, which in its turn differs according to the age, so that the form had better be considered in a later chapter which treats of its structure. Its size also depends in a certain degree upon its age,

but exceptionally even a young one is enormously large; for example, I found on *Cryptomeria* a haustorium, only one year old, which measured 8 mm. in diameter (Fig. 2 *b*), while, generally, haustoria of such age measure only 3–4 mm. On the other hand the largest haustorium which I have ever obtained was found on *Abies*: it had attained 14 mm. in diameter, at an age of perhaps more than twenty years.[1]

The surface of the haustorium is smooth when young (Fig. 5 *a*), but becomes gradually coarser, owing partly to the development of the corky layer and its splitting off in scaly sheets as in the epidermis of stem and root, and partly to the formation of concentric stripes around the haustorium, which become more numerous as the latter becomes older.

In respect to their position on the mother-root, we can distinguish two kinds of haustoria—the *lateral* and the *terminal*. The lateral haustoria occur most frequently and in the young plant the haustoria seem to be exclusively of this kind, looking like the nodules on the roots of Leguminosæ (Fig. 1). However, the haustoria in the older part of the root are generally terminal and they make an appearance as if the root tips of the parasite were penetrating directly into the host-root (Figs. 2 *b, c*; 3 *t*: 4; etc.). This difference in the position of haustoria leads us to inquire their origin, their manner of formation and their morphological nature. The occurence of a terminal haustorium in a perennial parasite has been already noticed by HEINRICHER who regarded this position in *Lathræa* as being caused by the breaking off of a part of the mother-root and added: "Ja, ich glaube auf Grund eingehender Beobachtung sagen zu können, dass die Wurzel-

1). The largest haustorium among Santalaceæ was mentioned by SCOTT in the *Santalum album*, as being 8 Linie (18 mm.) in diameter. SCOTT, loc. cit., p. 148.

spitze sich nie zum Haustorium umbildet, sicher aber geschieht das nur selten."[1]

The terminal position of the haustoria of *Buckleya* may be ascribed to the same cause, and this may be most clearly proved by a comparison of the various stages of their development, which display an apparent transition from the lateral to the terminal position. Considering at first a young haustorium, which lies laterally to the mother-root, we frequently find that the part of the mother-root beyond the point where the haustorium is emitted is retarded in growth (Fig. 5. *a*, *pr*), and this difference of development beyond and behind that point becomes more obvious as the root advances in age. That portion of the mother-root which lies beyond the haustorium and is retarded in growth, comes to obliterate gradually until at last it is cast away, leaving behind merely a small process or a scar at a certain point of the haustorium (Fig. 5. *b*, *c*). But in an old, vigorously grown haustorium, even such a scar becomes indistinguishable and thus the haustorium becomes apparently terminal (Figs. 2 *b*, *c*; 3 *t*; 4). The above stated facts regarding the position of the haustorium are not difficult to understand, if we examine its anatomical construction. The fact of the modification of the root-tip to the haustorium seems questionable to me, though HEINRICHER, as above cited, stated that this takes place in some rare cases, because the young root of *Buckleya* is provided, as usual, with a root-cap as well as with root-hairs.

Sometimes it is not easy to mark off exactly the connecting part of the haustorium from the mother-root, in the case of the

1). HEINRICHER, Biologische Studien an der Gattung Lathraea. Ber. d. deutsch. bot. Gesellsch. Bd. XI. 1893, p. 9. ——, Ueber Lathraea Squamaria. Sep.-Abdrk. aus d. Ber. d. naturwiss.-medic. Vereins in Innsbruck. XXI 1892/93.

terminal one. Such is the case when the axis both of the haustorium[1] and the mother-root run in the same direction, while the mother-root grows in thickness together with the haustorium, the thickening taking place gradually towards the haustorium (Fig. 4. *b*). The root thus thickened looks as if its tip, in the course of its longitudinal growth penetrated into another root, or as if its tip expands after it has come in contact with the root. Even where the haustorium is terminal, the exact limit between it and the mother-root is clearly distinguishable, if the mother-root be slender and the haustorium greatly developed, or if the direction of the axis of the mother-root be at a right angle or nearly so to that of the haustorium, as it should be if the haustorium were originally in a lateral position (Figs. 3; 5. *c*).

IV. ANATOMY OF THE HAUSTORIUM.

Let us now turn to the discussion of the inner structure of the haustorium. This is in general so similar to that of the Santalaceæ already investigated that it seems scarcely worth while to enter into details. But as the structure varies within certain limits according to age, it deserves special attention as to its modification. For the sake of convenience I shall discuss the structure of the young and old specimens separately. I shall first take up the young stage in order to show how closely the structure of the haustorium in this stage resembles that of other Santalaceæ in the main, endeavouring at the same time to make intelligible its later modification as well as the nature of some of its tissues, which have hitherto been misunderstood.

1). By the axis of the haustorium is meant that line which connects its point of origin and the front of the haustorium.

A haustorium is made up of the outer *cortical* and the inner *axial* parts. The cortical part, or briefly the cortex, is composed throughout of parenchymatous cells: those lying at the periphery are larger than the inner ones, roundish or tangentially stretched and loosely connected together, leaving numerous intercellular spaces between them, while those lying in the inner part arrange themselves regularly, are rich in plasm and are elongated in the longitudinal direction[1] (Fig. 8 *co.*). In the median region of the cortical parenchyma along the lateral sides of the haustorium,[2] some layers of cells, extending from the apex to the base,[3] collapse, lose their contents, and their wall becomes pressed together into a *striated band*, one on each side[4] (Figs. 7, 8, *st*). The band and those cells, which surround it and are soon to undergo the same fate, are clearly distinguishable by the absence of any trace of reserve starch-material, from the surrounding parenchyma, which are gorged with it at this period.

On the apex of the haustorium the cortical part goes into the formation of the *attaching-folds*, which overlap the host-root and lie in pairs on both sides of the haustorium itself, the younger folds being formed successively one after another inside the older (Fig. 7 *at*, *at'*). The development of these folds seems to depend upon the size of the host: for instance, when the latter is comparatively young and slender, generally a large thick fold is de-

1). *i. e.*, in the direction of the axis of the haustorium.

2). As the lateral side we designate each half of the haustorium divided by the plane which passes through the axis both of the haustorium and the host-root.

3). The end of the haustorium which adheres to the mother-root is called the base and the other end which terminates in the host the apex or the front of the haustorium.

4). CHATIN (loc. cit.) and PETRA (loc. cit. p. 15) incorrectly regarded such striation in *Thesium* as being composed of the prosenchymatous cells, but that the striation is really presented by the stretched cell-walls of parenchyma was afterwards shown by SOLMS (loc. cit. p. 559). In *Buckleya* it is composed of a very thin and delicate cell-wall presenting a fine cellulose reaction with chloroiodide of zinc.

veloped overlapping a greater part of the periphery of the host-root. The folds, in the majority of cases, are in two or three pairs, among which the inner ones are always smaller, appearing, in cross-section, like a pointed process frequently directed toward the interior of the haustorium (Fig. 7 *al'*). In *Thesium* PITRA[1] and SOLMS[2] mentioned the occurence of numerous folds in the case in which it had attacked some monocotyledonous roots, but only a single pair in the case of dicotyledonous roots. The latter author regards this difference in the number of the folds in monocotyledonous and dicotyledonous roots, to be the results of differences in the resistances exerted by the host-roots for the penetration of the haustorium and of difference in the degree of its development. He observed that the fold-formation ceases as soon as the apex of the haustorium has applied itself to the endodermis of the host, though many folds may, up to that time, have been formed successively.[3] He observed also that, in dicotyledonous roots, the penetration of the haustorium and its connection with the wood of the host are very easily accomplished, the differentiation of the tissue in the haustorium being early finished before the formation of any fold other than the primary one has taken place; while in monocotyledonous roots, as the resistance is greater, the haustorium is allowed to produce numerous folds till its tissue is completely differentiated.[4] So he regarded a certain cell-mass in the corner of the adult sucker in a dicotyledonous root as the rudiment of a secondary fold.[5] I have not yet obtained any haustorium of *Buckleya* on a monocotyledonous

1). loc. cit.
2). loc. cit.
3). loc. cit. p. 554.
4). loc. cit. p. 555.
5). loc. cit. Taf. XXXIII, Fig. 2 *m*.

root and so am unable here to compare the formation of the fold in the two kinds of roots. I can state only that in *Buckleya*, though numerous folds develope even on dicotyledonous and coniferous roots, they differ in certain respects from those of *Thesium* formed on monocotyledonous roots, for we find in the former that the outermost fold is always the thickest and the largest, adheres to the host-root even after the younger one is formed within, while on the other hand, in the case of *Thesium* it is generally the youngest fold which is the thickest and the largest, the older folds being lifted away from the host-root.[1] The cells constituting the folds differ a little from those which are found in the remaining part of the cortex. Here the outermost cells are arranged compactly and at right angles to the surface and are somewhat elongated in this direction, while the remaining cells do not keep up any definite form or size, being mostly round with wide intercellular spaces between them. Along the median region of all folds the cells seem to go into the formation of the striated bands which unite directly with the similar bands in the cortex (Fig. 7).

The surface of the cortical part, as has been stated above, is covered with corky layers (Fig. 8 *ck*). The thin and thick walled layers are formed alternately and the older part can be easily stripped off layer by layer. The layer extends not only to the exposed surface of the cortical part but also even further to that of the innermost folds, which adhere to the still active cortical tissue of the host, thus leaving only a small portion of the surface in contact with the host uncovered (Fig. 7). By this development of the cork on the contact surface, the passage of

1). PITRA, loc. cit. Fig. 12 and SOLMS-LAUBACH, loc. cit. Taf. XXXII, Fig. 1 and XXXIII, Fig. 3.

nutrient substance between the host and the parasite must be to a certain degree impeded.

Next let us consider the axial part of the haustorium. The axial part is that portion, which is composed mainly of vessels united into two strands with the parenchyma between them.[1] If we make a cross-section through the median region of the haustorium we will find a pair of semilunar *vascular strands* facing each other and running in the direction of the axis of the host (Fig. 8 *va*). The central parenchyma, which I propose to call by the name of *pith*, is composed of small polygonal or slightly elongated cells with large nuclei and rich plasm, almost all being of equal size. The pith after passing through certain places of the vascular strands goes up gradually into the cortex, there being no sharp line of demarkation between them (Fig. 8).[2]

In the strands we find also a few parenchymatous cells which are found irregularly scattered in the inner portion, while in the peripheral portion they constitute somewhat regular rows. The characters and functions of these cells will be discussed later on.

The longitudinal section of the axial part is similar in its form to a flask with rounded base and wide mouth opened at the apex of the haustorium, the strands then corresponding, as it were, to the sides of the flask, and the pith to its contents (Fig. 7 *ax*). The vessels are generally reticulated, rarely pitted. At the bottom of the flask as well as on the inner surface of its sides, the form and arrangement of the vessels appear very

1). The reader is requested to notice that the designation of this part of the haustoria of Santalaceae as given by previous authors is such as to include the innermost tissue of my cortical part with their inner part of the haustoria, which is called by PITTRA "Mittere Theil" (loc. cit. p. 14) and by SOLMS-LAUBACH "Kern" (loc. cit. p. 543).

2). PITTRA considered, in *Thesium*, the parenchyma between the two strands to be of the same nature as that which surrounds strands, and so he called them "Cambialgewebe." loc. cit. p. 14.

irregular ; besides they are short and have an oblique terminal wall. Otherwise the vessels are regular, much elongated and have slightly oblique terminal walls. The center of the bottom of the vascular strand or properly of the axial part being composed mainly, besides vessels, of parenchyma does not look so dense as in the other parts (Fig. 19 *c*, *d*). When the frontal extremity of the strand bends much towards its lateral side (Fig. 10), evidently on account of the pressure exerted by the host-root, the arrangement of the elements at that part will be greatly disturbed, the vessels being much shortened into a rhombic form (the same change takes place at the same time in the cortical elements, which directly surround the strand). In general, at the frontal end of the strand the vessels are divided into numerous bundles, of which only a few vessels abut directly on the wood of the host, while others seem to disappear among the parenchyma (Figs. 7, 19). It should be noticed that the ends of these bundles of vessels are directed mostly towards the lateral side in order to abut on the radial wall of the woody elements of the host, which have been produced since the haustorium attached to the woody part of the former.

In order to obtain a more definite idea of the form of the axial part and of the structure of the vascular strands, it is advisable to consult serial cross-sections passing through several points of a haustorium, as shown in Fig. 19. At first, the section through the bottom of the flask (*c*) shows that the axial part is roundish and that vessels are densely arranged at its periphery but scattered in its center. At a little higher level the section is similar in form with a similar arrangement of vessels, but it is somewhat laterally compressed (*d*). It is thus clearly seen from these two figures that the basal portion of the vascular

strand is composed of a complete ring of vessels. But in a section passing through the median portion of the haustorium, we see that the vessels are divided into two opposing masses (*e*), giving an elliptical outline to the axial part. We find here some notches in its contour which becomes deeper as we go down to the next section. Each vascular strand dissolves away finally into a certain number of vessel-groups (*f*), and in the cross-section passing through the apical region of the haustorium we will observe that these vessel-groups are represented in longitudinal section with their ends frayed into brushes, indicating thereby that they run transversally in this region (*g*).

Occasionally we find in the pith strands of vessels or in other cases numerous strings of isolated vessels (Fig. 19 *iv*), traversing it longitudinally. Sometimes in their course they unite with the main strand and sometimes disappear amidst the pith.

At the front of the haustorium the central part is distinguished from the surrounding attaching-folds by its projecting into the host, and is therefore to be called the *sucker* as in the haustoria of other Santalaceae. The sucker consists mainly of the axial part with a few layers of cortical parenchyma around it. There are no other particular characteristics to be noted, except that the constituent cells are somewhat elongated. It suffices to state here that the apical layer of parenchyma in the sucker, which, being free from coating of the corky layer, can be in direct contact with the host, exhibits the structure of the so-called absorbing tissue, *i. e.*, it is palisade-like, rich in granular plasm, and is furnished with a very thin wall and large round nuclei.

When we take into consideration this histological similarity of the sucker to the main part of the haustorium, and again

when we study the structure of the old haustoria, which will be described later on, the conclusion seems to be justified that *the sucker of the haustorium is nothing but a portion of its apex temporarily imbedded within the tissue of the host.*[1]

V. SECONDARY GROWTH OF THE HAUSTORIUM.

1. The Cambium.

In the foregoing pages we have described the general structure of the young haustorium, but it does not remain unaltered throughout: on the other hand, this primary structure undergoes secondary changes on account of the formation of new additional elements. Therefore in studying the secondary growth of the haustorium we must here fully treat of this tissue.[2] Notwithstanding the remarkable similarity of the structure of haustoria of all Santalaceæ to that of the same organ of *Buckleya*, yet the cambium

1). The sucker of *Thesium* was described by SOLMS-LAUBACH as follows (loc. cit. p. 745): "Der Saugfortsatz schliesst sich in seinem Bau so eng an den Haustorialkern an, dass eine davon gesonderte Betrachtung desselben nur aus Gründen grösserer Uebersichtlichkeit gerechtfertigt sein dürfte. Er besteht aus den directen Fortsetzungen aller im Obigen für den Kern des Haustorium betrachteten Gewebe. Dieselben haben jedoch hier sämmtlich in sofern eine Modification erlitten, als ihre Elemente bei weitem stärker in der Richtung der Längsachse des Haustorium gedehnt sind....."

2). On the meristem which occurs in the haustorium of *Pedicularis* VORKART says (loc. cit. p. 36): "Den Tracheidenstrang umschliesst ein embryonales Gewebe aus kleineren Zellen mit grossem Zellkern und starkem Protoplasmabelag der Wandungen." Heinricher also points out in Lathræa (loc. cit. p. 331): "Eine ebenfalls zweckdienliche Einrichtung im Aufbau des Haustoriums ist ferner die Ausgestaltung einer Meristemzone an beiden Längsseiten der Tracheidenplatte. Durch dieselbe ist dafür gesorgt, dass, wie die peripherischen Zellen des Fortsatzes im Wirthsgewebe neuen Raum gewinnen und hierdurch eine grössere Ausbreitung des Fortsatzgewebes ermöglichen, so auch eine Vermehrung der Elemente der Haustorialplatte, oder des sie umgebenden Parenchyms, vor sich gehen kann, wobei entsprechend einer vermehrten Leistung der aufnehmenden Zellen gewissermassen auch Vermehrung der leitenden gewährleistet wird. Uebrigens scheint dieses Meristem nur begrenzte Zeite hindurch thätig zu sein."

has ever been clearly mentioned in none of these. PITRA[1] has given a cambium-like tissue in *Thesium*, but in very ambiguous terms: "Zwischen diesen Gefässbogen den ganzen Raum einnehmend, ferner auf ihren äusseren Seiten, also die Gefässbündel rundherum umgebend, liegt ein Gewebe aus dünnwandigen, mit trüber Flüssigkeit erfüllten Zellen, welches die Gefässbündel in das Gewebe der Nährpflanze begleitet: es kann als Cambialgewebe der Saugwarze und der Saugwurzel betrachtet werden, ..." Thus judging merely from its structure, he regarded the central parenchyma which I call here the pith as being similar to the parenchyma outside the vascular strand, but he did not mention whether the function of these two parenchymas is idential or not. SOLMS-LAUBACH[2] observed on the outermost parenchymatous cells of the axis (Kern) in *Thesium*, "die gestreckter, dünnwandiger Zellen, wie sie in Weichbast und Cambium vorkommen." HIERONYMUS[3] also considered that parenchyma as the "an Cambium erinnernden Gewebe." Even in the case of haustorium of *Santalum* and *Osyris*, of which the secondary growth is more than probable, no particular attention has ever been paid to this feature. From my study in *Buckleya*, however, it is clear that the parenchyma immediately bordering the vascular strand is the *cambium* and it is almost certain that if the secondary growth occurs in the haustoria in other Santalaceæ it should be performed by means of this meristem interposed between the axial part and the cortex.[4] It must be understood then that this parenchyma should properly be separated from the

1). loc. cit. p. 14.
2). loc. cit. p. 543.
3). loc. cit. p. 205.
4). In fact SOLM-LAUBACH's figure of *Osyris* (Taf. XXXII, Fig. 7 and 8) shows that the haustorium is in at least its second year, for the sucker is imbedded in the wood of the host as in the case of an old haustoria of *Buckleya*.

axial part, though the previous writers included this tissue in that part of the haustorium (Figs. 8, 14, 16 *ca*).

The function of this cambium is of course to add the wood inside and the cortex outside. In order that the growth in thickness of the haustorium may go hand in hand with that of the mother-root to which it belongs, the cambium layer extends to the base of the haustorium and unites with that of the mother-root, as the branch does to the stem.

Besides, at the apex of the haustorium we find the union of the cambium with that of the host which, we think, is the most necessary and indispensable process. For, when two tissues of quite different plants like *Buckleya* and its host come into intimate contact and when it is necessary that a physiological communication should be maintained between them, an increase of the elements for enlarging the contact surface must take place at the corresponding places in both contact surfaces in order that the danger of their slipping from each other may be avoided. Accordingly if we make a longitudinal section of the haustorium, which at the same time cuts the host-root crosswise, we see that the cambium of the haustorium joins that of the host directly (Figs. 7, 9, 10, 11 *ca*). In this way we can obtain in section a dumb-bell shaped circuit of the cambium ring through the host, the haustorium and the mother-root, whereby the elements of the haustorium are so placed, as to be in the best possible position for uniting with the corresponding elements in the host- and mother-root (Figs. 7, 9, 10).

As the cambium above mentioned serves only for the growth in thickness of the haustorium we must next inquire into the means of its longitudinal growth. In the primary growth we have seen that the apical cells of the sucker, like all the other haustoria, grow further and further in the longitudinal direction

dividing themselves transversely, and after penetrating the cortex of the host, come into contact with the wood tissue. The primary growth of the haustorium seems generally to be arrested, when its apex thus reaches the lignified cells of the wood, since, they are so hard and so thick that they can resist against the penetration of the young thin-walled apical cells of the sucker. Only rarely some few apical cells can penetrate further into the wood tissue of the host, compressing in their course the lignified cells of less resistance, as, for instance, the medullary rays. But this local and limited prolongation is not to be considered as a longitudinal growth of the whole sucker.

Again we have ascertained very frequently that when the haustorium occurs on a young host-root in the first year of its development, the penetration and longitudinal growth of the sucker do not cease at the cambium zone of the host, but on the other hand as the tissue at this stage is still soft and thin walled the sucker easily makes its way further in.

Manifestly the parenchyma will be least resistent against the pressure exerted by the forward growth of the haustorium as well as the chemical action of the same.[1] So the sucker grows in this direction, dissolving and pushing aside and exending itself to the pith.

As the apical cells of the sucker, after the primary growth, become the permanent tissue, the occurence of the secondary growth will become impossible.

2. The Vascular Strands.

Having determined the occurence of the cambium layer and its distribution, we shall now study the vessels derived from it.

1). The action of the haustorium upon the host is worthy a special treatment which will be given in future.

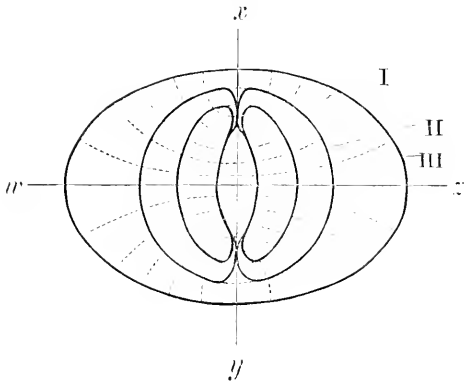
It has already been noticed that, on either transverse or longitudinal sections, vessels are arranged irregularly at the base as well as the inner region of the strand, and are short and irregular in form, while in the remaining part each succeeding prismatic vessel is so placed that the result is a radial row. It seems beyond doubt that the irregularity of the arrangement of the innermost vessels is due to their origin from the procambial cell-groups, directly differentiated in their own form just as we see in the formation of the primary wood in the fibro-vascular bundle. But immediately after the complete formation of the cambium ring, secondary elements being derived from the latter by its tangential division will be arranged in radial rows.

At the earlier period of growth, rows of vessels are directed laterally, being all approximately parallel, even at the extremities of both strands (Fig. 8). But as the activity of the cambium is greater laterally (*i. e.* in the direction of *wz*, Woodcut 1) than in the direction perpendicular to it (*i. e.* in the direction of *xy*), the diameter of the axial part increases greatly in the former direction and the rows of vessels derived henceforth begin to diverge from one another, and when the cambium becomes circular, they become radially arranged. The formation of vessels from the cambium being always strongest in the lateral direction, the vascular strand which has once become circular will then become longer laterally and take in section an elliptical shape with its minor axis placed in the direction of the major axis of the primary ellipse.[1]

The diagram here given will demonstrate the modification of the form of the axial part during its secondary growth (Woodcut 1). It indicates three types of form in cross-section, which the

1). As far as my observation goes pretty old haustoria mostly keep this form.

Woodcut 1.



haustorium takes in its youngest (I), middle (II), and oldest stages (III) respectively. An example of the quite similar mode of the secondary growth has already been given by SACHS[1] with a similar diagram of the wood of *Aristolochia Sipho*,[2] where the inner annual rings are at first elliptical in cross-section, then circular and finally oval. He concluded thereupon that the directions of the medullary rays are the orthogonal trajectories of those of annual rings, so in our case the latter and the rows of vessels stand to each other in similar relations.

These morphological changes of the axial part during the secondary growth take place also in its frontal part which lies in the so-called sucker. The fact that the sucker, as has been before stated, is primarily elliptically compressed along the axis of the host, might be explained as the most suitable arrangement for splitting the cortex of the host in order to make its way easily into the wood[3] (Fig. 5. *b*, *suc.*).

The vigorous increment of elements only in the lateral direction during the secondary growth must also be considered as

1). SACHS, Ueber Zellenanordnung und Wachsthum. Arb. d. Bot. Inst. in Wurzburg. Bd. II, 1882, p. 192.

2). loc. cit. Fig. 3.

3). If we take off the young haustoria from the host spindle-shaped scars will be seen on the central part of the contact surface. HEINRICHER is of the same opinion as to the reason why the tracheal plate in the sucker of *Lathraea* is placed longitudinally (Anatomischer Bau und Leistung der Saugorgane der Schuppenwurz-Arten. Cohn's Beitr. z. Biol. d. Pflz. Bd. VII, 1896, p. 331).

being very serviceable for the function of the haustorium. For in order to assume an intimate union of the elements between the host and the haustorium, it is evident that the growth in thickness of the latter must go on parallel with that of the host (*i. e.* in the direction of *wx*, Woodcut 1). But as no secondary longitudinal growth occurs in the host, there is needed in the haustorium no remarkable growth in the direction of the axis of the host (Woodcut 1, *xy*).

The natural consequence of such secondary growth in thickness especially in a lateral direction, unaccompanied by longitudinal growth, is that the axial part is reduced to a disc with various outlines, according to its age as well as to the proportion of its height to its breadth. In all haustoria, especially in old ones, the vascular strand has the concave bottom (Figs. 9, 10) and also in some vigorously growing haustoria, as, for instance, those which feed on *Abies*, the frontal portion of the strand is likewise concave (Fig. 9). So, if the haustorium is pretty old the axial part will take the form of a biconcave lens, or if it is somewhat younger it will assume the form of an amphicœlous vertebral bone (Fig. 9). In a less vigorous haustorium the front, being overlapped by the tissue of the host on account of the very vigorous growth of the latter, becomes convex and then the axial part assumes the form of an opistocœlous vertebral bone (Fig. 10). Not only are all these forms to be seen in sections, but also not infrequently they may be found even on the dead haustoria still resting upon the host; in this case, however, the cortical parenchyma having already decayed, only the hard lignified axis with concave base is barely exposed (Fig. 6 *hr*.).

3. The Cortical Part.

In the cortex there are no such remarkable changes as are seen in the axial part. The increment of elements here is exceedingly slight and at the same time as the older part of the cortex is gradually torn off by the formation of corky successive layers beneath, any noticeable increase in thickness is not observed.

The parenchymatous cells, which are the only elements of the secondary as well as of the primary cortex are regular both in form and arrangement. No differentiation whatever occurs in the same stage of development, all being similar in size, form and inner structure. Compared with the cortical elements of the mother-root, they are rather short.[1]

In the following lines I will discuss somewhat more minutely the nature of the cortical part in order to make clear its functions.

At first we will inquire whether the sieve-tube is present or not. The investigation of the haustorium of various parasites in this respect has cleared up their parasitic nature. PEIRCE[2] has studied the structure of the haustoria of some phanerogamic parasites and established the fact that sieve-tubes are present in the haustorium of the Convolvulaceae, Rafflesiaceae and Balanophoraceae, while in *Viscum album*, a green parasite, they are wanting. He then drew conclusion that the former kinds of parasites must depend absolutely upon their host for food, *i. e.*, that they obtain raw materials through the tracheid or tracheae

1). The cortex of the root of *Buckleya* consists principally of parenchyma, sieve-tubes, companion-cells and a few bast-fibres.

2). PEIRCE, On the Structure of the haustoria of some Phanerogamic Parasites. Ann. of Bot. Vol. VII, 1893, p. 291.

and elaborated materials through sieve-tubes; while in *Viscum* the absorption of food from the host is limited to crude materials in aqueous solution. Quite recently CANNON[1] has ascertained also the absence of sieve-tubes in the haustorium of *Phoradendron villosum*, a green parasite. Of course we can not establish the physiology of nourishment merely from the structure of the elements; experiments are always necessary.[2] But as this investigation is beyond the scope of the present paper I will here confine my remarks as to *Buckleya* to a description of the histological structure of the cortical part. I have examined with great care to ascertain whether the walls of any parenchymatous cells are furnished with callus, which would prove them to be sieve-tubes. After the process of PEIRCE I have stained the sections from the haustoria, collected in various periods, with an aqueous solution of anilin-blue and examined them in glyceline after washing with water. A part of the bast of the mother-root was treated in the same manner for the purpose of control. The presence of callus was evidently proved in the mother-root, but never in the haustorium. The following facts are also in the favour of the view that there are no sieve-tubes in the cortical part of the haustorium :—

1). Throughout their life, the parenchymatous cells of the cortex retain their nuclei, which are large and round or oval when young but spindle-shaped when old.

2). In these cells the slightly granular plasm accumulates around the nuclei and forms plasmic strings, while, on the contrary, in the sieve-tubes the plasm with granular contents is to be found only adhering to the cell-wall.

1). CANNON, loc. cit.

2). See PFEFFER's remark in his Pflanzenphysiologie 1897, Bd. I, 2 Aufl. p. 355.

3). They store up starch during the resting season in the same way as other parenchymatous cells.

4). The apical cell of the cortical part does not differ at all from that of the axial part, both being nucleated and rich in plasm (Fig. 11).

These facts led me to conclude that no elements for the translocation of the plastic substances, at least in the form of sieve-tubes, exist in the cortical part of the haustorium of *Buckleya*.

4. The Medullary Rays.

So far as I know, we are not yet aware that medullary rays occur in the haustorium of any parasite. In *Buckleya*, in which the vascular strands grow to a considerable thickness, the development of such tissue is to be expected, when we consider its physiological importance in stems and roots. It originates very early, when the vessel begins to be differentiated from among the procambial cells. Then we notice that some cells retain their parenchymatous nature, while others develop into vessels; but since the arrangement of the former at this early stage is very irregular, it can not yet be decided whether they have the property to differentiate into medullary rays or not (Fig. 14). (*Cf.* p. 13). Indeed SOLMS-LAUBACH[1] observed also parenchymatous cells in the vascular strand of *Thesium*, but about these he recorded merely that they are of the same kind as those of the cortex bordering the axial part. It is only after the full development of vessels, that the arrangement of these cells into definite *medullary rays*, which pass through the vascular strands

1). loc. cit. p. 544.

radially, is completed. The cells of the medullary rays are usually rectangularly prismatic, often with rounded corners, somewhat comparable to bricks, for they chiefly elongate in one direction and lie with their greatest diameter directed horizontally and radially. The form of rays can be learned from the sections of the haustorium in its transverse, longitudinal and tangential directions: they are flat bands, like ordinary rays, composed of many layers of cells transversely, but only a few layers thick (Fig. 16). They occur pretty abundantly; some originate from the pith and traverse the thick bundle of vessels, while others take their start in the bundle itself and proceed toward the cortical part. Even in older haustoria the wall of the cells remains *unlignified*, delicate, and capable of yielding to pressure and tension,[1] so that they are often laterally compressed, sometimes with abundant reserve starch (Fig. 16. *a*).

We may ask then how the medullary rays behave themselves when they enter the cortical part. When they penetrate the cambium layer and run among the cortical parenchyma, they are difficult to be distinguished, their structure being similar to that of the cortical parenchyma. Their outer form and the direction of their longer axis alone serve for their distinction (Fig. 15). Not infrequently does it happen that in cross-section both elements are so similar in their form that they are hardly distinguishable from each other, but in radial section the medullary rays can always be plainly recognized as such by their horizontal course, the axis being elongated in this direction, while the cortical parenchyma runs longitudinally.

Having thus ascertained the existence and distribution of the

1). For the sake of comparison I have examined the medullary rays in the wood of the mother-root. The cell-wall is lignified and thickened, having numerous pits.

medullary rays in the cortical and axial parts, their function as the reservoir, and also as the way of conduction, of nutritive substance (mainly carbohydrate) at certain times, becomes quite evident.

5. The Annual Rings and the Formation of the Duramen.

In the stem as well as in the root of *Buckleya* we can distinguish the annual rings very clearly, though in the latter they are somewhat irregular and sometimes somewhat obscure.[1] As regards the haustorium the arrangement and structure of elements do not go so far as to make clear the difference of spring and autumn wood. Yet we can faintly recognize alternate zones of denser and less dense regions; a demarkation between the two is scarcely observable under the microscope of high power, but is visible to the naked eye as an obscure line (Fig. 20).

Of these zones, the denser are formed of vessels with lumen which is radially narrower than that of vessels of the other zone; while between the vessels of both zones there is no noticeable difference in the thickness of the cell-wall, as is usually the case.

When the haustorium attains a sufficient thickness, we can observe in the successive zones of growth or annual rings, the differentiation into the *alburnum* (younger rings) and the *duramen* (older rings). Externally the two parts are distinguished by their

1). Solms-Laubach (loc. cit. p. 539) has given as a general remark on the structure of the Santalaceous plants, "Seine (Holz) Jahrringe sind äusserst undeutlich." Chatin (loc. cit.) however has already shown clearly the annual rings in the stem of *B. distichophylla*.

colour and density as in the stem of many trees (Fig. 21). As is well known, the duramen of the stem is characterized, not by any modification in the original structure and thickness of the cell-wall, but merely by changes of the material properties of the wall, as well as of the contents. The original physiological work (the purely mechanical function is not here considered) of the cells is rendered impossible by the accumulation of deposits of some infiltrating bodies or by the formation of thyloses within the vessels and cell-lumina. The change into duramen in the haustorium is in most respects similar to that in the stem, but here it is accompanied by a process of disorganization.

The cell-walls of the pith as well as of the medullary rays in the haustorium under consideration become lignified, and at the same time they swell up to a considerable thickness (Fig. 18), until at last they come to fuse together into a yellowish mass. The walls of the vessels are also disorganized into a yellowish substance, which fills up the lumen of the vessels and so makes the performance of their functions impossible (Fig. 17). The further essential change, which takes place during the formation of the duramen, consists in the disappearance of starch which characterises the living cell. Thus the essential physiological functions of this part, which consist in translocating and storing up the nutrient substances, become entirely impossible to be performed.

It is noticeable here that the formation of the duramen in the haustorium stands in close connection with the condition of the tissue of the host-root, with which the duramen of the haustorium is connected. For, in the cases investigated, I have found that, in the cross-section of the host (*Abies*), just under

the haustorium provided with the duramen, certain changes occur in the wood in front of the sucker. For instance, here the medullary rays contain very little, if at all, of the reserve starch (Fig. 21).

These modified tissues of the host extend just so far as the duramen of the haustorium extends. In one instance, I have seen that not only in the part of the host just beneath the haustorium, but even in the part far removed from it, the older rings undergo the same modification into a kind of duramen. It seems therefore probable that the formation of the duramen in the old host-root necessarily induces the same change or process of disorganization in the older tissues of the haustorium, that are connected with the duramen of the host.

This formation of the duramen in the haustorium was found exclusively in old specimens, which were, judging from the number of the annual rings of the host-root, from fifteen to twenty years old, or thereabout. These old haustoria were discovered by me only on *Abies*, and the occurence of such a modification in the haustoria found on other hosts still remains undetermined.

6. The Attaching-Fold, Sucker and Striated Band.

As has been stated above, the haustorium acquires a few pairs of the folds already during its primary growth; but afterwards we can observe no increase in their number, and generally, in the old haustorium, they disappear entirely. The question then arises, how do they disappear? To clear up this question, haustoria of various ages were examined and compared with one another. In the young haustorium the folds adhere, as

was before stated, firmly to the cortex of the host, compressing and deforming the tissue of the latter between the sucker and themselves (Fig. 7).

The cortical tissue of the host, however, continues, during the secondary growth, to die away gradually from the outside and becomes replaced by its new layers formed from the cambium; and when the outermost decayed tissue of the cortex comes to be cast away, the folds, which were formerly in contact with it, detach naturally from the surface of the host, imitating a roof projecting laterally on the lateral side of the haustorium, as will be seen in the advanced stage of Fig. 10 (*at*). While the fold is thus gradually removed from the host, another change occurs which causes the disappearance of the fold.

Generally towards the end of the primary growth, the formation of the corky layer is observed under the superficial parenchyma of the fold, and those cells lying outside this corky layer will ultimately detach from the fold and be cast off. As the age advances, therefore, the folds are no longer sharply edged as before and form only rounded elevations.

At last after the successive formation of the corky layer, the folds disappear entirely, so that the surface of the haustorium becomes homogeneous throughout (Fig. 21).

While the modification of the structure is thus going on in the frontal portion of the cortex, the shape of the central part at the apex of the haustorium will gradually deviate from that which it had at first. Now the frontal part of the haustorium, when young, is distinctly divided into the peripheral and the central portion, which are respectively the attaching-folds and the sucker; the latter however being simply imbedded in the tissue, especially in the cortex, of the host shows no difference

whatever in its structure from other parts behind it, as I have already pointed out (*cf.* Sec. IV). During the secondary growth, the cambium of the sucker, after having joined with that of the host, produces new elements in the same direction and nearly with the same activity as the cambium of the host produces its own elements, and so the sucker is able to expand more and more laterally. Hence, after the obliteration of the primarily formed cortical tissue of the host-root, which at first had enveloped the lateral sides of the sucker, the parallel growth in thickness of both haustorium and host no longer allows the cortex of the host to grow over the sucker, or the cortex of the haustorium to overlap the host, as it was the case in the primary growth (Figs. 4, 9, 10).

In this way the contact surface, which is at first wavy and irregular, especially when the folds are numerous, becomes simple, the greater part of the surface being now occupied by the front of the sucker (Figs. 9, 10). At the same time the sucker, which we have conveniently distinguished as such in the primary growth by its being imbedded in the host, can no longer be distinctly separated from the other part in such an old haustorium.

Together with all these changes we see that on the whole surface of the haustorium, excepting however a small portion of its apex, the corky layer is formed successively under the superficial layer of the cortical parenchyma, which is torn off sooner or later from the haustorium, while new cortical parenchyma is constantly produced by the cambium. During this process the striated band, which is originally situated along the median portion of the cortex, changes its position gradually towards the periphery, until finally it is cast off together with the corky layer and the cortical parenchyma.

7. The Connection of the Haustorium with the Mother-Root.

The final point in the structure of the haustorium, to which I wish to refer, concerns the connection of the haustorium with the mother-root. This was omitted in the description of the young haustorium, as I deemed it better to study the point on the haustorium which had already undergone the secondary growth. To begin with the wood of the mother-root, it is composed of pitted vessels, wood-parenchyma and thin-walled wood-fibres, traversed by medullary rays with lignified walls. At the place where the haustorium occurs, vessels are exceedingly numerous and some of them are directed towards the haustorium as a massive strand of reticulated vessels, which, after passing a certain distance, comes in contact with the bottom of the vascular strand of the haustorium (Fig. 7 *ne*). This part which thus connects the haustorium with the mother-root, is called the *neck* of the haustorium. In the median portion of the neck the vessels are exceedingly small in number, mostly forming isolated chains of vessels among the parenchyma. The longitudinal and cross sections reveal very clearly the course of the vessels in this region (Fig. 12).

In a section taken near the mother-root the vessels are arranged compactly in radial rows (Fig. 19 *a*); and again near the bottom of the vascular strand they unite and fuse together into those of the strand.[1)]

1). A similar structure was ascertained in *Lathræa* by HEINRICHER. He says (Cohn's Beitr. loc. cit. p. 334): "Die Tracheen verlaufen in derselben in bogigen Curven, welche mehr minder senkrecht an die Tracheen des Wurzelstranges ansetzen, da und dort finden sich zwischen den Tracheen noch parenchymatische Elemente eingeschaltet." (Compare his illustration, Taf. VII, Fig. 2). In the young haustorium of *Buckleya*, in which the develop-

In Fig. 12. *b* the course of the vessels of the neck is shown in longitudinal section. The vessels in this part are more or less stretched, so that they are longer than those of the axial part. They have transverse walls at both ends (Fig. 13).

Generally, as the vascular strand of the old haustorium surpasses in growth the woody part of the mother-root, the vessels in the neck diverge towards the bottom of the haustorium.

As to the cortical part of the neck, it will not be worth while to say more than that it has the same structure as that of the main part of the haustorium, consisting entirely of parenchymatous cells of equal size and arrangement, but with no striated bands among them (Fig. 12. *a*).

The study of the secondary growth of haustorium makes it very easy for us to understand its outer form, which is mainly determined by the form and arrangement of the constituent tissues. In the young stage the axial part is comparatively smaller than the cortical part, so that it is the latter that chiefly determines the form at that stage; for instance, the development of the attaching-fold determines the form, according to the degree of its thickness and size.

The length of the neck is also a factor in determining the form of the young haustorium: the shape of the latter is that of a long cone if the neck be moderately long. In one case I have found a conical-shaped haustorium 3 years old, and 7 mm. in height, of which 3,5 mm. formed the length of the neck—the

ment of vessels is not yet completed, the part of the so-called neck is only with difficulty distinguishable. In *Osyris* judging from the figure given by Solms-Laubach (loc. cit. Taf. XXXII, Fig. 7), one pair of arch-shaped strands of vessels composing the axial part of the haustorium seems to start directly from the sides of the wood of the mother-roots.

longest neck I have ever seen. During the secondary growth, however, the axial part is the most important factor in determining the outer form of the haustorium, for, as it was before explained, this part is the most changeable in form, so that the form of the haustorium mainly accords with that of the axial part. When the axial part becomes rounded in cross-section, the outline of the haustorium is also rounded; and when in the old haustorium the axial part becomes again elliptical, the surface view of the haustorium is also elliptical. The chief modification of the cortical part is then that the attaching-fold obliterates, leaving in a certain stage concentric furrows along the front of the haustoria, which however disappear in very old specimens. That the old haustorium does not increase at all in its height and thus becomes flat, has already been briefly stated.

VI. GENERAL REMARKS AND SUMMARY.

It would be of much interest to inquire how far the above described structure of the haustorium of *Buckleya* resembles that of the same organ in the allied species, and to what extent my interpretations concerning the nature of its tissue can be applied to others; but, as to the latter point, I can refer only to a few species, since in many parasitic Santalaceæ the structure of the haustorium has not yet been studied. In *Thesium*, *Santalum*, *Osyris* and *Buckleya* the structure of the haustoria is the same in general respects; thus we find in all of them the cortical and axial part,[1] including the sucker, attaching-fold and striated

1). The part called by me the axial part does not correspond to the "Kern" defined by other authors in other species, for they included in "Kern" the cambium as well as parenchyma, which latter must be properly included in the cortical part according to my designation above given.

band. Judging from the haustoria of *Buckleya*, I think I may venture to say that some points in the interpretations of the tissues and the comparative studies on the structures of haustoria of different species, made till now by various authors, are more or less imperfect. Our knowledge of the anatomy of *Thesium*-haustoria we owe especially to CHATIN, PITRA and SOLMS-LAUBACH. The last author has also studied the haustoria of *Osyris* and mentions the following characters as distinguishing that genus from *Thesium* :—

a). A very small and narrow axial part (Kern).

b). A sucker which does not differ markedly from the axial part histologically.

c). The more extensive growth of the border of the sucker.

It appears to me that in comparing the structure of the haustoria of various species, the age and the secondary growth in thickness must be considered. In all haustoria it may be conjectured that the "Kern" or the axial part should be very small in the earlier stages of development. Since, in *Buckleya*, the thickness and general form of the vascular strand in the youngest haustoria are nearly similar to those of the adult haustorium of *Thesium*, it would not be improper to conclude that in *Osyris* the comparatively thin vascular strand, as was mentioned by SOLMS, should be found only in a young haustorium.

As regards the sucker, its existence was ascertained in Rhinanthaceæ, *Lathræa*, Santalaceæ, etc., each having its characteristic structure. In all these cases, the name sucker was given to that portion of the haustorium, which is imbedded in the host. In Santalaceæ it seems that its tissue was distinguished by SOLMS-LAUBACH[1] from that of the "Kern" by its more elongated cells;

1). loc. cit. p. 556.

so, he remarks that the sucker of *Osyris* is somewhat different from that of *Thesium* in that its tissue is composed of unelongated cells, "unmittelbare Fortsetzung derer des Haustorialkernes."[1] When we take into consideration the structure of the haustorium of *Buckleya*, it seems to me to be needless to distinguish the so-called sucker from the "Kern," since the "Kern" itself must be regarded as an absorbing organ, and since, further, it is impossible to find any anatomical difference whatever between the sucker and the "Kern." This view will seem quite natural when we examine the haustorium which is advanced in age and possesses a discoidal shape (Figs. 9, 10).

SOLMS-LAUBACH has pointed out in *Osyris* that the sucker, after having reached the wood of the host-root, expands along it, thus lifting up the bast, and that in some cases the lateral sides of the sucker are divided into finger-shaped processes, which terminate in the bast with the wood of the host interposed between them.[2] He thought that this is due to marginal growth, but did not say whether this growth is primary or secondary; nor did he say anything about the growth of the entire haustorium, in spite of the existence of a meristematic zone bordering the vascular strand. In *Buckleya* I have only rarely found that the sucker is divided as in *Osyris*. In such a case, as the end of each division is applied to the bast of the host and its cells elongate themselves towards the bast, each division must increase its length correspondingly with the growth of the host-root. This instance confirms the existence of the marginal growth of the sucker in *Buckleya* also, but it must be considered as an abnormal case. I have also ascertained that the sucker, as soon as it meets

1.) loc. cit. p. 556.

2). Compare the figure of SOLMS-LAUBACH (loc. cit. Tal. XXXII, Fig. 8).

the wood of the host, grows laterally and tangentially along the cambium ring of the latter, imitating the marginal growth. We must, however, consider that this growth is confined only to the period of development, and no secondary growth of the margin is observed. Applying these features found in *Buckleya* to *Osyris*, which in every respect resembles the former, it is highly probable that the marginal growth of the sucker in *Osyris* occurs during its primary growth only, and that, on the other hand, the secondary growth concerns its thickness only.

SOLMS-LAUBACH has described in the haustorium of *Santalum album* that the sucker extends like a fish-tail: "Der colossale Saugfortsatz hat durch seine beiden sich zwischenschiebenden Lappen die Rinde zu $^3/_4$ vom Holz heruntergeschält, sie zwischen deren Oberseite und die Ansatzfläche einpressend."[1]

This way of extention of the sucker I could confirm in *Buckleya*, and in my case the degree of extention seems to depend upon the character of the host and, even in the same species of the host, upon the conditions of its growth. It is doubtful whether this extention in *Santalum* will continue during its further growth; for, from the description of SOLMS-LAUBACH, it may be inferred that the haustorium investigated by him was only one year old, *i. e.*, he could observe its structure only in its primary stage. If I am not mistaken, the sucker would not keep such a form for a long time,[2] but it will grow in thickness in the same manner as *Buckleya* and take a similar form (Compare Figs. 9, 10).

Though our knowledge of the structure of the haustorium of *Osyris* and *Santalum* is in some respects still insufficient, the

1). Foot-note by SOLMS-LAUBACH in SCOTT's loc. cit. p. 149.

2). From analogy it may be considered that the haustorium of *Santalum* may maintain a perennial growth.

similarity of the structure of the four species till now studied strengthens, in every respect, our view that the haustorium of the above two plants will follow the same fate as that of *Buckleya*, for instance, as to the mode of the secondary growth as well as to the modification of the structure then occuring, provided they will sustain their activity during many years.

Lastly I may here say something about the attaching-folds. Their number as well as form are different in different species of hosts; thus the haustorium of *Thesium* has numerous attaching-folds on Monocotyledons, but only a pair on Dicotyledons, while in *Osyris* and *Santalum* a single pair is always developed.[1] In *Buckleya* I found that numerous folds are developed on various dicotyledonous and coniferous roots. But the haustorium of *Buckleya* on monocotyledonous roots has not yet been found, nor has the number of the folds yet been decided. That the development of the folds differs with different kinds of hosts—that they are not formed during the secondary growth of the haustorium—that they disappear in old specimens—all these facts lead us to regard them as organs subordinate in their functions and to consider their function of attachment to be but of minor importance.[2]

The results of the studies on the structure of the haustorium of *Buckleya* are briefly as follows:—

1. The haustorium is provided with a cambium ring between its cortical and axial parts, whereby a continued growth

1). Whether the dovelopment of the single pair of the folds in *Osyris* and *Santalum* is found on monocotyledonous or dicotyledonous root is unknown to us.

2). HEINRICHER (loc. cit. p. 323) has already published the same view on the haustorium of Lathraea: "Wir haben also vor allem gezeigt, dass die „replis préhenseurs," die Zangenfortsätze, eine ganz nebensächliche Erscheinung an den Haustorien sind."

in thickness is accomplished. The cambium of the haustorium joins that of both the host and the mother-root, and together forms a complete thickning ring. Any noticeable secondary growth in length does not take place.

2. The form and structure of the haustorium are changeable according to age.

3. In earlier stages the axial part of the haustorium has an elliptical form in cross-section, which has its major axis coincident with the longer axis of the host-root, but after a certain stage, owing to the more vigorous growth in lateral direction it becomes circular and then again takes an oval shape, with its major axis in the place of the minor axis of the former ellipse.

4. The haustorium possesses medullary rays.

5. The existence of sieve-tubes cannot be definitely ascertained.

6. The striated band in the cortex disappears in the older haustorium.

7. The attaching-folds undergo the same fate. At first, they detach from the host-root, project at the margin of the apex of the haustorium and sometimes produce striations or furrows.

8. The sucker, easily distinguishable in the younger stage, loses its demarkation from the part behind it after a certain degree of growth.

9. So long as the host-root is alive, the haustorium may be active and can maintain its life during many years.

10. Demarkations between the zones produced in each period of growth are visible, though faintly, in the vascular strand of the haustorium.

11. In the older haustorium the older part of its axis goes into the formation of the duramen.

In conclusion I wish to express my heartiest thanks to Professor Dr. M. MIYOSHI and Professor Dr. S. IKENO for much valuable advice and criticism throughout the work. I am also greatly indebted to Professor Dr. M. SHIRAI for useful information, which he has given me during his stay in Berlin.

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May, 1902.

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Fig. 2.—Round haustoria on *Cryptomeria japonica*, nine years old. *a*, six years old ; *b*, one year old ; *c*, six years old ; *d*, young one on small rootlet. Nat. size.

Fig. 3.—Old haustoria on *Carpinus japonica*, twelve years old. 3. *a*, frontal view showing the elliptical shape of the haustoria. 3. *b*, side view. *l*, lateral haustorium ; *t*, terminal haustorium ; *oh*, obliterated haustorium. Nat. size.

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Fig. 7.—Longitudinal section of haustorium two years old on *Cryptomeria*, drawn semidiagrammatically. From cross-section of the host-root it may be learned that the haustorium attached itself to the host at the end of its second year. *co*, cortical part ; *ax*, axial part ; *ca*, cambium ; *suc*, sucker ; *at*, *at'*, primary and secondary attaching-

folds; *st*, striated band; *va*, vascular trand; *ne*, vessel in the neck; *mo*, wood of the mother-root; *ck*, corky layer; *pi*, pith; *w*, wood of the host; *ba*, bast of the host. ×25.

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Fig. 9.—Diagram of longitudinal section of an old haustorium on *Abies*. It attacked the host at an early stage, thus the wood of the host is greatly hypertrophied. *mo*, wood of the mother-root; *va*, vascular strand; *bt*, bottom of the axial part; *at*, attaching-fold; *w*, *b*, wood and bast of the host. ×3.

Fig. 10.—Diagram of longitudinal section of the haustorium five years old, given in Fig. 3 *t*; it has a rhombic outline with the vascular strand diverging in a lateral direction. The growth of the host surpasses that of the haustorium and so the contact surface becomes convex towards the host. *mo*, etc., as before. ×3.

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Fig. 12. *b*.—Longitudinal section through the neck of the haustorium. *vs*, vessel; *mo*, branch of wood of the mother-root; *bo*, bottom of the axial part; *ex*, external and *in*, internal portion of the neck. ×70.

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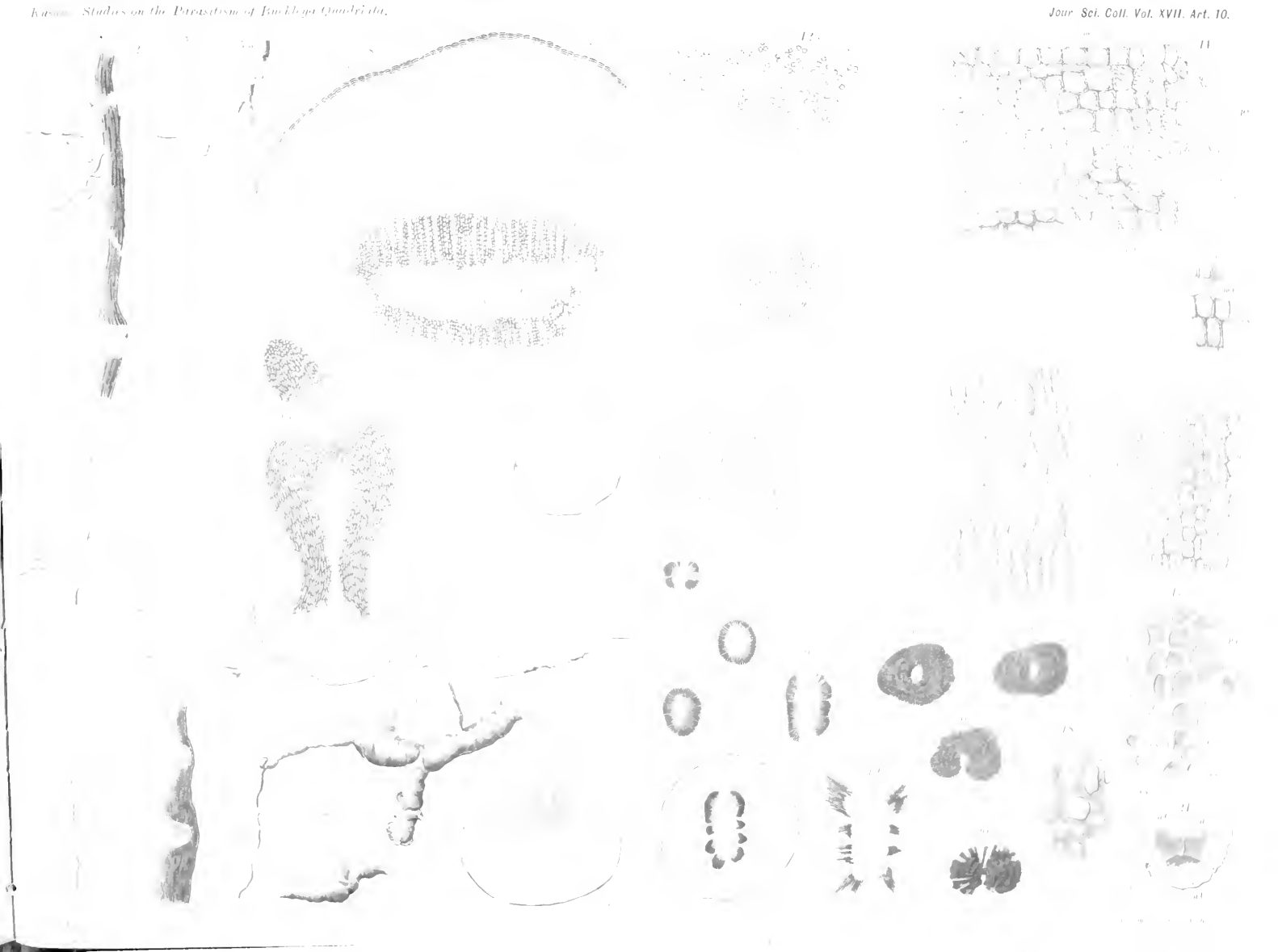
Fig. 18.—Cross-section of the pith of a very old haustorium. The wall swollen into yellowish substance. ×350.

Fig. 19.—Axial part of the haustorium two years old on *Rhododendron sinense*, diagrammatically shown in cross-section. *a*, neck of the haustorium near the mother-root, the vessels of which are faintly divided into two groups; *b*, middle portion of the neck with vessels scattered among parenchyma. Each vessel is shown by a dot; *c*, bottom of the vascular strand with compactly arranged vessels on the periphery and isolated vessels in the central part; *d*, base of the strand with an elliptical form; *e*, middle portion of the strand. The strand is divided into two parts, each assuming a flat band; *f*, more frontal portion. *co*, cortical part; *ax*, axial part; *st*, striated band; *g*, apical portion, the vessel in the periphery being shown in longitudinal section. *iv*, chains of vessels. The dot in all sections stands each for an isolated vessel. ×8.

Fig. 20.—Cross-section of the axial part of a very old haustorium, showing annual rings. *a*, basal; *b*, middle; *c*, frontal; *d*, apical portion. ×4.

Fig. 21.—Longitudinal and cross-section of an old haustorium and *Abies*-root (Fig. 4. *a*) respectively, showing the duramen in both of them. *al*, alburnum of the haustorium; *dr*, duramen of the haustorium; *al'*, alburnum of the host; *dr'*, duramen of the host. Magnified.



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Observations on the Japanese Palolo,

Ceratocephale osawai, n. sp.

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With 2 plates.

The investigation, the results of which are embodied in the following pages, was begun in the summer of 1896 and carried on at intervals during the three following years, partly in the Laboratory of the Zoological Institute and partly in a room hired for the purpose of facilitating the collection of fresh materials.

The subject was taken up at the suggestion of Professor IJIMA, who has also kindly undertaken the work of revising the manuscript. To him and also to Professor MITSUKURI I beg to express my sincere thanks for the help and advice which they have frequently extended to me in connection with my work. Further I am under deep obligation to Professor K. OSAWA of the medical college, who, being himself much interested in observing the breeding and swarming habits of the worm described in this paper, has favored me with valuable information and suggestions.

The worm in question is a Lycorid Polychæte which exhibits at a certain period of the year, a most remarkable swarming habit for the purpose of breeding, which so closely resembles that of the so-called "Palolo" worms of the South Pacific (*Eunice viridis**) and of the Atlantic (*Eunice fucata*†), that it seems not altogether inappropriate to give it the title of the Japanese "Palolo." However, our Japanese "Palolo," as will soon be shown, is systematically quite distinct from both the South Pacific and the Atlantic form; it is referable to the family Lycoridæ, not to the Eunicidæ. It further shows certain differences in circumstances connected with the process of swarming.

All the three forms mentioned seem to burrow in the bottom during the immature stage. With the attainment of sexual maturity and under certain peculiar conditions, the part of the body with sexually developed segments swarms out. In the Pacific and the Atlantic "Palolo," the sexual segments are in the posterior portion of the body; this portion becomes detached from the anterior and is known to do the breeding-swarm. Contrariwise in the Japanese "Palolo," the sexual segments are confined to the anterior portion, which alone does the swarming after shedding off the posterior, shrunken, non-sexual segments.

As regards the period of the year when the swarming takes place, it is known that in the "Palolo" of both the Atlantic

* For accounts of this worm, see: S. J. WHITMEE, On the habits of Palolo viridis. Proc. Zoöl. Soc. London, 1875, P. 493–502.—W. C. MCINTOSH, Report on Annellida. Chall. Rep. XII., 1885, pp. 231–235 (*Staurocephalus*); pp. 257–261 (*Palolo viridis*).—A. COLLIN, Ueber den Palolowurm. Appendix to A. Kramer's Ueber den Bau der Korallenriffe, etc. Kiel u. Leipzig, 1897.—B. FRIEDLANDER, Ueber den sogenannten Palolowurm. Biol. Centralblatt, XVIII., 1898, pp. 337–357.—A. AGASSIZ, Islands and Coral Reef of the Fiji Group. Am. Jour. Sci., ser. 4, V., 1898, p. 123.—A. G. MAYER, An Atlantic Palolo, etc. Bull. Mus. Comp. Zool., XXXVI., 1900, pp. 1–14.

† For the latest account of this worm, see A. G. MAYER, The Atlantic Palolo. Science Bulletin, Mus. Brooklyn Institute of Arts and Sciences, I., 1900, pp. 93—103.

and the South Pacific the process occurs in the mornings upon or near the day of the last quarter of the moon, but the former in June-July, and the latter in the months of October and November. Whereas, in the case of the Japanese "Palolo," the swarming takes place during nights closely following the new and the full moon in October and November. Details of my observations of this phenomenon will be given further on, and as to particulars about the breeding habits of the other "Palolo" the reader is referred to the literature of the subject.

With respect to the systematic position of the Japanese "Palolo," it seems to come nearest to *Ceratocephale* MGRN., which genus has hitherto been represented by a single species from Swedish shores, *C. loveni* MGRN.* Among its points of agreement with that genus and species, are the facts that the proboscis is provided with papillae but not with paragnathea, and that the parapodium lacks the upper ligula on the dorsal ramus and shows similarly shaped setose bristles on the inferior ramus. All these characters may serve to distinguish it from the closely related *Nereis*, although it resembles this genus, in disagreement with *Ceratocephale loveni*, in the fact that the ventral cirri are simple instead of being bifid. Taken as a whole, I have preferred, tentatively at least, to refer "the Japanese Palolo" to MALMGREN'S genus *Ceratocephale* rather than to *Nereis* or to creating a new genus for its reception.

At all events it is certain that the species is a new one, and I take pleasure in naming it *Ceratocephale osawai* in honour

* A. G. MALMGREN, Annulata Polychaeta, 1867.—Possibly another species of the genus is represented by the specimens from the Gulf of St. Lawrence, noted upon by MCINTOSH (Ann. and Mag. Nat. Hist., ser. 7, X., 1902, p. 258).

of Professor K. Osawa, whose interest in the study of the worm I have already had occasion to mention.

The characters of the species may be summarily stated as follows:

Immature worm (Pl. I., fig. 1).—Number of segments as many as 300. Præstomium subhexagonal with broad base; anteriorly concave on the sides; provided with a pair each of tentacula and subtentacula, and a pair of eyes. Peristomium with four pairs of tentacular cirri. Proboscis protrusible, with soft papillæ and a pair of jaws. Parapodia nearly similar throughout the body, being biramus; only the lower ligula of the dorsal ramus is present. Both the dorsal and the ventral cirri are simple. Pygidium with a pair of slender anal cirri.

Mature worm (Pl. I., figs. 6, A, B).—This is the head-bearing anterior portion of the original worm. The segments number 78 or less. They are distinguishable into those of the thorax and of the abdomen. Head and thoracic segments remain unchanged in character, except that the eyes are now more conspicuous than before. Abdominal segments enlarged; their bristles, originally of the ordinary form, are now replaced by paddle-shaped ones.

Ceratocephale osawai is one of the most common littoral Annelids in Tokyo and vicinity, where it is extensively used in both immature and mature phases as bait. The immature phase is locally known under the name of "Itome" and the mature phase under that of "Bachi."

The species is also known to me from Miya in the Province

of Owari, from Shimizu Harbor in Suruga, from Ito in Izu, and from Matsushima and Hachinohé on the east coast of Northern Japan.

With these remarks I proceed to record the fuller details of my observations in the following order: 1) on the immature phase, 2) on the mature phase and 3) on the breeding-swarm.

1. Observations on the Immature Phase.

Habitat.—The immature worms or the atoca of the species (Pl. I., fig. 1) occur in great abundance between the tide marks along the Sumida River, on which Tokyo is situated, for a distance of about six miles from its mouth, and also in the adjoining parts of the Gulf of Tokyo. They also extend some distance into the tributaries, canals and ditches, which empty into the waters just mentioned.

At the ebb-tide they are found burrowing in the mud or sand to a depth of a foot or more. The entrance of the burrow is usually indicated by a small round hole on the surface, the margin of which is always slightly raised above the level. With the flood-tide, irrespective of the hour of the day, they leave their retirement and creep about on the bottom. They are then very active and voracious, feeding on various aquatic animals and plants. Often they are seen to dip with the head end into the bottom mud or sand in search of food.

Size and general shape.—The body is long, slender and dorso-ventrally compressed (Pl. I., figs. 1 and 2). Since we have to do with immature growing worms, it is but natural that the

dimensions should be exceedingly variable. It may in general be said that they attain a length of 200–250 mm. and a breadth of 3 or 4 mm. The number of segments varies of course according to the size of the specimens; in a large one there may be as many as 300. The addition of new segments during growth takes place, one by one, invariably in front of the pygidium.

Gradually from the head end backwards the breadth increases slightly to about the tenth segment, then it continues nearly the same to about the 40th or 50th segment, beyond which the body again begins to taper very gradually towards the hindmost region, which may be said to be very slender.

The size of the segments stands in direct proportion to the breadth of the region to which they belong. In respect of their structure, both internal and external, the segments are all essentially alike so that a demarcation into thoracic and abdominal segments can not be carried out.

Color.—In the living state of average specimens, the dorsum (Pl. I., fig. 1) in the anteriormost region is dusky brown with a purplish iridescence. In the main portion of the body, this color gradually passes posteriorly into a deep red, which again gradually becomes somewhat lighter towards the hind end. On the præstomium the bluish brown pigments are developed in small irregular, sometimes eye-like, blotches. The parapodia are always of a much lighter hue than the segments to which they belong. The dorsal median blood-vessel is prominently visible as a deep red line, in which the postero-anteriorly directed peristalsis may be distinctly observed.

Seen on the ventral side, the peristomium and a few succeeding

segments may be said to present a light pinkish color. For the rest the ventral surface is of a pale flesh color.

In the specimens preserved in alcohol, the coloring matter has dissolved away, leaving only a light bluish hue on the anterior dorsal surface of the body.

Præstomium (Pl. II., fig. 9).—This is small and flat, but well developed. The shape may be said to be subhexagonal, consisting of a transversely elongate, eye-bearing section attached by a broad base to the peristomium, and of an anterior section which somewhat narrows towards the bases of the tentacula at the front edge. The sides of the head at the junction of the two sections are concave. The anterior section is divided into two halves by a median groove.

The tentacula, present in a pair and situated close together on the anterior edge of the head, are small, being of a length less than half that of the entire head. Their base is slightly pigmented, the remaining part being colorless.

The subtentacula, likewise in a pair, are attached to the sides of the head at their anterior concave part as well as to the peristomium. They are thick and fleshy, with a small, round, somewhat retractile boss at the tip. Except at this end, which is colorless, they are of a brownish color.

The eyes, of which there are two pairs, are situated close to the lateral borders of the hind section of the head. In the anterior pair they are more widely separated from each other than in the posterior. Moreover, in the former they are directed forwards and outwards, while in the latter their direction is upwards and outwards. In alcoholic specimens the eyes are scarcely visible, so that with only such specimens one might be misled into assuming their absence.

Peristomium.—The peristomium or the first segment shows much longitudinal folding. On each side it is provided with four tentacular cirri, two dorsal and two ventral. The anterior ventral cirrus is the shortest, and the posterior dorsal the longest. The latter, when laid down backwards, reaches to about the the middle of the fourth segment. Each cirrus consists of a basal, more or less pigmented, section and of a colorless distal section pointed at the free end.

Proboscis.—Pl. II., figs. 10 and 11, show respectively the dorsal and the ventral views of the proboscis in its protruded state. It is furnished with a pair of strong chitinous jaws and a number of soft papillæ. The blackish brown but translucent jaws are slightly curved and their inner concave edge shows 7–9 teeth. Structurally, the jaws consist of a peripheral colored layer and an inner colorless mass. The latter is concentrically laminated and incloses two longitudinally running canals, which open externally on the points of the first and the second teeth. The canals contain a substance which in its staining capacity resembles the secretion of the "Spinndrüsen," a gland found in the dorsal ramus of certain parapodia. In the fully protruded state of the proboscis the points of the jaws are apart; these close and become crossed as it is withdrawn. They serve not only for capturing prey but also for burrowing into the bottom-ground.

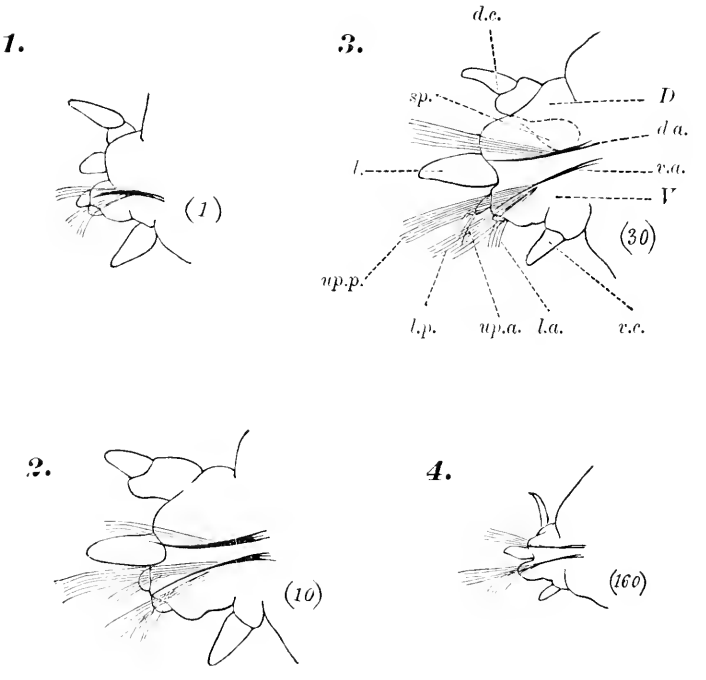
The surface of the proboscis, when protruded, is divided by a ring-groove into a posterior and an anterior ring, both of which are again subdivided into a number of small areas by grooves which run in the main longitudinally. The posterior ring is entirely destitute of papillæ, these being confined to the anterior ring. This latter ring (Pl. II. fig. 10), seen on the dorsal side,

shows in the middle a transverse row of three papillæ, situated one in each of the three areas known as the anterior median and the right and left anterior submedian. Further it shows two more papillæ of quite insignificant height, each situated near the lateral border, i. e., in the areas known as the anterior lateral. The ventral aspect of the anterior ring (Pl. II., fig. 11) bears a varying number of papillæ, arranged in irregular rows. These seem to increase in number with the growth of the individual. In the larger specimens their number varies from 17 to 27.

The paragnathea, which are of much common occurrence in the Lycoridæ and offer points of considerable systematic importance, are not found in this species as is also the case in *Ceratocephale loveni*.

Typical Parapodia.—The parapodia are best developed where the segments are largest, say in segments X. to XL. As an illustration of a typical parapodium I may take that shown in the annexed Woodcut 3, which represents the 30th parapodium belonging to the XXXI. segment.

It consists of two rami, the dorsal (*D*) and the ventral (*V*). From the upper side of the dorsal ramus arises the dorsal cirrus (*d.c.*), consisting of an elongate conical distal, and a thick basal, section. The latter, in the parapodium in question, is broad but of only about half the length of the former, and is well marked off by constrictions from this as well as from the ramus bearing it. The dorsal ramus lacks the upper ligula but possesses the lower ligula (*l.*), which arises from a point slightly ventral to its distal end and is somewhat longer than the dorsal cirrus. The ligula may be said to be conical in shape and more or less



Woodcuts 1–4. Parapodia of the atocous phase of *Ceratocephale osawai*, from the left side. All in posterior view. The figures in brackets indicate the serial number of each parapodium as counted from the first.

D, dorsal ramus; *d.a.*, dorsal acicula; *d.c.*, dorsal cirrus; *l.*, ligula; *l.a.*, lower anterior bunch of bristles; *l.p.*, lower posterior bunch of bristles; *sp.*, Spinndrüsen; *up.a.*, upper anterior bunch of bristles; *up p.*, upper posterior bunch of bristles; *V.*, ventral ramus; *v.a.*, ventral acicula; *v.c.*, ventral cirrus.

flattened in the antero-posterior direction; it is richly supplied with a vascular network.

The dorsal ramus is further provided with an acicula and a bundle of bristles. The acicula (*d.a.*) is of the usual simple shape and of a blackish color. It lies imbedded in the ramus, except its pointed tip which projects outwards between the ligula base

and the outer end of the ramus in question. The bristles are exclusively of the setose kind (Pl. II., fig. 12) with the blade finely serrated on one edge. They are placed in a bunch, dorsal to both the ligula and the acicula mentioned above.

The ventral ramus of the parapodium has no ligula, but is supplied with two small and rounded terminal lobes, the upper and the lower. Both are directed outwards and downwards, Further there is present on the ramus the ventral cirrus (*v.c.*). This compares well both in size and shape with the distal section of the dorsal cirrus; but the basal section, clearly distinguishable in the latter, is here only indistinctly indicated in that it shows no bounding constriction against the body proper of the ramus. The acicula (*v.a.*) is quite similar to that of the dorsal ramus, while the bristles are here of two kinds, the falcate and the setose, and are grouped in four bunches. Of these the lower-anterior bunch consists only of the falcate bristles (Pl. II., fig. 14). The upper-anterior bunch consists of setose bristles, whose blades are short and finely serrated on one edge (Pl. II., fig. 17). The lower-posterior bunch consists of setose bristles whose blades are long and rather coarsely serrated on the convex edge (Pl. II., fig. 15). This form of bristles closely resembles that from the ventral ramus of *Ceratocephale loreni*. The upper-posterior bunch is composed of setose bristles, the longest and most slender of the kind in the entire parapodium (Pl. II., fig. 16). The blade is slightly curved, with very fine serration.

Variation of the Parapodia.—The parapodia in different regions of the body are subject to certain variations as regards their size and the relative development of their parts. From the first parapodium (belonging to the second segment) backwards to

about the tenth, they increase in size gradually and successively, along with the increase in breadth of the body in that region. Then they keep up approximately the same size for some distance further backwards; and in the hind region of the body, they again become gradually smaller and smaller, until the very last parapodium, occurring in the præanal segment, is represented by quite a simple and insignificant elevation. To illustrate in a way the variation above indicated, I have shown in the Woodcuts 1–4 (p. 10) respectively the 1st, 10th, 30th and 160th parapodia, taken from an average-sized individual with 178 segments.

In the first parapodium (Woodcut 1) the superior ramus is always entirely destitute of the acicula and the bristles, and is simply provided with the dorsal cirrus and the ligula. The former is well developed, being about twice as long as the latter. Its basal section, making up about one-fifth of the entire length, is marked off by constrictions from both the distal section and the ramus bearing it. The ligula is small and simple in appearance, and has a rounded tip. In the much more complicated inferior ramus the two terminal lobes are of about the same size. The upper lobe projects slightly farther out than the ligula, while the lower is directed outwards and downwards. The ventral cirrus is nearly similar in shape and size to the dorsal, but differs in having no basal section marked off. The ventral acicula present and the bunches of bristles occuring in association with it are rather weakly developed.

Generally speaking the second parapodium and sometimes even the third are essentially like the first, the dorsal ramus being characterized by the absence of both the acicula and the bristles.

The next parapodium—that is, generally the third, but

sometimes the second or the fourth—is of a somewhat more complicated structure, being supplied with the aciculæ and the bristles in both rami. Especially the ventral ramus is more strongly developed than in the preceding parapodium. The ligula is larger, and extends a little beyond the ends of the terminal lobes of the ventral ramus. The dorsal cirrus differs from that of the first parapodium in that its basal section is longer and takes up nearly one-third of the entire length.

The series of successive parapodia beginning with the one last described and reaching down to about the 150th or thereabout, possesses essentially the same parts, which however show a very gradual variation in the following manner:

The distal portion of the dorsal cirrus grows relatively shorter posteriorly to about the 100th parapodium, from which point farther backwards it again becomes somewhat longer but more and more slender. The basal section of the cirrus increases in size posteriorly until about the 10th parapodium (Woodcut 2), where it attains its maximum size, and thence backwards it again gradually becomes shorter. The ligula continues to grow larger but flatter until about the 20th parapodium, in which it attains its greatest size. From that point backwards it gradually becomes smaller again. The setose bristles of the dorsal ramus are longest in about the 60th parapodium, in which the blade is $2\frac{1}{2}$ times as long as that of the same bristle in the 30th parapodium.

The aciculæ vary in length in proportion to the general size of the parapodium to which they belong. Abnormally two or three aciculæ may occur together in either of the two rami. Such cases have been found most frequently in the first two or three parapodia.

The terminal lobes of the ventral ramus increase in size from

the anteriormost parapodium to about the 20th and then begin to become smaller posteriorly until in about the 140th they become united together, finally to disappear altogether some distance still farther posteriorly.

The blades of the falcate bristles, which constitute the lower-anterior bunch of the ventral ramus, grow stronger from the anteriormost parapodium to about the 20th, in which the maximum development is reached (being about 1½ times the length of the 30th parapodium). Then they become smaller again to about the 25th or the 26th, after which they remain of nearly the same size until the posterior region of the body is reached.

The length of the setose bristles of the lower-posterior bunch is subject to much variation. It continues to increase from the front region posteriorly until the maximum length (about 1⅓ times that in the 30th parapodium) is reached in about the 60th parapodium, and then the same length is maintained to about the 100th or so, whence posteriorly it again diminishes. The variation in length of the bristles forming the upper-posterior bunch generally coincides with that of the bristles of the bunch just described.

In the upper-anterior bunch, the bristles vary not only in size but also in form. In the first 23–24 parapodia, the bunch consists exclusively of falcate bristles, just like those in the lower-anterior bunch. From about the 20th parapodium, their number in each bunch begins to decrease, while the shorter setose bristles in the bunch constantly increase in number, until in the 28th–30th the latter entirely replace the falcate variety. The setose bristles as they occur together with the falcate in the same bunch in the 20th–26th parapodia are slightly longer than the latter. Posteriorly they continue to increase in length to about

the 30th parapodium, in which they reach the maximum length (the blade length being about twice that in the 30th parapodium); after that they again gradually become shorter towards the posterior end of the body.

The ventral cirrus is best developed in the anteriormost segments, gradually becoming smaller in those more posteriorly situated.

The "Spinndrüsen" of EHLERS are found in the superior ramus. This gland begins to appear in the 22nd–25th parapodium. In Woodcut 3 the outline of it is indicated by a dotted line. It is somewhat pear-shaped and occupies a great portion of the ramus. In the more posteriorly situated parapodia it may even extend into the body beyond the basal constriction of the parapodium. Externally it opens by a short duct at the base of the dorsal cirrus. It is largest in about the 35th parapodium.

Here I may state that the locomotion of the worm is effected by alternate forward and backward movements of the parapodia on both sides of the body, assisted by forcible protrusions of the chitinous appendages, which are retractile to a certain degree. It has seemed to me that in the forward motion of the worm the setose bristles come more into operation than the falcate, and *vice versa* in the backward motion, as for instance in the act of retreating into the burrow.

Pygidium.—This is about as long as it is broad, the length being about equal to that of the three preceding segments taken together. The center of the rounded hind end is occupied by the anus, which shows a radial wrinkling around it. The pygidium is devoid of parapodia but is furnished with a pair of colorless, slender and delicate anal cirri, which, arising from its ventral posterior end, are directed backwards.

The Change of the Immature (Atoca) into the Mature Phase (Epitoca)—This begins to take place in the early part of September. All individuals that have passed the summer do not, however, pass nearly simultaneously into the epitoca. At the period of the year just mentioned, the worms still vary in size and it is especially the larger ones, as might be expected, which first begin to manifest symptoms of the change. These are however by no means of a uniform size. They are undoubtedly those which are destined to swarm out under the first favorable circumstances during the following month. Others which swarm later in the season undergo the change at a correspondingly retarded period. Probably some of the smallest found in the season do not become at all ready for swarming until the autumn of the following year; for throughout the winter after the last swarming has taken place in November, there are still to be found small immature worms, though in an incomparably smaller number than in the spring and summer.

The successive stages of the change into the epitocous phase are illustrated in Pl. I., figs. 1–5. To start with, fig. 1 shows a well-grown representative atoca, before the change has set in. In such specimens the sexes can not be determined by external observation. This becomes however possible as the worm grows markedly stouter, indicating the beginning of the change. The sexes then begin to present a difference in color. The worms in fig. 2,—in which figure, as also in several following figures, the letters A and B stand respectively for the male and the female, —the male (2 A) will be observed to be more dusky or more brownish than the female (2 B), which is for the most part of a bright red color, while both have grown considerably in breadth, especially in the anterior region of the body as compared with

the stage (fig. 1), in which the sexual color difference could not be perceived. As before indicated, such larger worms begin to be observed from the early part of September. During the course of that month the change proceeds further and the worms acquire the appearance of those shown in figs. 3 and 4. Though the head end remains of nearly the same size as in an early stage, most of the segments in the anterior portion of the body, have undergone a considerable growth; they are now much broader and more plump-looking than before, owing to the development of sexual products within. That portion of the body, composed of approximately one-third the total number of segments, is the part which goes to make up the body of the epitoca. It is of a reddish color much lighter than before, the female being distinguished by a yellowish tint which grows deeper as the days advance. The posterior portion of the body, comprising about two-thirds of the total number of segments, is destined shortly to be cast off. It stands in contrast with the anterior part not only in that its segments have remained stationery in size and consequently are very much narrower, but also in the marked change which has taken place in the color of that part. Here the reddish color has disappeared, having given place to a dirty brown on the sides and to a pale streak in the middle. The dorsal vessel presents an interrupted appearance, preparatory to becoming sooner or later quite imperceptible. The posterior region in question is evidently undergoing a degenerating process.

For some time, this region is seen to pass gradually into the anterior epitocous region; but as the change into the epitoca nears its completion, the two regions become quite abruptly demarcated (fig. 5).

The worms represented in fig. 5 are those in which the

anterior epitocous region has attained full development. So far as that region is concerned, there is observable no difference whatever from the swarming epitoca (fig. 6) either in the proportions of dimensions or in the color presented by the sexes. But the worms still drag behind them the degenerated posterior portion which has now become darker in color than before and has somewhat shrunk in thickness. It is usual that more or less of the posterior natural end of this portion is missing, it evidently having become torn off and lost.

Worms in the state above described are met with a few days—say, for a period of about a week—before the swarming is to be expected. I have found them in greatest abundance in the beginning of October, not only in the natural habitat but also in the aquarium in which only atocous worms had originally been placed. They are known to fishermen under the name of "Hori-bachi" or the "dug-out bachi," so named because the Bachi, found swimming a few days later, are in this period obtained by digging in the mud.

It seems more than probable that the degenerated posterior portion, now greatly loosened in the consistense of its tissues, becomes more and more torn off at the end, as it is being dragged along by the burrowing worm. And what may remain of of it at the time of swarming, may easily be detached by the first swimming movements. However, it sometimes happens that, among the individuals that have swarmed out, there are seen such as still possess the shrivelled tail-like appendage in varying lengths. Fishermen call these "Ya-bachi" or the "Arrow-Bachi," evidently from the remote resemblance they bear to a flying arrow.

At all events the degenerated posterior portion is destined to be sooner or later completely cast off. This leaves an open

aperture to the continuous body-cavity at the hind end of the epitoca, and the genital products may find their way outwards through that aperture, assisted without doubt by the muscular exertions required for swimming. Only an insignificant quantity of genital products is extruded through the nephridial openings, as I know from direct observations. At any rate, there can be no doubt that the nephridial organs play quite an unimportant rôle as genital outlet in comparison with the rent at the posterior end or with the ruptures which subsequently occur in the general body-wall.

2. Observations on the Mature Phase.

Ceratocephale osawai, on attaining the epitocous phase (Pl. I., figs. 6, 7), i.e., the stage of sexual matruity in which it swarms out for the purpose of breeding, differs in general appearance from the immature or atocous worms so considerably that it may at first sight easily be considered as specifically distinct. But my direct observations on the life-history of the species, as also the occurrence of forms representing intermediate transitional stages, have placed the developmental relation of the two phases beyond the reach of doubt.

The best method of capturing the swimming worms is to use a hand-net. If the night be rough or rainy, the worms do not come quite to the surface of water. In such a case I have preferred to use the sort of a fine-meshed hand-net, which is commonly employed in Tokyo for the capture of *Sarane microdon* in the spring. This net, as set in frame, is triangular, with sides of about six feet length each. It is dipped into the water

somewhat vertically, and by a sweeping motion the worms, as they come along with the ebbing tide, can be scooped up with convenience.

The swimming worms are attracted in numbers by the light of a lantern, which greatly facilitates the collecting. In the aquarium, a candle-light readily attracts them. Whereas, in the case of non-swimming atocous worms I have found that the same light exercised no such influence.

The epitocæ, after being captured, can be kept alive for a week or two in shallow wooden vessels placed in a shaded place and with a small quantity of the river-water taken at the time of high tide, just enough to cover the worms. The water must be changed once or twice a day, taking care to remove all the rent or otherwise injured worms as soon as possible, without which precaution the water will soon become so filled with discharged sexual products as to be detrimental to the general health. Too much water, as also much light, induces the worms to motion and thus increases the chance of receiving injuries to their body.

As might be expected, the worms are apt to fall prey to fishes while swarming. On one occasion I chanced to capture two *Leuciscus hakuensis*, which were evidently in pursuit of the swimming worms. On dissection, they were found to contain the worms in the stomach or sticking in the throat.

Size.—The dimensions of the epitoca vary considerably in different individuals, as the direct result of the fact, already mentioned, that differently sized atocæ undergo the change into epitocæ.

I have found males measuring between 40 mm. and 130 mm.

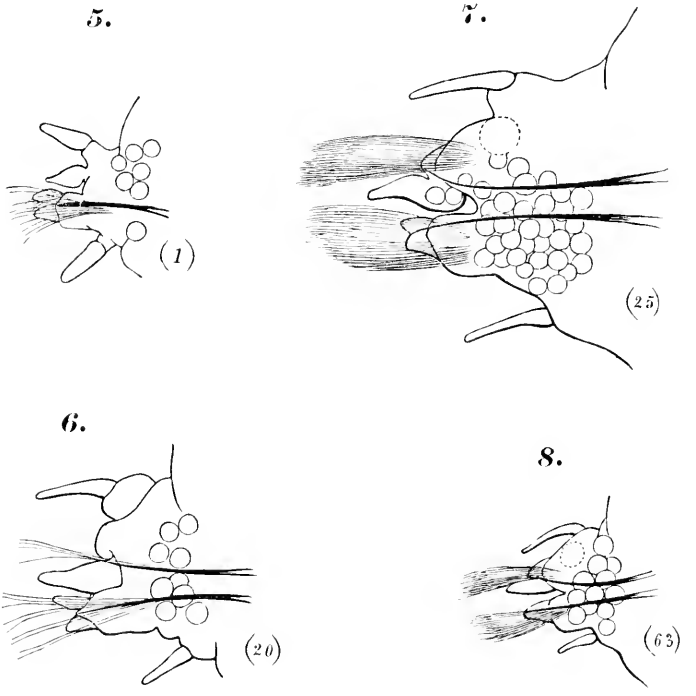
in length, and 3–11 mm. in breadth at the broadest part, while the number of segments varied from 60 to 78. In the females, the length varied from 35 mm. to 120 mm., and the breadth within nearly the same range as in the males; the number of segments was 60–74. In general it may be said that the atoca in developing into the epitoca increases about three-fold in greatest breadth, but decreases by nearly one-half in length, while the number of segments is reduced by approximately two-thirds as already indicated. The segments, taken singly, have grown not only in breadth but also considerably in length, and consequently have become more distinct than before.

Color.—In the epitocous phase, the sexes are as before mentioned easily distinguishable in color. In the females the body is at first of a yellowish color (Pl. I., figs. 6 B and 6b), but after swimming about for a while in exposure to light, the color changes to a light greenish (Pl. I., fig. 7). The males are pinkish white blending into a deep pink at the bases of the parapodia (Pl. I., figs. 6 A and 6 a).

Cephalic Region.—There exists no remarkable difference in the cephalic region in the two phases, except in regard to the eyes. It will therefore not be amiss here to give first a short description of an eye in the atocous phase. It consists of the three chief parts: retina, lens and cornea. The retina is composed of pigmented cells, each being in possession of a clear refractive end, the rod. The retinal cells collectively make up a pigmented single-layered epithelium, forming the wall of a deep cup, the aperture of which represents the pupil. This is not conspicuously discernible from the outside, being covered over by the cuticula

and the thick epidermis. The nuclei of the retinal cells are situated close to the outer end of the cells, forming a zone. The dark blue pigment is especially densely developed at the inner end, along the line demarcating the rods from the cell-bodies proper. The peripheral end of each cell tapers out into a process continuous with a nerve fibre, which can be traced uninterruptedly for some distance into the opic nerve. The clear refractive rods form, as indicated, an inner lining to the pigmented retina ; on the internal aspect they are indistinctly marked off from a clear central mass, the lens. They are longest in the fundus, and become shorter towards the rim, of the retinal cup. The lens not only fills up the entire cavity of the cup, but even projects slightly out of it at the pupil, thus coming here in direct contact with the epidermis. The name of cornea is given to that portion of the epidermal layer which intervenes between the lens and the cuticula. The latter is directly continuous with that over the entire head and is in no way modified over the eyes.

In adult worms the eyes have grown considerably in size ; the retinal cup is now about twice as large in diameter as in immature worms, while the diameter of the pupil has increased about four times. The lens now projects outwards to a much greater degree than before, pressing against and reducing the thickness of both the cornea and the cuticula. The former may sometimes become even entirely pressed aside, thus becoming perforate in the center and bringing the lens into direct contact with the cuticula. The result of these changes is that the eyes are now very conspicuously visible in surface view (Pl. II., fig. 18), in both the living and the preserved state of specimens. They are especially distinct in specimens killed with picrosulphulic acid, in which they appear as whitish spots surrounded by a black ring, the margin of the



Woodcuts 5–8. Posterior views of the left side parapodia of epitocous *Ceratocephale osawai*. Small circles in the figures indicate the outlines of eggs contained in the paradodia. The figures in brackets show the serial position of each parapodium as counted from the anteriormost. Magnified 20 ×.

pigmented retinal cup. The greater development of the eyes in the epitoca evidently stands in relation with the free-swimming habit.

Thoracic and Abdominal Regions.—In the epitocous worm we may speak of the thoracic and the abdominal regions of the body. The boundary between the two regions varies somewhat in different individuals. It lies between the 22nd and the 25th segments in females, and between the 24th and the 27th in males.

In both sexes, the segments of the thoracic region gradually enlarge antero-posteriorly to about the 10th segment and then remain nearly the same in size down to the abdominal region. The parapodia of that region are essentially similar to those in the corresponding body-part of immature worms (Woodcuts 5–6), except in this respect that the dorsal and the ventral cirri are generally much more slender and longer. The aciculæ as well as both the falcate and the setose bristles remain, in the thoracic parapodia, in much the same condition as before.

In the abdominal region, the segments begin again to gradually broaden and continue to do so to about the 40th in the female, and to about the 45th in the male; then they narrow backwards to the posterior end. The parapodia in this region show a somewhat sudden enlargement within the anterior two or three segments; and after that they are seen to increase posteriorly gradually in size until the 40th (female) or the 45th (male) segment is reached. Beyond this they again become gradually smaller towards the hind end of the body.

In all the abdominal parapodia (Woodcuts 7–8), the distinction between the dorsal and the ventral rami is more pronunced than in the atocous stage; the cirri are more elongated, and their basal portion much shorter, being sometimes scarcely distinguishable as such. The ligula has become somewhat more slender. The "Spinndrüsen" now show a rounded outline. The extension of the body-cavity into the parapodium is full of mature eggs or spermatozoa according to the sex. Sometimes the sexual products are found even in the interior of the ligula. The aciculæ present simply a larger size in correspondence to the general growth undergone by the parapodia themselves.

In the anterior two or three abdominal segments, the paddle-

shaped bristles are found together with the ordinary bristles, but in the more posterior segments, the latter disappear altogether. Only exceptionally are a few ordinary or setose bristles again found in both the dorsal and the ventral rami in the last one or two abdominal segments.

The paddle-shaped bristle (Pl. II., figs. 19 and 20) consists of a shaft, which shows regular transverse striations, and of a blade, which is very finely serrated on one edge. It remains the same in form throughout the same individual but differs somewhat according to sexes. The body-region in which the above mentioned transition in the character of bristles, from the setose to the paddle-shaped, occurs,—that is, approximately the boundary between the thoracic and the abdominal parts,—nearly coincides with that region of atocous worms in which the falcate bristles become replaced by the setose.

The Hindmost Segment.—In external appearance the hindmost segment of the epitocous worm is just like any of those which precede it. Its parapodia are directed postero-laterally, and of course no anal cirri are present. Examined in longitudinal sections, the hypodermis of this segment is seen to be greatly thickened, especially in the dorsal part. At the posterior end of the segment it is four times or more as thick as at the anterior end or in any other segment. Further, the hypodermis can be distinctly made out as being discontinuous with the wall of the intestine, in contrast to the condition in the hind end of atocous worms, in which the hypodermis cells pass gradually and continuously into the cells of the intestinal wall at the anus. The epithelial cells of the intestine in the hind end of epitocous worms present some signs of atrophy, in that the cell-outlines

are indistinct. Elongation of the hypodermis cells and a consequent thickening of the layer they compose also occur close to an artificial cut by which the hind part of a worm has been removed, but in this case, the cells of the intestinal wall do not show any sign of atrophy.

The circular muscle of the dermal musculature is strongly developed in the last segment, especially in its posterior part. The longitudinal muscles end abruptly in this segment ; so likewise the ventral nerve cord, its fibres showing some signs of degeneration. There is found no ring vessel connecting the ventral longitudinal vessel with the dorsal, which ring-vessel is one of the points characteristic of the anal segment in atocous worms ; both the dorsal and the ventral blood vessels terminate freely and apparently blindly. From what has been said it clearly follows that the hindmost segment of the epitocous worm is not the anal segment of the atocous. On the contrary there can be no doubt whatever of its being an ordinary body segment, which has undergone some change, as the result of the shedding away of the more posterior segments.

Some Other Points of Anatomical Differences between the Atoca and the Epitoca.—The integument becomes thinner as the worm passes into the epitocous phase. The cuticula measures 1–1½ μ in thickness in the epitoca, while in the atoca it is 2–3 μ hick. The hypodermis is 10–13 μ thick in the epitoca, but 20–25 μ in the atoca. The thinning is evidently due to the expansion of the general body cavity, in consequence of the development of reproductive elements.

Apparently for the same reason, both the dorsal and the ventral longitudinal muscles in the epitoca are greatly thinned

out and are pressed against the inner side of the body-wall; while in the atoca these muscles are much thicker and occupy a more internal position in the body. The circular muscle layer is also much reduced in thickness.

The communicating apertures between consecutive segments become larger as the worm approaches sexual maturity, and admit of a free movement of the genital element from one segment into another. As before indicated, the general body cavity, more or less filled up with either eggs or spermatozoa, extends into the parapodia and sometimes even into the ligula. The main body of the nephridium is pushed into the parapodial cavity by the mass of the genital product.

The alimentary canal, caudad from the transitional region between the proboscis and the intestine, is much reduced in caliber. The intestinal wall is now much poorer in capillary vessels than before.

Sexual Products.—The eggs or the spermatozoa are discharged while the epitocous worms are actively swimming near the surface of the water. The eggs then sink down gradually to the bottom. They are each surrounded by a thick and transparent gelatinous envelope, so that when found in masses, they are separated from one another by a considerable space. They are spherical in shape, with a diameter of 120–150 μ. In color they vary from yellow to greenish blue.

The vitellus, surrounded by a delicate membrane at first in direct contact with it, is finely granular and consists of the protoplasm inclosing at least three distinguishable kinds of matter, viz., large oil-drops, small oil-drops and deutoplasmic spheres (Pl. II., fig. 22). The large oil-drops, of which approximately

from twenty to thirty are present in each egg, vary much in size and are situated in the vegetative half of the vitelline mass. This pole of the egg is therefore lighter than the opposite and is thus always turned upwards in the natural position of the egg in water. The second kind of oil-drops are minute and highly refractive spheres, found scattered throughout the entire vitellus. I am unable to say whether they are chemically of exactly the same nature as the larger kind. There exist no intermediate sizes linking together these two kinds of drops. The deutoplasmic spheres may be said to stand in point of size intermediate between the two forms of the oil-drops. They are at first uniformly distributed in the vitellus together with the smaller oil-drops.

The spermatozoa, soon after expulsion from the body, are found adhering in large numbers to the gelatinous envelope of the ovum. Each consists of an ellipsoidal head and of a long slender filiform tail. The head is 3 μ long and 1.7 μ broad. The tail measures 35–45 μ in length ; it gradually tapers towards the hind end.

Artificial fertilization by bringing together the eggs and spermatozoa taken from mature worms can easily be effected, provided the precaution be taken to keep the water at the same temperature and the same degree of salinity as that at high tide in the river during the swarming period. Forty to fifty minutes after fertilization, the vitellus contracts and aquires an irregular surface, which is separated here and there from the vitelline membrane by vacant spaces. Sometime afterwards, the vitellus again assumes a perfectly spherical shape, and is then separated throughout from the membrane by a narrow perivitelline space.

Meanwhile certain changes, preparatory to the formation of polar bodies, take place in the animal pole. The deutoplasmic

spheres migrate away from the animal pole, leaving there a clear protoplasmic area, which henceforth slowly enlarges. The oil-drops mostly press themsleves into the vegetative half of the egg, but a few small oil-drops may still be seen scattered in the hyaline animal pole. The greenish blue color of the egg is now most intense in the vegetative pole; it gradually fades away towards the animal pole.

About an hour after fertilization, the first polar body is extruded; the second follows fifteen or twenty minutes later. In this stage (Pl. II., fig. 22) the vitellus appears somewhat flattened at the upper pole and is separated from the membrane by a considerably wider space than before. The main axis, connecting the animal and the vegetative poles, measures 110–140 μ in length. So far as I have been able to follow the cleavage process in the living ova, it shows a general agreement with that described by WILSON* for *Nereis limbata;* only it proceeds much more slowly than in that species.

The larva of the species I have not been able to observe.

3. OBSERVATIONS ON THE SWARMING.

With respect to the swarming habit of the "Bachi" or the mature *Ceratocephale osawai*, it has long been known from the experience of fishermen in the locality that the swarming occurs during the months of October and November, usually in four different periods, each lasting a few days; that the period falls on nights close to the days of the new and the full moon; that it invariably takes place in the evening just after the flood-tide;

* WILSON, E. B.—The Cell-lineage of *Nereis*. Jour. of Morph. Vol. VI. 1892.

and further, that in some years it occurs in two or three, instead of four periods and rarely only once during the months mentioned.

In order to make observations for myself, I spent many evenings on the river, and it was not long before I became convinced of the general accuracy of the fishermen's predictions as to when the swarming was to be expected. On October 8th 1896, I had the satisfaction of observing the swarming for the first time. It was the first swarming of that year. On that day the worms that swarmed out were not numerous, but on the following day the swarm proved to be one of the largest I have ever known.

As an illustration I may describe my experience on that particular day (Oct. 9th, 1896). In good time to see the beginning of the swarming, I was on the river in a boat manned by two fishermen and provided with a lantern, nets of various kinds and such other utensils as might be required for observing, capturing and preserving the worms. About half past six in the evening, the place was reached which was considered likely to be favorable for the accomplishment of my purpose. The flood was to occur at 6.54 p.m. While we were waiting for the beginning of the swarming, a crowd of other boats, each provided with a light to attract the worms, assembled near mine. They had come to catch the "Bachi," which, as I have said, are much used as bait in fishing and therefore are a marketable commodity. About 7 o'clock or a little later, the first swimming worms were observed. It was only individuals of small size, measuring 30–40 mm. in length, that were seen in the first part of the swarming; about 15 minutes later, larger ones began to join the swarm, in which a few individuals were noticed still trailing the shrivelled posterior portion of the body, such as are shown in fig. 5, Pl. I. ("Ya-bachi"). About half an hour

from the beginning, the swarm was thickest; full-sized individuals were now seen in abundance together with smaller ones. All swam about rapidly, somewhat after the manner of eels, darting in all directions. I ascertained that the swarm reached to a depth of three or four feet from the surface of the water. Within that extent, the worms in the height of the swarming, were so plentiful that one could not dip his hand into the water without touching some. About an hour and a half from the beginning, the larger worms first began to gradually disappear and as the end of the swarming approached, it was only the smaller specimens that could be found swimming. Two hours after the beginning of the swarming (at 9 p.m.), there were none to be found swimming.

The above account, except as it concerns the hours of the day, may in general be considered to hold good for all observed cases in which the swarming took place in large numbers.

Possibly a part of the worms after the swarming sink to the river-bottom in an exhausted condition. At the same time it seems certain that a large number of them are carried down stream and some distance out into the sea by the ebbing tide. On more than one occasion I have followed the shifting swarm to a distance of two or three miles from the river-mouth—to the neighborhood of the old forts off Shinagawa,—but always to lose sight of it gradually and altogether at the end.

In the aquarium, in which I had kept a number of large atocæ and in which the natural conditions were imitated as far as possible, the worms changed, into the epitocous phase and began to swim almost simultaneously with those at large. The ebb and the flood of each day were imitated in the aquarium at proper hours, the former by gradually drawing off the water so

as to almost expose the mud at the bottom and the latter by slowly adding fresh water to a depth of about two feet. In the river, the bottom-temperature was found to rise 1–2°C. higher during the flood than at the ebb, the surface maintaining the same temperature all the time. This periodical change in bottom-temperature could not very well be introduced into the aquarium, but this seemed to exercise no influence upon the swarming process. I regret that I omitted to ascertain by experiment whether or not the worms, when left in standing water that showed nothing like the ebb or the flood, would have swarmed out at the right time.

The epitocæ that have swarmed out in the aquarium continue to swim about for a longer or shorter period, sometimes for several hours, though the swimming may at times be interrupted by pauses in which they sink to the bottom and remain motionless. In many cases the distended body-wall becomes rent during the exertions of swimming; this soon puts an end to the swarming career. In other cases, they may remain for a considerable while uninjured save at the posterior torn end. Such individuals become gradually less energetic in their movements, finally to rise no more from the bottom, but soon to become ruptured in the body-wall. It may fairly be said that in twenty-four hours at the longest the energy of the worms becomes completely exhausted in all cases, unless, as before mentioned, their movements be restricted by denying them enough water to swim in, so that it seems exceedingly probable that the worms which have once swarmed out in the river never join in the swarm of the day following. I see no ground to doubt that the fate which I have observed to be fall the swarming epitocæ in captivity, is in general the same as that which happens to those under natural conditions.

Since taking up this subject of research, I have made a series of observations on the time and periods of the swarming, on the Sumida River in Tokyo. In both the years 1896 and 1897, the swarming occurred in four periods in the months of October and November. The following tables record my observations for these years:

Table I.

1896.

Periods.	Date and phase of moon.	Time of flood in the evening.	Hour and duration of swarming.	Size of swarm.
I.	Oct. 7th, New Moon.	5.25, P.M.	— —	None.
	„ 8th.	6.08, „	6.30-8., P.M.	Few.
	„ 9th.	6.54, „	7.-9., „	Very abundant.
	„ 10th.	7.41, „	7.40-9.20. „	Abundant.
	„ 11th.	8.31, „	8.40-9., „	Very few.
	„ 12th.	9.25, „	— —	None.
II.	Oct. 21st.	4.53, P.M.	— —	None.
	„ 22nd, Full Moon.*	5.26, „	About 6., P.M.	Very few.
	„ 23rd.	5.56, „	6.30-8.40, „	Abundant.
	„ 24th.	6.29, „	7.20-8.40, „	Few.
	„ 25th.	7.05, „	— — —	None.
III.	Nov. 5th, New Moon.	5.05, P.M.	— —	None.
	„ 6th.	5.54, „	6.30-8.20, P.M.	Abundant.
	„ 7th.	6.42, „	7.-8.40, „	Abundant.
	„ 8th.	7.30, „	About 8., „	Very few.
	„ 9th.	8.18, „	————	None.
IV.	Nov. 20th, Full Moon.†	5.05, P.M.	————	None.
	„ 21st.	5.40, „	————	None.
	„ 22nd.	6.17, „	About 6.40, P.M.	Very few.
	„ 23rd.	6.55, „	— — —	None.

* Moonrise at 4.53 P.M.

† Moonrise at 4.02 P.M.

Table II.

1897.

Periods.	Date and phase of Moon.	Time of flood in the evening.	Hour and duration of swarming.	Size of swarm.
I.	Oct. 11th, Full Moon.*	5.27, P.M.	————	None.
	„ 12th.	5.57, „	6.20–7., P.M.	Few.
	„ 13th.	6.29, „	6.50–7.20, „	Few.
	„ 14th.	7.03, „	————	None.
II.	Oct. 25th.	4.35, P.M.	————	None.
	„ 26th, New Moon.	5.19, „	6.–6.10, P.M.	Very few.
	„ 27th.	6.07, „	6.10–8.20, „	Very abundant.
	„ 28th.	6.56, „	7.05–9.20, „	Very abundant.
	„ 29th.	7.45, „	8.–8.40, „	Abundant.
	„ 30th.	8.39, „	————	None.
III.	Nov. 9th. Full Moon.†	5.03, P.M.	————	None.
	„ 10th.	5.36, „	6.–7.20, P.M.	Abundant.
	„ 11th.	6.11, „	6.40–7.10, „	Few.
	„ 12th.	6.45, „	————	None.
IV.	Nov. 24th, New Moon.	5.07, P.M.	————	None.
	„ 25th.	5.59, „	6.25–8., P.M.	Abundant.
	„ 26th.	6.49, „	About 7.20, „	Very few.
	„ 27th.	7.39, „	————	None.

The watch for the swarming was kept on several other days than on those mentioned in the above tables, but with negative results; so it may be confidently stated that no other swarms occured in the years 1896 and 1897. In both these years, from July to October I examined every week the atocous worms, freshly dug out of the mud. By the immature condition they presented it could be foretold that the swarming was not close at hand.

* Moonrise at 5.6 P.M.

† Moonrise at 4.11 P.M.

As before mentioned, forms transitional to the epitoca appeared at the end of September and became very numerous in the beginning of October. After that a sharp look-out for the swarming was of course daily maintianed on the river, at the same time continuing the examination of fresh specimens obtained every other day. In this way, I could approximately foresee the approach of the swarming, quite independently of the forecast made by the fishermen as there sult of the experience of many years.

After each swarming period the transitional half-epitocous forms, such as are shown in Pl. I., fig. 5, totally disappear for a time from among the worms collected from the river-bottom. However, in about ten days,—that is to say, a few days before the next swarming period,—a plenty of the half-epitocous worms are again met with in the mud; it is needless to say that these are to take part in the next swarming. After the last swarming at the end of November, there are to be found in the river-bottom only small atocous worms, which probably attain sexual maturity in the autumn of the following year.

In the years 1898 and 1899, I made only occasional observations on the swarming so that my records for those years are not so complete as for the two preceding years. Nevertheless, it can be confidently stated that the swarming in the years mentioned took place quite in accordance with our previous observations,—that is to say, it occurred at periods and hours, which could be foretold. The number of swarming worms was variable as before, and sometimes very small.

To give in general terms the results derived from my observations:

1.—The epitocous worms swim out four times a year, in the months of October and November.

2.—Each swarming period extends from one to four consecutive days, immediately following the days of the new and the full moon.

3.—The largest swarms occur within three days after the day of the new and the full moon.

4.—The swarming is greater after the new moon than after the full moon.

5.—The swarming invariably takes place just after the flood in the evening.

6.—The swarming continues generally from one to two hours.

7.—On warm cloudy nights, the swarming seems to be generally larger than on clear chilly nights.

As to the tidal conditions in the mouth of the Sumida River during the two months of October and November, it is known: 1) that spring-tides occur in the evening within three days following the day of the new and the full moons; and 2) that the spring-tide following the new moon is higher than that after the full moon. There is then noticeable a parallelism between the occurrence of the densest swarm and the highest spring-tide during the months concerned. Noteworthy seems also the fact that in the present species the time of swarming closely follows both the new and the full moon.

Postscript.

Professor K. Osawa presented before the V. International Zoologists' Congress (Berlin, 1901) a paper entitled "Ueber die japanischen Palolo," in which he, as I am told by him, dealt principally with the swarming habit, leaving the worm undescribed. The paper should have already appeared, in the "Verhandlungen," accompanied with a plate. Unfortunately the said "Verhandlungen" had not arrived in Japan before the time when I had to send my manuscript to the press; so that much to my regret, I could neither refer to nor benefit by his paper. It is more than probable that some facts already known through him have been unnecessarily redescribed by me in this contribution, for which repetition I herewith beg to offer my apology.

Tokyo, May 27th, 1903.

PLATE I.

Plate I.

Fig. 1. *Ceratocephale osawai* in the atocous or immature phase, commonly known under the name of "Itomé." (Nat Size).

Figs. 2–5. Immature specimens in different stages of transition into maturity or the epitocous phase. *A*, males. *B*, females. (Nat. size). In figs. 2*A* and 2*B*, it will be seen that the sexes differ slightly in color of the body, which is now considerably stouter than in the stage of growth represented in fig. 1. In figs. 3*A* and 3*B*, the anterior portion of the body has undergone much more enlargement, while the posterior portion remains nearly the same; the sexes are easily distinguishable on account of their different colors. Figs. 4*A* and 4*B* represent worms somewhat advanced in the change into the epitocous phase. Figs. 5*A* and 5*B* represent nearly full-grown worms, in which the anterior portion of the body, that is the epitoca about ready to swim out, is abruptly marked off from the shrivelled and discolored posterior portion. Some posteriormost segments have already been torn off and lost.

Fig. 6. Epitocous phase, or the so-called "Buchi." Figs. 6*A* and 6*B* represent respectively a male and a female epitoca of representative dimensions. Fig. 6*a* and 6*b* represent a male and a female of unusually small size.

Fig. 7. An epitocous female, showing the change of color into the greenish, after swimming about for a while in exposure to light. (Nat. size).

Fig. 8. Dorsal view of that part of the body, in which the anterior epitocous portion of the worm passes over into the posterior portion which is eventually shed off.


1


2 A


2 B


4 A


4 B


5 A


5 B


6 A


7

6 B


6 a 6 b


8


3 A


3 B

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PLATE II.

Plate II.

Fig. 9. Dorsal view of the anterior end of an atocous worm. 10 ×.
Fig. 10. Dorsal view of same, with protruded proboscis. 15 ×.
Fig. 11. Ventral view of same. 15 ×.
Fig. 12. A bristle from the dorsal ramus of 30th parapodium, from an atocous worm. 390 ×.
Fig. 13. A bristle from the upper-anterior bunch of the ventral ramus, from the same. 390 ×.
Fig. 14. A bristle from the lower-anterior bunch from the same. 390 ×.
Fig. 15. A bristle from the lower-posterior bunch from the same. 390 ×.
Fig. 16. A bristle from the upper-posterior bunch from the same. 390 ×.
Fig. 17. A bristle from the upper-anterior bunch of the ventral ramus of the 35th parapodium, from the same. 390 ×.
Fig. 18. Dorsal view of the head of an epitocous worm. 10 ×.
Fig. 19. A paddle-shaped bristle of the 30th parapodium, from a female epitoca. 390 ×.
Fig. 20. A paddle-shaped bristle of the 30th parapodium, from a male epitoca. 390 ×.
Fig. 21. Last two segments of an epitocous worm. 10 ×.
Fig. 22. Lateral view of a fertilized egg, just after the extrusion of the second polar body. Drawn from a living specimen. 390 ×.

9

10

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19 20

13 14

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On the Fossil Echinoids of Japan.

By

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With 4 plates.

Although the number of fossil echinoids hitherto found in Japan is small, yet there are several interesting forms amongst them. The Palæozoic strata has yielded no echinoids, while in the Mesozoic five genera have been found, one of which, however, is doubtful. They are *Pygurus*, *Toxaster*, *Cidaris*, *Pseudocidaris*, and *Hemicidaris* (?). *Pygurus*, up to the time of its discovery in Japan, had been found only in the Oolite and Cretaceous of Europe, and in the Cretaceous of Northern and Western Africa and N. America (?). In Japan it is found in the so-called Torinosu-limestone, respecting which it is still uncertain whether it is Cretaceous or Jurassic. One feature, however, that leads us to think that it may be Cretaceous, is the presence of a species of *Toxaster*, which has been hitherto restricted to the Cretaceous of Europe, N. Africa and Asia (Syria). Many specimens of *Cidaris* and *Pseudocidaris* have been collected from the same limestone, and also from a similar one in several other localities such as Tokano and Yokodani in the Province of Tosa and Itsukaichi in the Province of Musashi.

The genera found in the Cainozoic formation are the following: *Salenia*, *Coptosoma*, *Temnopleurus*, *Strongylocentrotus*, *Fibularia*, *Clypeaster*, *Laganum*, *Echinarachnius*, *Echinodiscus*, *Astriclypeus*, *Ilarionia*, *Echinolampas*, *Linthia*, *Schizaster*, *Prenaster*, *Hypsospatangus* and *Brissopsis*.

Among these, *Prenaster*, *Ilarionia* and *Hypsospatangus* are forms not living in the recent seas; and all except the last, which was collected from the Neogene Tertiary of Hokkaidō, were found in the Eocene of the Bonin Islands.

Astriclypeus is a genus restricted to Japan and China, being represented by a single living species *A. manni* VERILL. This genus differs from all others in possessing five large lunules. The fossil is a new species, and was found in the Miocene of Kai, Formosa and Riukiu (Loochoo Is.)

Coptosoma has only one living species, *C. crenulare* A. AG., also found fossil in the Pliocene of the Province of Sagami.

Echinodiscus is represented in Japan by one living (*E. laevis* KLEIN) and one fossil form (*E. formosus* YOSH.), the latter having been collected by myself from the Miocene of Formosa and Riukiu.

Linthia, which is no longer living in Japan, shows in the fossil form a wide distribution, being known from Kanazawa in Kaga; Sakae, Hidaka and Ikari in Shinano; Tsurushi and Hazzaki in Hitachi; Anrakujō in Uzen; and Asahigawa in Ugo. The formation in which it is found is probably Pliocene.

Schizaster has two or perhaps more species. One is *S. nummuliticus* TOK. found associated with *Nummulites* in the Eocene of the Bonin Islands; another is *S. recticanalis* YOSH. found together with *Linthia nipponica* YOSH. in Sakae. Besides the above two, I have found two indeterminable species of the

same genus in the Tertiary of Formosa (north of Ratō near Suō) and in the Pliocene of Yokohama.

Echinolampas, which in our country is represented by a single living species *E. oviformis* GMEL., has one interesting fossil species, *E. yoshiwarai* P. DE LORIOL, collected in the Pliocene of Kanaya in Kazusa together with several reef corals.

The wide-spread genus *Salenia* has also one fossil representative in our country which was described by P. DE LORIOL as *Salenia* (*Pleurosalenia*) *hakkaidoensis*.

Echinarachnius mirabilis BARN, a very common living echinoid in Japan, was living in the Diluvial time in Hitachi, Shimōsa and in the environs of Tokyo. *Echinarachnius parma* LAM., which is said to be living in Japan (locality uncertain), is found in the Neogene Tertiary of Australia as well as in that of Etchu, Echigo and Sado.

As to the genus *Laganum*, *L. decagonalis* LESS. is widely distributed in the fossil form in several countries, such as Japan (Diluvium), Java (Miocene) and Australia (Eocene). *L. fudsiyama* DÖD., which is now living in the Sagami Sea, has been collected by me in the Pliocene of Sagami.

Clypeaster testitudinarius GRAY has been found in the Miocene of Java, in the Tertiary of Australia and in the Diluvium of Loochoo and Tokuno-shima; *Brissopsis luzonica* GRAY, in the Miocene of Java and the Neogene Tertiary of Iwaki; and *Temnopleurus toreumaticus* KLEIN, in the Miocene of Java and the Diluvium of Ōji and Shinagawa. Lastly, indeterminable specimens of *Strongylocentrotus* have been collected from the Pliocene beds of Izu and Sagami.

NOTES ON THE SPECIES.

SALENIA (PLEUROSALENIA) HAKKAIDOENSIS

P. DE LORIOL.

Pl. II. Fig. 1.

LITERATURE CONSULTED :—

P. DE LORIOL, Notes pour servir a l'étude des Echinodermes, II. série, Fasc. I., 1902.

LOCALITY :—

Ekimomaanoro, a branch of the Anoro river in Yūbari coal-field, Prov. of Ishikari, the age of which is probably Pliocene (collector K. JIMBŌ).

COPTOSOMA CRENULARE (A. AG.)

SYNONYMS : *Glyptocidaris crenularis* A. AG.
Phymosoma crenulare A. AG.

LITERATURE CONSULTED :—

A. AGASSIZ, Revision of Echini.—Illust. Catalogue Mus. Comp. Zool. Harvard Coll., No. VII., 1872–74.

P. M. DUNCAN, A Revision of the Echinoidea from the Australian Tertiaries.—Quart. Journ. Geol. Soc. London, XLIII., 1877, p. 411.

P. M. DUNCAN, A Revision of the Genera and great Groups of the Echinoidea.—Journ. Linn. Soc. Zool., Vol. XXIII., 1889.

LOCALITIES :—

Koshiba, Prov. of Sagami (Pliocene) (coll. S. TOKUNAGA).

Living at :—Hakodate (Museum of Science College, Tokyo; and coll. by W. STIMPSON); Kominato-wan, Prov. of Mutsu (coll. I. IKEDA).

TEMNOPLEURUS TOREUMATICUS (KLEIN).

SYNONYMS : *Cidaris toreumatica* KLEIN.
Echinus toreumaticus GMEL.
Echinus sculptus LAM.
Temnopleurus bothryoides AGASS.
Temnopleurus reevesii A. AG.
Temnopleurus hardwickii GRAY.
Temnopleurus japonicus MART.
Toreumatica hardwickii GRAY.
Microcyphus elegans A. AG.
Temnotrema sculpta A. AG.

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K. MARTIN, Die Tertiärschichten auf Java, 1879–80.

F. J. BELL, Observations on the Characters of the Echinoidea. —Proc. Zool. Soc. London, 1880, p. 423.

A. AGASSIZ, Report on the Echinoidea dredged by H. M. S. Challenger, etc.—Challenger Report, Vol. II., 1881.

L. Döderlein, Seeigel von Japan und den Liukiu-Inseln.— Archiv. f. Naturg., I. Bd., 5 Jahr., 1885, p. 73.

J. E. Ives, Echinoderms and Arthropods from Japan.— Proc. Acad. Nat. Sc. Philadelphia, 1891.

Localities :

Java (Miocene) (described by K. Martin) ; Ōji and Shinagawa in the environs of Tokyo (Diluvium) (coll. S. Tokunaga).

Found living in the following localities : Hakodate (Mus. Sc. Coll. Tokyo ; also coll. by W. Stimpson) ; Sendai Bay (coll. St. John) ; Tokyo Bay (coll. L. Döderlein); off Yokohama (Challenger Exp.) ; Misaki, Prov. of Sagami (Mus. Sc. Coll. Tokyo) ; Dōketsuba in Sagami Sea (coll. I. Ijima) ; 4 miles west of Aburatsubo near Misaki (coll. A. Owston) ; Yokohama (Mus. Berlin) ; Jedo (Mus. Berlin) ; Wakano-ura, Prov. of Kii (Mus. Sc. Coll. Tokyo) ; Bay of Tango (coll. L. Döderlein) ; Maizuru, Prov. of Tango (coll. L. Döderlein) ; Miyatsu, Prov. of Tango (Mus. Sc. Coll. Tokyo) ; Tsuruga, Prov. of Echizen (Mus. Sc. Coll. Tokyo) ; Kasaoka, Prov. of Bitchū (coll. A. Izuka) ; Tomo, Prov. of Bingo (Mus. Sc. Coll. Tokyo) ; Usuki-wan, Prov. of Bingo (coll. T. Terasaki) ; Onomichi, Prov. of Bingo (coll. A. Izuka) ; Hiroshima, Prov. of Aki (Mus. Sc. Coll. Tokyo) ; Kōbe (Challenger Exp.) ; Hosojima-wan, Prov. of Hyūga (coll. T. Terasaki) ; Nagasaki (Mus. Berlin) ; Kagoshima (coll. W. Stimpson) ; East coast of Nippon (coll. W. Stimpson) ; Japan (coll. Salmin) ; Korea ; Kamtschatka ; Arafura Sea ; Philippine Is.; North China Sea ; Hongkong ; Siam ; E. Indies ; West of New Guinea ; Bombay ; Singapore ; Gulf of Persia ; Karrak Is.; Unalaska ; Tanjong ; Kling near Malacca ; C. Rachado ; Macclesfield Bank ; Coromandel Coast; Entrance to Palk Strait ; Ceylon ; all coasts of Australia.

FIBULARIA ACUTA Yoshi.

Pl. II. Figs. 5 and 6.

Literature consulted :—

S. Yoshiwara, Preliminary Notice of new Japanese Echinoids.—Ann. Zool. Japon., Vol. II., Pars. II., 1898, p. 57.

S. Yoshiwara, On some new Fossil Echinoids of Japan.—Journ. Geol. Soc. Tokyo, Vol. VI., No. 65, 1899.

Localities :—

Ōji near Tokyo (Diluvium) (coll. S. Tokunaga). Living at : Misaki, Prov. of Sagami (Mus. Sc. Coll. Tokyo); Shigajima, Prov. of Chikuzen (Mus. Sc. Coll. Tokyo); Asami-wan, Prov. of Tsushima (Coll. M. Namiye).

CLYPEASTER TESTITUDINARIUS (Gray)

Synonyms : *Echinanthus testitudinarius* Gray.
Echinanthus australasiæ Gray.
Clypeaster tumidulus Müll.
Clypeaster speciosus Verill.
Clypeaster desorii Mich.
Clypeaster australasiæ Mich.

Literature consulted :—

L. Agassiz, Monographies d'Échinodermes vivant et fossiles, 1838–42.

A. Agassiz, Revision of Echini.—Illust. Cat. Mus. Comp. Zool. Harvard Coll., No. VII., 1872–74.

A. Agassiz, Report on the Results of Dredging etc.—Bull. Mus. Comp. Zool. Harvard Coll., Vol. V., 1878, and Vol. VIII., 1880.

K. Martin, Die Tertiärschichten auf Java, 1879–80.

P. M. Duncan, A Revision of the Echinoidea from the Australian Tertiaries.—Quart. Jour. Geol. Soc. London, XLIII., 1887, p. 411.

L. Döderlein, Seeigel von Japan und den Liukiu-Inseln.—Archiv. f. Naturg., I. Bd., 51 Jahr., 1885, p. 73.

F. J. Bell, On the Echinoderms collected during the Voyage of H. M. S. Penguin etc.—Proc. Zool. Soc. London, 1894.

R. Kœhler, Catalogue raisonné des Échinodermes etc.—Mem. Soc. Zool. France ; Tome VIII., 1895, p. 374.

Localities :—

Unten in Okinawa Is., Loochoo (Diluvium) (coll. S. Tokunaga) ; Ishigaki-jima in Loochoo (Diluvium) (coll. S. Tokunaga) ; Java (Miocene) (described by K. Martin) ; Lindenow, Mitchell river in Australia (Tertiary) (described by P. M. Duncan).

Found living in the following places : Hakodate (coll Dall); Japan (Bonn Museum) ; New Holland ; Red Sea ; la Paz ; Sandwich Is.; off Twofold Bay in Australia ; off entrance to Port Philip in Australia ; Gulf of California.

LAGANUM DECAGONALIS (Less).

Synonyms : *Scutella decagonalis* Less.
Legana decagona Less.
Rumphia lesueuri A. Ag.
Polygaster elegans Mich.

Michelinia elegans Duj Hupé.
Laganum decagonum Less.
Laganum lesueuri Val.
Laganum elongatum Agass.
Laganum australe Gray.

Literature consulted:

L. Agassiz, Monographies d'Échinodermes vivant et fossiles, 1838–42.

A. Agassiz, List of Echinoderms sent to different Institutions in Exchange etc.—Bull. Mus. Comp. Zool. Harvard Coll., Vol. I., 1863.

A. Agassiz, Synopsis of the Echinoidea collected by Dr. W. Stimpson on the North Pacific Exploring Expedition etc.—Proc. Acad. Nat. Sc. Philadelphia, No. 7, Decem. 1863, p. 352.

A. Agassiz, Revision of Echini.—Illust. Catalogue Mus. Comp. Zool. Harvard Coll., No. VII., 1872–74.

J. E. Tenison-woods, On some new Australian Echini.—Proc. Linn. Soc. New South Wales, Vol. IV., Part. III., 1879, p. 282.

K. Martin, Die Tertiärschichten auf Java, 1879–80.

J. E. Tenison-woods, On the Habits of some Australian Echini.—Proc. Linn. Soc. New South Wales, Vol. V., 1880–81, p. 193.

A. Agassiz, Report on the Echinoidea dredged by H. M. S. Challenger etc.—Report on the Scientific Results of the Voyage of H. M. S. Challenger, Vol. II., 1881.

G. Pfeffer, Die *Clypeastriden* des Hamburger Museums.—Verhand. Naturw. Vereins von 1880, 1881.

L. Döderlein, Seeigel von Japan und den Liukiu-Inseln. —Archiv. f. Naturg., I Bd., 51 Jahrg., 1885, p. 73.

Jack and Etheridge, Geology and Palæontology of Queensland and New Guinea, 1892.

J. W. Gregory, Further Additions to Australian Fossil Echinoidea.—Geological Magazine, Dec. III., 9, 1892, p. 433.

F. J. Bell, On the Echinoderms collected during the Voyage of H. M. S. 'Penguin' etc.—Proc. Zool. Soc. London, 1894.

R. Koehler, Catalogue raisonné des Échinodermes etc.—Mem. Soc. Zool. de France, Tome VIII., 1895, p. 374.

T. H. Heming, Administration Report of the Marine Survey of India for the official year 1898–99, 1899.

F. P. Bedford, On Echinoderms from Singapore and Malacca. —Proc. Zool. Soc. London, 1900, p. 271.

Localities :—

Ōji and Shinagawa near Tokyo (Diluvium) (coll. S. Tokunaga) ; Usui, Prov. of Shimōsa (Diluvium) (coll. S. Matsuda) ; Shark's Bay in west Australia (Eocene) ; Java (Miocene) (described by K. Martin) ; Yule Island in New Guinea (Lower Pliocene ?).

Living in the following places : Ōmori, Prov. of Musashi (coll. S. Tokunaga) ; Tokyo Bay (coll. L. Döderlein) ; Misaki, Prov. of Sagami (Mus. Sc. Coll. Tokyo), Sagami Bay (coll. L. Döderlein) ; Wakano-ura, Prov. of Kii (Mus. Sc. Coll. Tokyo) ; Ōita, Prov. of Bungo (coll. T. Terasaki) ; Kagoshima Bay (coll. L. Döderlein) ; Loochoo Is. (coll. S. Tokunaga) ; Hōkotō in Formosa (coll. T. Tada) ; Japan (Mus. Copenhagen) ; Hongkong ; Canton ; Philippine Is.; Singapore and Malacca ; New Caledonia ;

Gasper Strait; Freemantle Bay in Australia; Port Denison and Port Jackson in Australia; Bay of Bengal; Torres Strait; Amboyna; Tongatabu; Papeete Harbour in Tahiti; near Flores; Arafura Sea; NE and W of New Guinea.

LAGANUM FUDSIYAMA Död.

Literature consulted:—

L. Döderlein, Seeigel von Japan und den Liukiu-Inseln. —Archiv. f. Naturg., I. Bd., 51 Jahr., 1885, p. 73.

Localities:—

Koshiba, Prov. of Sagami (Pliocene) (coll. S. Tokunaga). Living at: Sagami Sea (Mus. Sc. Coll. Tokyo, also coll. by L. Döderlein); Yamakawa-oki in Kagoshima Bay (coll. K. Mitsukuri and J. Hara).

ECHINARACHNIUS MIRABILIS (Barn).

Synonyms: *Scaphechinus mirabilis* Barn.
Chætodiscus scutella Lütk.
Scutella japonica Mart.
Echinarachnius pacificus Pfeffer.

Literature consulted:—

A. Agassiz, Synopsis of the Echinoids collected by Dr. W. Stimpson on the North Pacific Exploring Expedition etc.—Proc. Acad. Nat. Sc. Philadelphia, No. 7, Decem. 1863, p. 352.

A. Agassiz, Revision of Echini.—Illust. Cat. Mus. Comp. Zool. Harvard Coll., No. VII., 1872–74.

G. PFEFFER, Die Clypeastriden des Hamburger Museums.—Verh. Naturw. Vereins von 1880, 1881.

L. DÖDERLEIN, Seeigel von Japan und den Liukiu-Inseln.—Archiv. f. Naturg., I. Bd., 1885, 51 Jahr., p. 73.

LOCALITIES:—

Jōchū, Prov. of Hitachi (Diluvium) (coll. S. TOKUNAGA); Ōji and Shinagawa near Tokyo (Diluvium) (coll. S. TOKUNAGA); Narita, Prov. of Shimōsa (Diluvium) (coll. S. TOKUNAGA).

Living at: Otaru, Prov. of Nemuro (Mus. Sc. Coll. Tokyo); Hakodate (coll. W. STIMPSON); Asamushi, Prov. of Mutsu (coll. A. IZUKA); Yedo (coll. MARTINS); Kanagawa (coll. MARTINS); Yokohama (Mus. Berlin, Smith. coll.); Misaki, Prov. of Sagami (Mus. Sc. Coll. Tokyo); near Murono-hama, Prov. of Mikawa (coll. T. KŌYAMA); Tomo, Prov. of Bingo (Mus. Sc. Coll. Tokyo); Japan (coll. SALMIN AND WESSEL); San Francisco; Aleutian Is.

ECHINARACHNIUS PARMA (LAM).

Pl. III. Fig. 2.

SYNONYMS: *Scutella parma* LAM.
Scutella trifaria SAY.
Scutella rumphii BLAINV.
Echinodiscus parma BLAINV.
Echinarachnius rumphii AGASS.
Echinarachnius atlanticus GRAY.
Echinarachnius asiaticus MICH.
Echinarachnius australis MICH.
Echinarachnius undulatus MICH.

LITERATURE CONSULTED :—

L. AGASSIZ, Monographies d'Échinodermes vivant et fossiles, 1838–42.

A. AGASSIZ, List of Echinoderms etc.—Bull. Mus. Comp. Zool. Harvard Coll., Vol. I., 1863.

A. AGASSIZ, Synopsis of the Echinoids collected by Dr. W. STIMPSON on the North Exploring Expedition etc.—Proc. Acad. Nat. Sc. Philadelphia, No. 7, Decem. 1863, p. 352.

A. AGASSIZ, Revision of Echini.—Illust. Cat. Mus. Comp. Zool. Harvard Coll., No. VII., 1872–74.

J. E. TENISON-WOODS, On some new Australian Echini.—Proc. Linn. Soc. New South Wales, Vol. IV., Part III., 1879, p. 282.

J. E. TENISON-WOODS, On the Habits of some Australian Echini.—Proc. Linn. Soc. New South Wales, Vol. V., 1880–81, p. 193.

W. L. TOWER, An abnormal Echinoid.—Zool. Anz., Bd. XXIV., No. 640, 1901, p. 188.

LOCALITIES :—

Tagawa-mura and Konade-mura in Tonami-gōri, Prov. of Etchū (Neogene Tertiary) (Imp. Hous. Mus. Tokyo) ; Funabashi-mura in Mishima-gōri, Prov. of Echigo (Neogene Tertiary) (Imp. Hous. Mus. Tokyo) ; Sado Is. (Neogene Tertiary) (Imp. Hous. Mus. Tokyo) ; Australia (Tertiary).

Living in : Japan (Challenger Exp.) ; Kamtschatka ; Avatscha Bay ; Aleutian Is., New Holland ; Vancouver ; Labrador ; New Jersey ; Long Island Sound ; Gay Head ; Nova Scotia ; Nantucket Is.; South Shoals, Mass.; Cape Cod, Mass.; Massachusett Bay ; St. George's Bank ; Trenton Pt. M.; Eastport ; Grand Menan ;

Gaspé; Mingan Is.; Straits' Bell Is.; Gilky Harbour, Meine; N. Australia; Red Sea; India.

ECHINODISCUS FORMOSUS Yosh.

Pl. I. Fig. 1 and 2. Pl. II. Fig. 2.

Literature consulted:—

S. Yoshiwara, On the Geologic Structure of Riukiu Is. etc. —Journ. Coll. Sc. Imp. Univ., Tokyo, Vol. XVI., Part I., 1901, p. 62.

Diam.	Length odd petal.	Width odd petal.	No. of pores in odd petal.	Length ant. paired petal.	Width ant. paired petal.
100 mm.	25 mm.	11.5 mm.	75.	22.5 mm.	11.5 mm.

No. of pores in ant. paired petal.	Length post. paired petal.	Width post. paired petal.	Width of poriferous zone.	Distance from the extremity of petal to lunule.
67.	22.5 mm.	6.7 mm.	4 mm.	5 mm.

Test thin, very slightly raised dorsally; broadly ovoid, widest posteriorly, not so strongly truncated as in *Echinodiscus bioculatus* Ag.; the largest specimen having a diameter of 140 mm.

Apical system nearly central; madreporite central, polygonal; four genital pores existing in the basal plates.

Petals nearly closed, the anterior being the longest.

Lunules two, one in each posterior ambulacral space, large and elliptical; 13.5 mm. in length and 9 mm. in width in a specimen 100 mm. in diameter; the longer axis of the lunule making an angle of about 30° with the median line of the ambulacrum; distance from the extremity of the petal to the lunule only 5 mm.

Peristome central, very small; groove single near peristome, and soon bifurcating.

LOCALITIES :—

Hatto near Kelung, Formosa (Miocene) (coll. S. TOKUNAGA) ; Iriomote-jima, Loochoo Is. (Miocene) (coll. S. TOKUNAGA).

ASTRICLYPEUS INTEGER YOSH.

Pl. I. Figs. 3 and 4. Pl. II. Figs. 3 and 4.

SYNONYM : *Astriclypeus integris* YOSH.

LITERATURE CONSULTED :—

S. YOSHIWARA, On some new Fossil Echinoids of Japan.— Journ. Geol. Soc. Tokyo, Vol. VI., No. 65, 1899.

S. YOSHIWARA, Geologic Structure of Riukiu Curve etc.— Journ. Coll. Sc. Imp. Univ. Tokyo, Vol. XVI. Part I., 1901, p, 61.

Diam.	Length of petal.	Greatest width of petal.	Greatest width of porif. zone.	Greatest width of interp. zone.	Distance of lunule from the extremity of petal.
110 mm.	23 mm.	12 mm.	4 mm.	4 mm.	15 mm.

Test thick, with a circular or elliptical outline, regularly conical, sloping uniformly from vertex to the ambitus.

Apical system similar in form and structure to *Astriclypeus manni* VERILL.

All the petals nearly equal in length and breadth ; poriferous zone broad, broadest near the extremity of petals which are nearly closed at the extremity ; number of pores 37 in a specimen measuring 110 mm. in diameter ; thus differing from living *Astriclypeus*.

Each of five lunules oval, very wide (14 mm.: 9 mm.), constituting the characteristic feature of this species.

Lower surface flat, teeth similar to those in the allied species. Actinal groove and tubercles not preserved.

LOCALITIES :—

Mizuho-mura in Minamitsuru-gōri, Prov. of Kai (Miocene) (coll. T. HIRABAYASHI AND S. TOKUNAGA); Hatto near Kelung, Formosa (Miocene) (coll. S. TOKUNAGA); Iriomoto-jima, Loochoo Is. (Miocene) (S. TOKUKAGA).

ILARIONIA YOSHIWARAI P. DE LORIOL.

LITERATURE CONSULTED :—

P. DE LORIOL, Notes pour servir a l'étude des Échinodermes, II. serie, Fasc. I., 1902.

The genus has hitherto been restricted to the Eocene of Europe and Sind (Asia).

LOCALITY :—

Nishi-ura in Haha-jima, Bonin Is. (Eocene) (coll. S. TOKUNAGA).

PYGURUS ASIATICUS TOK.

Pl. III. Figs. 3–6.

Length.	Width.	Height.	Length ant. paired petal.	Length post. paired petal.	Distance of apical system from the ant. edge.	Distance of peristome from the ant. margin.
88 mm.	80 mm.	63 mm.	46 mm.	52 mm.	36 mm.	20 mm.
60 mm.	60 mm.	44 mm. (?)			28 mm. (?)	

Test large, with undulating marginal outline, grooved anteriorly and broadly ridged posteriorly; very highly conical, concave actinally.

Apical system at the conical apex, eccentric in front.

Ambulacra flush dorsally, unequal; open petaloid part lance-

olate, tending to converge on the extremity, and continued over the margin as narrow lines of small pores in pairs; the greatest width of this part in a specimen having a diameter of 88 mm. measured at about $\frac{1}{3}$ distance from the apical system to the extremity, and respectively 7 mm., 11.5 mm., and 10.5 mm. in the odd, anterior paired and posterior paired ambulacra; poriferous zone about 3.5 mm. wide in the paired ambulacra, which is about $\frac{1}{3}$ as wide as the width of the whole petal; pores very numerous, about 90 in the anterior paired ambulacra in the petaloid part; width at the extremity of the petaloid part only 3.5 mm. in the paired ones; narrow lines of pairs of small pores passing over the ambital margin and continued to the peristome; ambulacral portion of the actinal side deeply and broadly grooved.

Peristome eccentric, pushed anteriorly; periproct close to the posterior edge of the actinal side of the test.

Locality :—

Torinosu in Sakawa, Prov. of Tosa (probably Cretaceous) (coll. B. Kotô).

ECHINOLAMPAS YOSHIWARAI P. de Loriol.

Literature consulted :—

P. de Loriol, Notes pour servir a l'étude des Échinodermes, II. serie, Fasc. I., 1902.

Locality :—

Kanaya, Prov. of Kazusa (Pliocene) (coll. S. Hatta & S. Tokunaga).

TOXASTER TOSAENSIS P. DE LORIOL.

LITERATURE CONSULTED :—

P. DE LORIOL, Notes pour servir a l'étude des Échinodermes, II. serie, Fasc. I., 1902.

LOCALITY :—

Torinosu in Sakawa, Prov. of Tosa (Cretaceous) (brought to S. TOKUNAGA).

LINTHIA NIPPONICA YOSH.

Pl. I. Figs. 5–7.—Pl. III. Fig. 1.

LITERATURE CONSULTED :—

S. YOSHIWARA, On some new Fossil Echinoids of Japan.—Journ. Geol. Soc. Tokyo, Vol. VI. No. 65, 1899.

Length.	Width.	Height.	Length of odd petal.	Length of ant. paired petal.	Length of post. paired petal.	Width of petal.	Width of interp. area.
87 mm.	86 mm.	38 mm.	48 mm.	46 mm.	32 mm.	7 mm.	2.2 mm
64 mm.	60 mm.	25 mm.	31 mm.	31 mm.	20 mm.	5.5 mm.	2 mm.
59 mm.	58 mm.	21 mm.		25 mm.	18 mm.	5.5 mm.	2 mm.
58 mm.	54.5 mm.	23 mm.	27 mm.	26 mm.	18 mm.	5.5 mm.	2 mm.
58 mm.	57 mm.	22 mm.					
64 mm.	64 mm.	30 mm.					

Test thick, large, somewhat cordiform; tumid dorsally, slightly concave actinally, a portion of apical system sunken; anterior groove very wide and shallow; posterior extremity vertically truncated.

Apical system and vertex nearly central or slightly eccentric towards the front in old specimens: basal plates four, perforated; madreporite large, central.

Ambulacra diverse; the anterior one in a broad groove, pores not obliterated, fewer in number than those of other petals, round and small; interporiferous area thickly provided with secondary tubercles. The lateral ambulacra sunken and slender; the anterior making an angle of 35° with the odd ambulacrum, straight and reaching almost to the ambitus; the posterior shortest and also straight. Poriferous zone wide, and the interporiferous area shallow, occupying nearly one third of the width of the petals. It is to be noticed that all the petals are nearly straight and retain the same width throughout their whole length, except near the vertex. The number of pores in the anterior lateral petal 39, and in the posterior one 28 in the largest specimen; interporiferous area of the paired ambulacra very narrow, leaving no space for tubercles.

Peripetalous fasciole narrow, angular with a deep reëntering angle in the anterior and posterior lateral ambulacra; undulating at the anterior ambulacrum and deeply entering outside, but almost nearly straight in the posterior interambulacrum. The lateral fasciole sloping toward the ambitus, abruptly curved near the posterior edge, and passing under the anus.

Actinal surface flat; actinostome situated at about one fourth the distance from the anterior edge. Anus elliptical, visible only in a side view. The tubercles of the abactinal surface very small and uniform in size, those of the actinal surface large and also uniform.

The living species of *Linthia* are now found in Australia, Tasmania, the Arafura Sea and the West Indies; fossils have been discovered in the Cretaceous of Europe, Africa, N. America, and in the Tertiary of Europe, Africa, W. Sind and the West Indies. The general outline, the shallow anterior groove, and the long antero-lateral petals, are characters which justify us in placing this species under *Linthia* rather than under *Schizaster*.

LOCALITIES :—

Kanazawa, Prov. of Kaga (probably Pliocene) (Mus. Sc. Coll. Tokyo); Sakae, Prov. of Shinano (probably Pliocene) (Mus. Sc. Coll. Tokyo); Ikari, Prov. of Shinano (probably Pliocene) (Mus. Higher Normal School Tokyo); Tsurushi and Hazzaki, Prov. of Hitachi (Pliocene) (coll. C. KOCHIBE); Hidaka-mura in Kamimizuta-gōri, Prov. of Shinano (probably Pliocene) (Tokyo Imp. Hous. Mus.); Anrakujō-mura in Mogami-gōri, Prov. of Uzen (Pliocene) (coll. K. INOUE); Asahigawa-mura in Minamiakita-gōri, Prov. of Ugo (coll. D. SATŌ).

SCHIZASTER RECTICANALIS YOSH.

Pl. IV. Figs. 1–3.

LITERATURE CONSULTED :—

S. YOSHIWARA, On some new Fossil Echinoids of Japan.—Journ. Geol. Soc. Tokyo, Vol. VIII. No. 65, 1899.

Long. diam.	Trans. diam.	Height.	Length of odd petal.	Length of ant. paired petal.	Length of post. paired petal.
48 mm.	53 mm.	36 mm.	30 mm.	30 mm.	12 mm.

Test thick, cordiform, angular, posterior side not so elongated as in many other species; anterior groove shallow.

Apical system almost central; from the vertex situated behind the apical system the test curves suddenly to the truncated posterior extremity.

Odd ambulacrum very wide and shallow with about 23 small double pores. Antero-lateral ambulacra broad having about 35 pores, almost straight, not closed at the extremities, and very long, reaching almost to the ambitus; postero-lateral ambulacra

very short, curved, round and closed at the extremities, number of pores 21.

Peripetalous fasciole strongly recurved in postero-lateral interambulacra, and retreating far from the centre in the odd posterior interambulacrum. Lateral fasciole meeting with the former at the extremity of the antero-lateral ambulacrum.

Actinostome situated about $\frac{1}{3}$ diameter distance from anterior edge.

Tubercles on abactinal side very small, but large on actinal side.

This species is characterized by having posteriorly a not very elongate shell, a shallow anterior groove and straight antero-lateral ambulacra.

Locality :—

River bank of the Saigawa near Kanazawa, Prov. of Kaga (Pliocene) (brought to O. Yoshida).

SCHIZASTER NUMMULITICUS Tok.

Pl. IV. Figs. 4–6.

Literature consulted :—

S. Yoshiwara, Geological Age of Bonin Is. etc.—Geol. Magazine, London, Dec. IV., Vol. IX., 1902, p. 298.

Long. diam.	Trans. diam.	Height.	Length of odd petal.	Length of ant. paired petal.	Length of post. paired petal.
61.5 mm.	60 mm.	46 mm.	35 mm.	31 mm.	15 mm.
58.5 mm.	56 mm.	44 mm.		29.5 mm.	15 mm.

Test thin, high, cordiform; the broadest part lying a little anterior to the apical system, and the highest point being in that system; anterior groove broad and deep; median posterior keel not very prominent.

Apical system posteriorly 42 mm. wide in a test 61.5 mm. long, separate from the anterior edge; madreporite extending centrally and posteriorly; only one large genital pore in the basal plate adjoining the right postero-lateral interambulacrum and three small indistinct pores in the plates adjoining the remaining lateral ambulacra.

Odd ambulacrum with distinct paired pores on each side; these are 30 in number in a test 61.5 mm. long: interporiferous area closely covered with granules. Paired ambulacra petaloid, deeply sunken, bare; petals not increasing in width at the extremity as in *Schizaster japonicus* A. Ag., but closely converging, the greatest width being in their middle portion; anterior paired flexuous, extending forwards, pores 38 in number; posterior petals forming an angle of about 75° between them, pores 22.

Peristome eccentric towards front, 12 mm. from the anterior edge, semilunar, with a projecting posterior labrum. Posterior paired ambulacra of actinal side very narrow. Periproct in a truncated posterior margin, which is deeply sunken.

Peripetalous fasciole narrow, deeply re-entering at the antero-paired interambulacral spaces, running almost on the edge of petal in the postero-paird interambulacra, and slightly re-entering at the posterior interambulacrum. Lateral fasciole narrow, meeting with the former at some distance behind the extremity of the antero-lateral petals.

Tubercles close, larger on actinal side.

Locality :—

Nishi-ura, in Haha-jima, Bonin Is. (Eocene) (coll. S. Tokunaga).

PRENASTER BONINENSIS P. DE LORIOL.

LITERATURE CONSULTED :—

P. DE LORIOL, Notes pour servir a l'étude des Échinodermes, II. serie, Fasc. I., 1902.

The genus has hitherto been restricted to the Eocene of Europe, N. Africa and W. Sind (Asia).

LOCALITY :—

Nishi-ura in Haha-jima, Bonin Is. (Eocene) (coll. S. TOKUNAGA).

HYPSOSPATANGUS JAPONICUS P. DE LORIOL.

LITERATURE CONSULTED :—

P. DE LORIOL, Notes pour servir a l'étude des Échinodermes, II. serie, Fasc. I., 1902.

LOCALITIES :—

Wakkanai, Prov. of Kitami (Neogene Tertiary) (coll. K. JIMBŌ) ; near Sanbōshi, Prov. of Kushiro (Neogene Tertiary) (Tokyo Imp. Hous. Museum).

BRISSOPSIS LUZONICA (GRAY).

SYNONYM : *Kleinia luzonica* GRAY.

LITERATURE CONSULTED :—

J. E. GRAY, Descriptions of some new Genera and Species of *Spatangidæ* in the British Museum.—Ann. Mag. Nat. Hist., II. series, Vol. 7, No. 38, 1851, p. 130.

A. Agassiz, Revision of Echini.—Illust. Cat. Mus. Comp. Zool. Harvard Coll., No. VII., 1872–74.

K. Martin, Die Tertiärschichten auf Java, 1879–80.

A. Agassiz, Report on the Echinoidea dredged by H. M. S. Challenger during the years 1873–76.—Report on the Scientific Results of the Voyage of H. M. S. Challenger, Vol. II., 1881.

L. Döderlein, Seeigel von Japan und den Liukiu-Inseln. —Archiv. f. Naturg., I. Bd., 51 Jahr., 1885, p. 73.

A. R. S. Anderson, On the Echinoidea collected during the Season 1893–94.—Natural History Notes from H. M. Indian Marine Survey Steamer "Investigator" etc., Series II. No. 16.

R. Koehler, Catalogue raisonné des Échinodermes recuilles par K. Korotnev aux iles de la Sonde.—Mem. Soc. Zool. de France, Tome VIII., 1895, p. 374.

T. H. Heming, Administration Report of the Marine Survey of India for the official year 1898–99, 1899.

Localities :—

Shiroyama near Taira, Prov. of Iwaki (Neogene Tertiary) (coll. D. Satō) ; Java (Miocene) (described by K. Martin).

Found living in the following localities : Sagami Sea (Mus. Sc. Coll. Tokyo, also coll. by L. Döderlein) ; Formosa (Mus. Godeff) ; Philippine Is ; Siam ; Banca Strait ; Coromandel coast ; North of Timor ; West of New Guinea ; New Zealand ; New Caledonia ; East Indies ; Indian Ocean.

SPINES OF INDETERMINABLE SPECIES OF CIDARIS AND PSEUDOCIDARIS.

CIDARIS *a* sp.

Pl. II. Fig. 7.

Spine about 14 mm. long and 3.5 mm. in greatest diameter, club-shaped, narrowed near the base, where it is quite smooth; shaft striated with about 9 or more longitudinal bands which are all coarsely granulated; some striations do not reach the base of the shaft, but are replaced with other shorter bands. Base plain and hollowed.

Locality :—

Iwasa in Tokano, Takaoka-gōri, Prov. of Tosa (probably Cretaceous).

PSEUDOCIDARIS *a* sp.

Pl. II. Fig. 11.

Spine 15.5 mm. long and 12.5 mm. in greatest diameter, pear-shaped, widest at the lower part and acuminated near apex with no narrowed or smooth portion near the base; longitudinally closely striated, the number of striations being more than 40, and each striation being very closely granulated, so that it is usually impossible to trace the lines of striation near the apex; granules more than 15 on each striation.

Locality :—

Torinosu in Sakawa, Prov. of Tosa (probably Cretaceous).

PSEUDOCIDARIS β sp.

Pl. II. Fig. 12.

LITERATURE CONSULTED :—

E. NAUMANN UND M. NEUMAYR Zur Geologie und Paläontologie von Japan.—Denk. Mathem.-Naturw. Classe Kaiser. Akad. der Wissenschaften, Bd. LVII., 1890.

Spine 19 mm. long and 11.5 mm. in greatest diameter, club-shaped, acuminated near apex and narrowed near the base, very finely striated longitudinally; each striation closely granulated, the granules being larger and coarser near the apex.

This specimen was collected from the same locality as the preceding one, and probably belongs to the same species which E. NAUMANN and M. NEUMAYR named *Cidaris cfr. glandifera* GOLDF.

CIDARIS β sp.

Pl. II. Fig. 8.

A spine 25 mm. long and 13.5 mm. in greatest diameter, rather cuneiform, the greatest diameter lying nearer the summit than in the preceding species (*Pseudocidaris α sp. and β sp.*), narrowed near the base; striations and granulations similar to the above.

LOCALITY :—

Tarusawa near Itsukaichi, Prov. of Musashi (Jurassic or Cretaceous).

CIDARIS δ sp.

Pl. II. Fig. 10.

A very large specimen 28 mm. in greatest diameter near the summit, club-shaped, abruptly narrowed at the smooth portion of the collar; granules very numerous, longitudinal striations almost unrecognizable on account of the bad state of preservation.

Locality :—

Itsukaichi, Prov. of Musashi (Jurassic or Cretaceous).

CIDARIS ε sp.

Pl. II. Fig. 9.

Literature consulted:—

E. Naumann und M. Neumayr, Zur Geologie und Paläontologie von Japan.—Denk. Mathem.-Naturwiss. Classe Kaiser. Akad. der Wissenschaften, Bd. LVII., 1890.

This is a spine broken near the base, club-shaped, 21 mm. in greatest diameter near the base; the longitudinal striations are very numerous, and their granules close together; tolerably regular fine horizontal striations are traceable on the whole surface. This spine probably belongs to the same species as the specimen already described as a new species of *Cidaris* by E. Naumann and M. Neumayr.

Locality :—

Yokodani in Shiraishizawa, Prov. of Tosa (probably Cretaceous).

PLATE I.

Plate I.

Figs. 1-2. Abactinal view of *Echinodiscus formosus* Yoshi. From Hatto near Kelung, Formosa.

Fig. 3. Abactinal view of *Astriclypeus integer* Yoshi. From Mizuho-mura in Minamitsuru-gōri, Prov. of Kai.

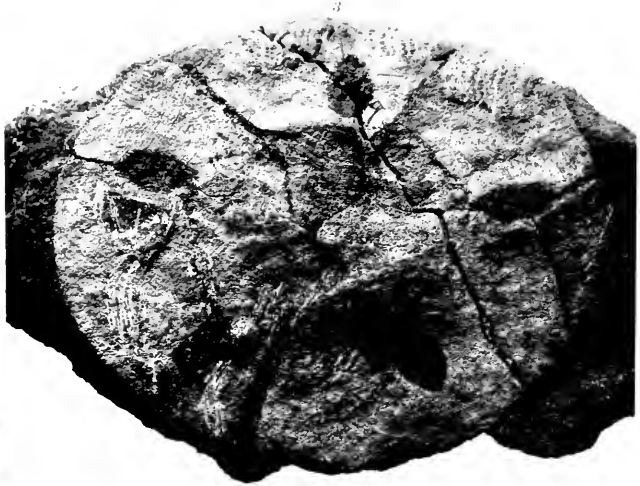
Fig. 4. Abactinal view of *Astriclypeus integer* Yoshi. From Hatto near Kelung, Formosa. (Surface eroded).

Fig. 5. Abactinal view of *Linthia nipponica* Yoshi. From Hidaka-mura in Kamimizuta-gōri, Prov. of Shinano.

Fig. 6. Abactinal view of *Linthia nipponica* Yoshi. From Sakae-mura, Prov. of Shinano.

Fig. 7. Actinal view of another specimen of *Linthia nipponica* Yoshi. From Sakae-mura.

(All figures in natural size).



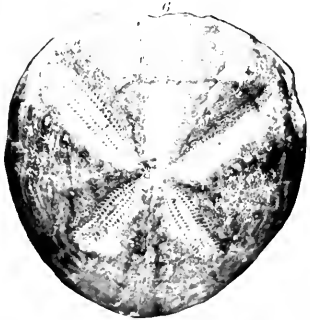


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PLATE II.

Plate II.

Fig. 1. Abactinal view of *Salenia hakkaidoensis* P. DE LORIOL. From Yūbari, Prov. of Ishikari. 2 ×

Fig. 2. Abactinal view of *Ehinodiscus formosus* YOSH. Showing petals and lunules. 2 ×

Fig. 3. Abactinal view of *Astriclypeus integer* YOSH. Showing petals and lunules.

Fig. 4. Actinal view of *Astriclypeus integer* YOSH. Showing lunules and teeth. (Surface eroded).

Fig. 5. Actinal view of *Fibularia acuta* YOSH. Drawn from a living specimen. (Magnified).

Fig. 6. Abactinal view of *Fibularia acuta* YOSH. Drawn from a living specimen. (Magnified).

Fig. 7. Spine of *Cidaris α sp.* From Iwasa, Prov. of Tosa. (Magnified).

Fig. 8. Spine of *Cidaris β sp.* From Itsukaichi, Prov. of Musashi.

Fig. 9. Spine of *Cidaris ε sp.* From Yokodani, Prov. of Tosa.

Fig. 10. Spine of *Cidaris δ sp.* From Itsukaichi, Prov. of Musashi (Restored).

Fig. 11. Spine of *Pseudocidaris α sp.* From Torinosu, Prov. of Tosa.

Fig. 12. Spine of *Pseudocidaris β sp.* From Torinosu, Prov. of Tosa.

(All figures except figs. 1, 2, 5, 6 and 7, in natural size).

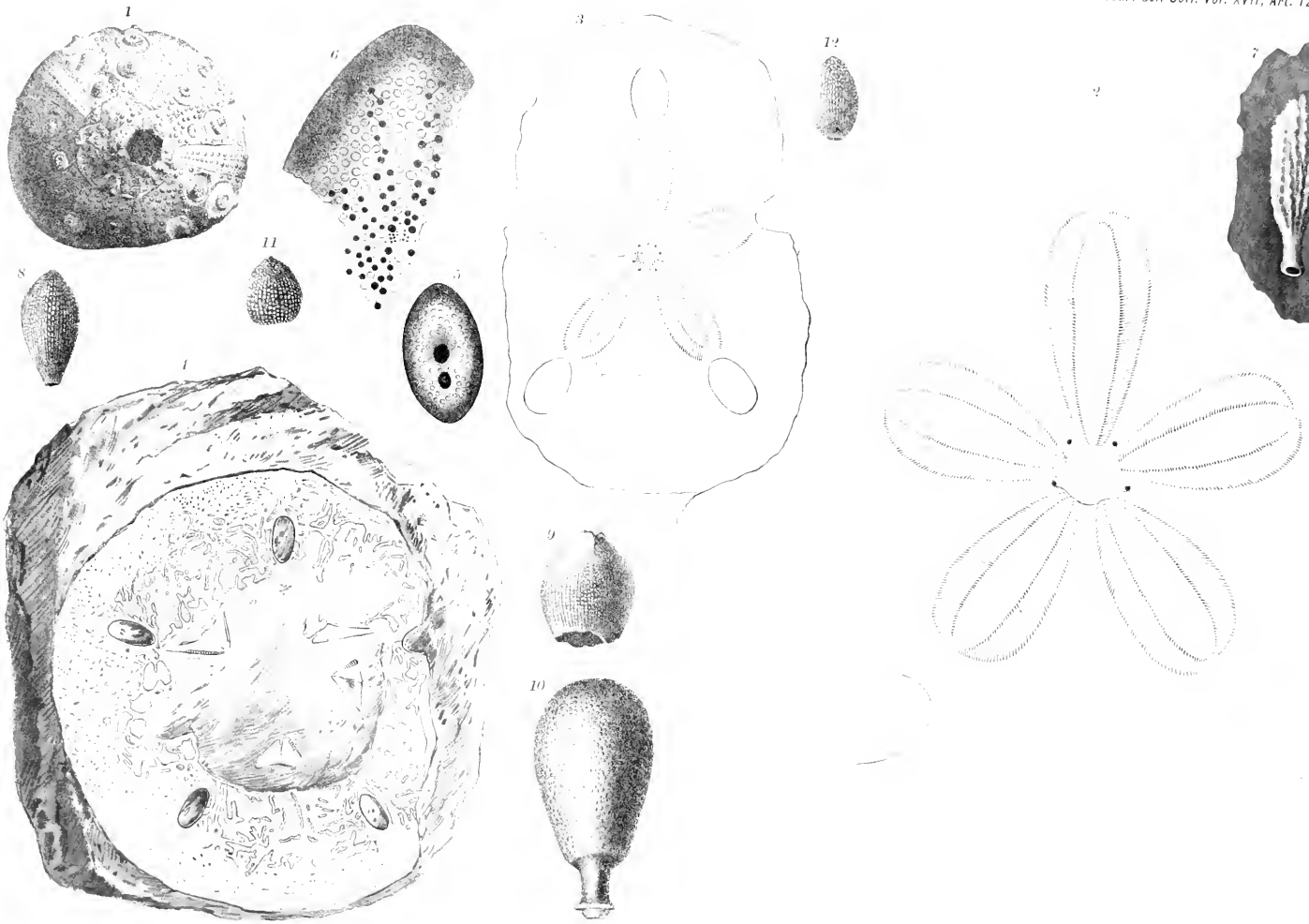

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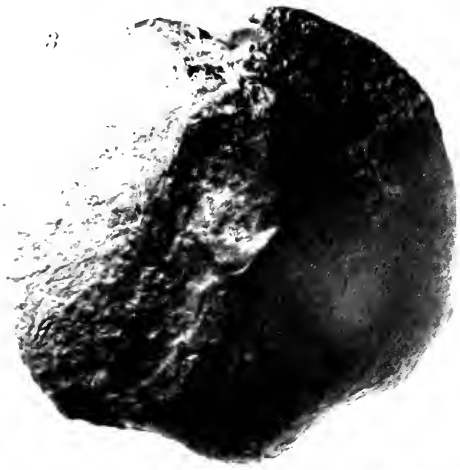
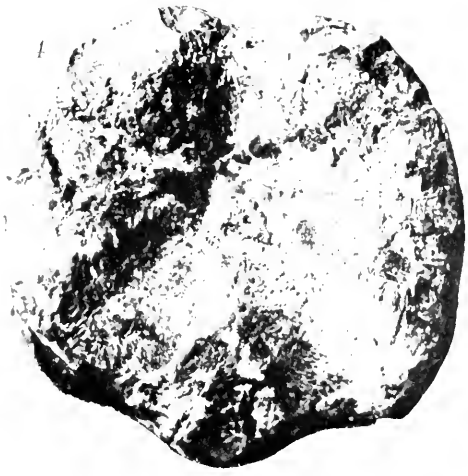
PLATE III.

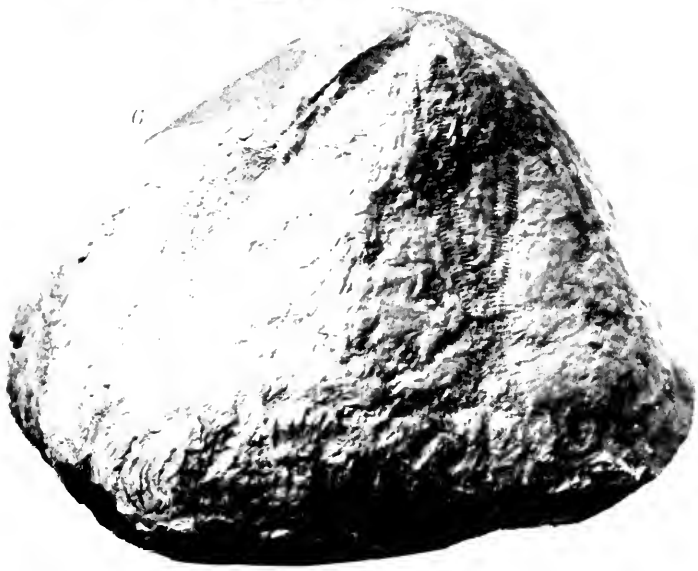
Plate III.

Fig. 1. Cast of *Linthia nipponica* YOSH. From Sakae, Prov. of Shinano.
Fig. 2. Abactinal view of *Echinarachnius parma* LAM. From Sado Is.
Figs. 3–5. Abactinal, actinal and profile views of *Pygurus asiaticus* TOK. From Sakawa, Prov. of Tosa. (Apical portion destructed).
Fig. 6. Profile view of another specimen of *Pygurus asiaticus* TOK.

(All figures in natural size).







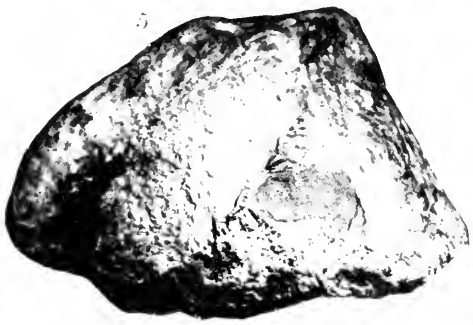


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PLATE IV.

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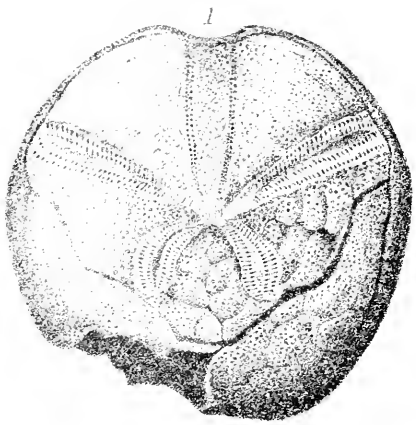
Figs. 1–3. Abactinal, actinal and profile views of *Schizaster rectecanalis* Yosh. From the environs of Kanazawa, Prov. of Kaga.

Figs. 4–5. Abactinal and actinal views of *Schizaster nummuliticus* Tok. From Bonin Is.

Fig. 6. Same specimen of *Schizaster nummuliticus* Tok., showing the periproctal portion.

(All figures in natural size).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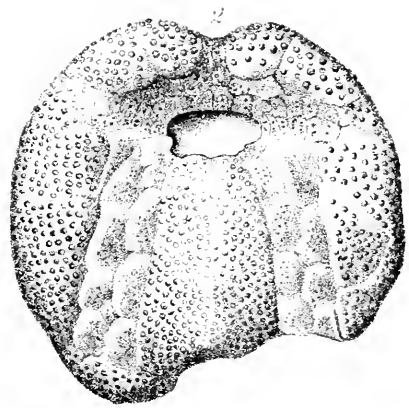
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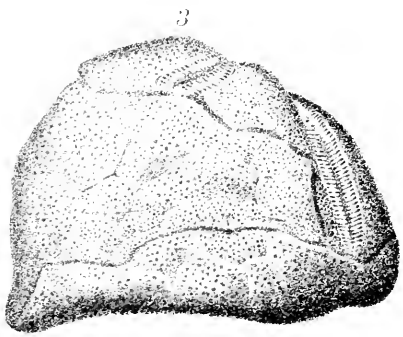
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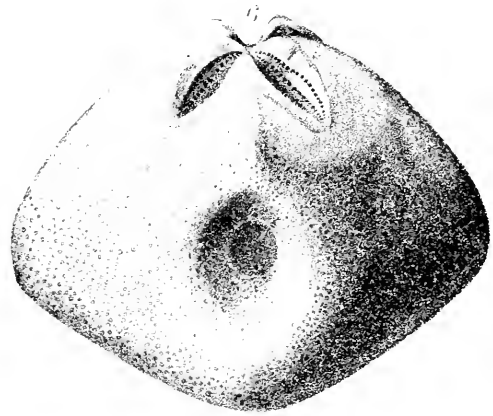
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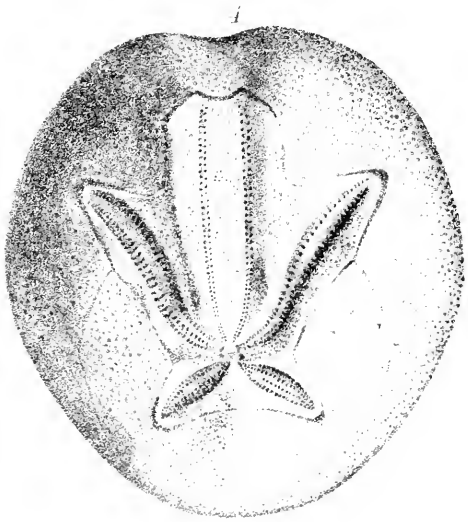
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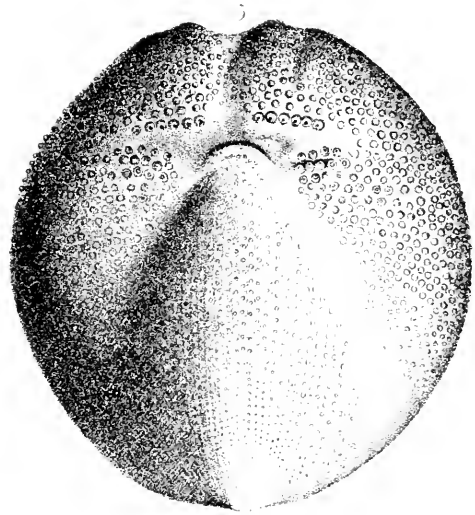
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Published Articles of Vol. XVI.

Art. 1.—Notes on the Raised Coral Reefs in the Islands of the Riukiu Curve. (*With* 2 *Plates*). By S. Yoshiwara.—Publ. Dec. 8th, 1901.

Art. 2.—Geologic Structure of the Riukiu Curve, and its Relation to the Northern Part of Formosa. (*With* 5 *Plates*). By S. Yoshiwara.—Publ. Dec. 8th, 1901.

Art. 3.—Corallinæ veræ Japonicæ. (*With* 7 *plates*). By K. Yendo.—Publ. May 30th, 1902.

Art. 4.—Revisio Umbelliferarum Japonicum. (*With* 3 *plates*). By Y. Yabe.—Publ. May 30th, 1902.

Art. 5.—Revisio Alni Specierum Japonicarum. (*With* 4 *plates*). By J. Matsumura.—Publ. May 30th, 1902.

Art. 6.—Notes on a New Fossil Mammal. (*With* 3 *plates*). By S. Yoshiwara and I. Iwasaki.—Publ. June 30th, 1902.

Art. 7, now under preparation.

Published Articles of Vol. XVII.

Art. 1.—Ammonium and other Imidosulphites. By E. Divers and M. Ogawa.—Publ. Dec. 13th, 1901.

Art. 2.—Nitrilosulphates. By E. Divers and T. Haga.—Publ. Dec. 13th, 1901.

Art. 3.—Contributions to the Embryology of Amphibia:—The Mode of Blastopore Closure and the Position of the Embryonic Body. (*With* 4 *plates*). By S. Ikeda.—Publ. June 12th, 1902.

Art. 4.—On the Development of *Lingula anatina*. (*With* 8 *plates*). By N. Yatsu.—Publ. June 12th, 1902.

Art. 5.—Notes on Histology of *Lingula anatina*. (*With* 2 *plates*). By N. Yatsu.—Publ. June 12th, 1902.

Art. 6.—On Some Fossils from the Islands of Formosa and Riukiu. (*With* 4 *plates*). By R. B. Newton and R. Holland.—Publ. May 28th, 1902.

Art. 7.—Some New Scyphomedusæ of Japan. (*With* 2 *plates*). By K. Kishinouye.—Publ. June 26th, 1902.

Art. 8.—Preparation of Sulphamide from Ammonium Amidosulphite. By E. Divers and M. Ogawa.—Publ. June 23rd, 1902.

Art. 9.—Studies on the Hexactinellida. Contribution II. (The genera *Corbitella* and *Heterotella*). (*With* 1 *plate*). By I. Ijima.—Publ. July 3rd, 1902.

Art. 10.—Studies on the Parasitism of *Buckleya Quadriala*, B. et H., a Santalaceous Parasite, and on the Structure of its Haustorium. (*With* 1 *plate*). By S. Kusano.—Publ. Aug. 11th, 1902.

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第拾七冊第拾壹編

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Art. 11.—Observations on the Japanese Palolo, *Ceratocephale osawai*, n. sp. (*With* 2 *plates*). By A. IZUKA.—Publ. July 1st, 1903.

Art. 12, now under preparation.

Published Articles of Vol. XVIII.

Art. 1.—Studies on the Hexactinellida. Contribution III. (*Placosoma*, a New Euplectellid; *Leucopsacidæ* and *Caulophacidæ*). (*With* 8 *plates*). By I. IJIMA.—Publ. May 5th, 1903.

Art. 2.—Cretaceous Cephalopoda from the Hokkaido. Part I. *Lytoceras*, *Gaudryceras* and *Tetragonites*. (*With* 7 *plates*). By H. YABE.—Publ. June 8th, 1903.

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Art. 4, now under preparation.

Published Articles of Vol. XIX.

Art. 1.—An Orographic Sketch of Korea. (*With* 4 *plates*). By B. KOTO.—Publ. April, 23rd, 1903.

Articles 2—4, now under press.

Art. 5.—Ueber die im Bereiche der rationalen Zahlen Abel'schen Zahlkörper. Von T. TAKAGI.—Publ. May 21st, 1903.

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With the Article under this cover (**Vol. XVII., Art. 12**), **Vol. XVII.** of the Journal is closed, so that it may now be bound. Appended to this Article are the title page and the contents to that volume.

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Published Articles of Vol. XVIII.

Art. 1.—Studies on the Hexactinellida. Contribution III. (*Placosoma*, a New Euplectellid; *Leucopsacidæ* and *Caulophacidæ*). (*With* 8 *plates*). By I. IJIMA.—Publ. May 5th, 1903.

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Art. 6.—Rigidity of Rocks and Hysteresis Function. (*With* 22 *plates*). By S. KUSAKABE.—Publ. June 21st, 1903.

Art. 7.—Ueber einige Anhydrobasen aus Diaminen der Fettreihe. Von. T. Haga und R. MAJIMA.—Publ. June 25th, 1903.

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