On the Nature of the Excretory Processes in Marine Polyzoa.

By

Sidney F. Harmer, M.A., B.Sc.,
Fellow and Assistant Tutor of King's College, Cambridge.

With Plates II and III.

The observations described below were made during an occupation of the Cambridge University table at the Zoological Station at Naples, in the course of the Easter vacation, 1891. The results obtained by Kowalevsky[1] on the excretion of carminate of ammonia and of indigo-carmine in various Invertebrates had suggested an inquiry, by means of the same methods, into the physiological meaning of the periodic formation of "brown bodies" in Ectoproctous Polyzoa, since there seemed reason to suppose that this process might serve as a means of excretion, a view which has been most definitely formulated by Ostroumoff.[2] My experiments made with artificial pigments confirm the view that the marine Ectoprocta are not provided with definite nephridia; and appear to show that the excretory processes are carried on principally by the "brown bodies," the funicular (connective) tissue, and the free mesoderm-cells contained in the meshes of the latter.

The species which, by reason of their transparency and of their abundance at Naples, were principally employed for these

[1] A. Kowalevsky, "Ein Beitrag zur Kennt. d. Excretionsorgane," 'Biolog. Centralblatt,' ix, 1889–90, pp. 33, 65, 127.

[2] A. A. Ostroumoff, "Cont. à l'Ét. Zool. et Morphol. des Bryozoaires du Golfe de Sébastopol," 'Arch. Slaves de Biol.,' t. ii, 1886, p. 339.

observations were Flustra papyrea, Pall., Bugula neritina, Linn., and Bugula avicularia, Linn.[1] Each of these species was exposed to the action of the following pigments dissolved in sea-water—(1) Indigo-carmine; (2) carminate of ammonia; (3) Bismarck-brown; and was further placed in sea-water containing carmine-powder in suspension. After leaving the animals for various periods, as explained below, in the solution of the pigment, the colonies were transferred to pure sea-water in a tank, and the changes undergone by the pigment were examined in the living animal from day to day. It may be at once remarked that the colonies remained living and apparently healthy, even after prolonged immersion in solutions of the pigment.

I have to acknowledge my indebtedness to Dr. Paul Mayer for the suggestion that Bismarck-brown might be expected to yield interesting results, a suggestion which I followed up with some success. Kowalevsky[2] has, however, pointed out that Bismarck-brown may be used with advantage in investigations of this kind, and implies that it is taken up by those parts of the excretory organs which absorb carminate of ammonia, a result which is largely confirmed by my own observations.

One of my most interesting results has been the observation of the fact that the tissues of different forms, even of two species of the same genus, do not necessarily react in the same

[1] For the synonymy of these species see Miss Jelly's 'Synonymic Catalogue of the Recent Marine Bryozoa' (London, 1889).

My specimens of Flustra papyrea correspond very closely with F. papyrea, var. Mazeli, Marion ("Draguages au large de Marseille," 'Ann. des Sci. Nat.,' 6e sér.; 'Zool.,' viii, 1879, article No. 7, p. 33). It appears to me that Marion's statement that the forms described by Busk ('Cat. of Marine Polyzoa, ... Brit. Museum,' part i, 1852) were composed of narrow linear segments was due to a misapprehension of the fact that the numbers of Busk's plates xlix and l have been transposed, as pointed out by Waters ('Ann. Mag. Nat. Hist.,' ser. 5, iii, 1879, p. 119); and that the "var. Mazeli" does not really differ from the normal form of F. papyrea.

For further remarks on the species see Hincks, 'Brit. Mar. Polyzoa,' vol. i, p. 124.

[2] Loc. cit., p. 76.

way to the action of the same pigment; and that the taking up of the pigment by particular tissues may have a definite relation to the normal pigmentation of those tissues.

The bearing of this fact on the results arrived at by Eisig,[1] with regard to the excretory value of certain normal pigments, will be subsequently pointed out.

I. The Normal Characters of the Living Zoœcium in the Species investigated.

a. Bugula neritina, L.

This species, which is well figured in its natural colour by Costa,[2] is very common at Naples; the specimens examined by me being, as described by most authors, invariably destitute of avicularia.[3] The colony, as is obvious from Costa's figures, possesses a dark colour which varies from yellowish brown to purple.[4] The characteristic colour is almost entirely due to the presence of a pigmented funicular tissue, already noticed by Costa.[5] This tissue is shown in Pl. III, fig. 17.

It will be noticed that the funicular tissue agrees with that

[1] H. Eisig, "Monographie d. Capitelliden d. Golfes v. Neapel," 'Fauna and Flora . . . . G. v. Neapel,' xvi Monogr., 1887 (see especially the "Physiologischer Theil").

[2] O. G. Costa, "Fauna del Regno di Napoli," 'Zoofiti,' Napoli, 1838, Tav. v, fig. 1 [belonging to the chapter headed "Ordine III. Cellariee"].

[3] A. W. Waters ('Ann. and Mag. Nat. Hist.,' ser. 5, vol. xx, 1887) describes an Australian form of this species in which numerous avicularia are present.

[4] For an account of the pigments of B. neritina see MacMunn, 'Quart. Journ. Micr. Sci.,' xxx, 1890, p. 78, and the reference there given to Krukenberg.

[5] Costa, loc. cit., p. 15. The zoœcia are described as being "ripiene di sostanza granellosa, tinta di rosso di tutte le gradazioni."

[6] See W. J. Vigelius, 'Biolog. Centralblatt,' iii, 1883-4, p. 708; and his larger work, "Die Bryozoen . . . . 'Willem Barents,'" 'Bijd. tot de Dierkunde,' 11e Aflev., 1884 ("Parenchymgewebe"), and other writers.

of other marine Ectoprocta[6] in forming a continuous network stretching through the cavity of the zoœcium. It usually forms a thin layer on the inner side of the ectocyst, and another thin layer on the outer surface of the alimentary canal and tentacle-sheath, the two layers being connected by the network which traverses the body-cavity. A special region of this network is often distinguishable as a definite tract which passes from the apex of the cæcum of the stomach, in company with the colourless retractor muscles, to the proximal end of the zoœcium. In young zoœcia the network is much denser, and the young polypide-buds are constantly enveloped by a specially dense concentration of the network. The pigment, which is readily soluble in fresh water, is contained for the most part in numerous minute granules scattered through the funicular tissue, and occurs in two principal colours—purple and yellowish brown, both of which may occur in the same zoœcium.

A similar pigment occurs in the growing-points (which are very densely pigmented), in the tentacles, round the apertures,[1] and in the ovicells (the young ones being, like the growing-points, densely pigmented). It is probable that the pigment in all these cases is contained in the funicular tissue, although some of it may possibly be in the ectoderm.

The meshes of the funicular tissue contain numerous transparent cells, which are colourless or faintly yellow. These cells consist of an aggregation of vacuoles filled with a transparent fluid, and they are often produced into long, fine processes (fig. 19[2]), by means of which they are suspended in the funicular network. They play an important part in the absorption of indigo-carmine, and although not exactly similar to ordinary white blood-corpuscles, they will be alluded to in the remainder of this paper as the leucocytes.

These so-called leucocytes have been well figured by

[1] S. delle Chiaje ('Mem. sulla Storia e Notomia degli Animali senza Vertebre del Regno di Napoli,' vol. iv, Napoli, 1829, p. 147): "Osculis margine subfusco cinctis."

[2] Representing these cells after exposure to the action of indigo-carmine.

Claparède[1] in Bugula avicularia, who describes them as "gelbe Tropfen" (p. 153), and states that they only occur in zooecia in which the polypide has degenerated. My own observations on the same species show that they are of normal occurrence in all the zooecia, and Claparède's failure to find them universally present was probably due to the fact that they are often colourless in the young zooecia. The yellow colour of these cells, so obvious in Claparède's figures, probably implies that they had taken up excretory pigments from other tissues during the degeneration of the polypide; and Claparède himself regards them as being excretory in nature.

These cells are mentioned by various other authors, as by Joliet,[2] who states that they are of very general occurrence in the Polyzoa. They are no doubt identical with some of the structures alluded to by Smitt[3] as "Fettkroppar" or as "floating cells," and are described by Cuénot[4] as "amibocytes." They are described by Prouho,[5] in the larva of Flustrella hispida, as very refringent aggregations of spherules, of a mulberry-like appearance, a description which I can confirm from my own observations.

Most of the alimentary canal has a pale, diffuse, yellowish-brown colour; but the walls of the stomach and cæcum contain numerous pigmented (brown) granules. A clear space, devoid of granules, always occurs at the base of the cæcum, in the position shown in fig. 16, which, however, refers to the next species.

[1] "Beitr. z. Anat. u. Entw. d. Seebryozoen," 'Zeits. f. wiss. Zool.,' xxi, 1870, Taf. viii, figs. 1 B, 1 C, *t*.

[2] L. Joliet, "Cont. à l'Hist. des Bryozoaires des Côtes de France," 'Arch. de Zool. Exp. et Gén.,' t. vi, 1877, p. 233.

[3] F. A. Smitt, "Om Hafs-Bryozoernas Utveckl. och Fettkroppar," 'Öfvers. af k. Vet.-Akad. Förh.,' xxii, 1865; and in other places.

[4] L. Cuénot, "Ét. sur le Sang," &c., 'Arch. de Zool. Exp. et Gén.,' 2e sér., t. vii, 1889; 'Notes et Revue,' p. III.

[5] H. Prouho, "Rech. sur la Larve de la Flustrella hispida," ibid., 2e sér., t. viii, 1890, p. 427.

b. Bugula avicularia, L.[1]

This form grows in short, bushy tufts, of a grey colour. The avicularia are usually large, although they vary a good deal in size; they are, in many cases, absent on the older parts of the colony, and are hence probably deciduous. The three spines on the upper margin of the aperture are very small in most of the specimens which I obtained at Naples. The Neapolitan form of this species has been well figured by Claparède.[2]

The funicular tissue contains no pigment. "Leucocytes" similar to those of B. neritina occur in the meshes of the connective tissue, and are either colourless or distinctly brownish grey. The colour, if any, is contained in a diffuse form in the vacuoles which compose most of the cell, and which sometimes contain, in addition, one or two minute granules. The connective tissue of this species will be described more fully in the later parts of this paper.

The pharynx and intestine are colourless. Yellowish-brown granules occur in the walls of the proventriculus, cæcum, and part of the stomach, the granules being most numerous in the cæcum. A saddle-shaped area at the base of the cæcum is devoid of granules (Pl. III, fig. 16[3]), just as was the case in B. neritina.

The walls of the rectum contain granules, which are either almost colourless or are faintly yellow in tinge.

c. Flustra papyrea, Pall.

The ordinary funicular tissue may be non-granular, or may

[1] This is probably referred to by Costa (loc. cit.) as one of the forms assumed by B. neritina. This "second generation" arises from the "piedi della prima," and is "bianchissima, translucida come il cristallo, fragillissima." The individuals figured by Costa on Tav. vi possessed avicularia, and presumably belonged to this species.

[2] 'Zeits. f. wiss. Zool.,' Bd. xxi, Taf. viii, figs. 1, 1 B.

[3] The granules are, indeed, not represented in this figure; but the blue or green colour, due to the indigo-carmine which had been taken up by this individual, gives a perfectly correct idea of their distribution.

resemble that of B. neritina in containing numerous granules, which are, however, quite colourless, instead of being deeply pigmented, as in that species. They are spherical in form, and are highly refractive. When treated with iodine they assume a colour precisely similar to that taken on by starch under the same conditions.[1] This observation was made just before my departure from Naples, and I was unable to investigate the subject with more care. Dr. A. Hansen has, however, been kind enough to undertake to examine the reactions of these granules, which differ from starch in being readily soluble in dilute alkalies or acids. I am inclined to regard the granules, which were noticed in the tentacles as well as in the general body-cavity, as a reserve supply of nutritive (perhaps carbo-hydrate) material. This is supported by the facts that they are most numerous at the growing edges of the colony, and that relatively few are present in any zoœcium which contains a brown body and an old embryo; indicating that the nutritive substance of the granules has been employed for the nutrition of the embryo. This granular funicular tissue is best developed on the sides and on the front wall of the zoœcium.

Depositions of orange pigment may be seen attached to various parts of the ectocyst, and enclosed in structures which are very similar to the "Excretbläschen" described by Eisig[2] in the Capitellidæ. They may assume the form of a number of minute granules or vesicles scattered through the substance of a cell, or of coloured granules contained in the interior of a more faintly coloured vacuole (fig. 13). They appear to be

[1] According to H. J. Carter ("On the Identity in Structure and Composition of the so-called Seed-like Body of Spongilla with the Winter-egg of the Bryozoa, and the Presence of Starch-granules in each," 'Ann. Mag. Nat. Hist.,' ser. 3, iii, 1859, p. 331), starch-grains are present in the statoblasts of a species of Lophopus; while K. B. Reichert ("Verg. anatom. Unt. üb. Zoobotryon pellucidus," 'Abhandl. k. Akad. d. Wiss. zu Berlin,' 1869, p. 281) describes, in the endocyst of Zoobotryon, certain bodies which he calls "Amyloidkugeln," which take on a "Granatfarbe," or even a violet colour, under the action of iodine.

[2] 'Fauna u. Flora G. v. Neapel,' xvi Monographie.

most numerous near the growing edges. On the formation of a brown body they are collected into much larger masses, which occur here and there in the zoœcium. Similar orange granules were also observed in the leucocytes.

Most of the tissues of the polypide, such as the tentacles and the several parts of the alimentary canal, have a diffuse yellow colour, the pharynx, the circumoral region, and the intestine being of a brighter orange-yellow colour. The first part of the stomach and the whole of the cæcum contain, in addition, pigmented granules, the number of which gradually shades off towards the region connected with the intestine, so that a zone between the two granular parts is devoid of granules.[1]

II. The Absorption of Various Pigments.

A. Indigo-carmine.

A saturated solution of indigo-carmine in sea-water was added to the sea-water containing the living colonies until the solution was of such a strength that the animals were not easily seen through a thickness of more than one and a half inches of the solution. After being left for two or three days in this solution the colonies were transferred to ordinary sea-water in a tank, and were examined from day to day. It may be pointed out that even after this treatment, and in the other experiments described below, the colonies remained perfectly capable of producing "brown bodies," fresh polypide buds, and new zoœcia at the growing edges of the colony.

a. Bugula neritina.

On examining a colony which has been immersed for some hours in indigo-carmine, it is at once obvious that while most

[1] The arrangement of these granules is well shown by Haddon in the same species ('Quart. Journ. Micr. Sci.,' xxiii, Pl. XXXVIII, fig. 12). Haddon has identified this species as F. carbasea, and the part of the alimentary canal which he has described as "intestine" (*int.*) is the part which I have alluded to throughout as "rectum."

of the tissues have remained uncoloured, the leucocytes have taken up a large quantity of the pigment. The appearance of a zoœcium after exposure to indigo-carmine is shown in fig. 17, where numerous coloured leucocytes are seen in the meshes of the pigmented funicular tissue. The whole of the blue pigment is contained in the vacuoles of the leucocytes (fig. 19); and, as in the analogous cases noticed by Kowalevsky,[1] the nucleus is unstained.

The bright blue colour is acquired only in the younger zoœcia, most of which possess functional polypides. It cannot, however, be supposed that the indigo-carmine has been first absorbed by the alimentary canal, and then passed on to the free mesoderm-cells, as the pigment is taken up quite readily by the leucocytes of zoœcia which possess no polypides, at the growing-points. Nor, for the same reason, can it be supposed that the blue colour is derived from pigment introduced into the body-cavity directly from the tentacle sheath of the polypide, in the manner described by Pergens.[2] I have, indeed, no observations which confirm Pergens' results with regard to the mechanism of the extrusion of the polypide, although I am not prepared to deny the accuracy of those results. The indigo-carmine observed in the leucocytes has probably been derived from traces of the pigment which have diffused through the walls of the zoœcia; the leucocytes absorbing the whole of the pigment which enters the zoœcium in this way, and so protecting the other tissues from the action of the pigment.

That the leucocytes of older zoœcia, with "brown bodies" developed or developing, are in a condition physiologically differing from that of the zoœcia which are nearer the growing-points is shown by the fact that the cells, in most of these cases, are of a distinct, though not very bright, green colour, instead of being blue.[3] The bright blue colour always appears

[1] 'Biolog. Centralblatt,' Bd. ix, p. 47, &c.

[2] 'Zoolog. Anzeiger,' xii Jahrg., 1889, p. 508.

[3] According to the results of Kitasato and Weil ('Zeits. f. wiss. Mikrosk.,' vii, 1890, p. 241), this would apparently point to the existence of the pigment in a reduced condition.

in the leucocytes situated in the growing-points or in the younger zoœcia, and sometimes in older zoœcia.

No part of the alimentary canal takes up any recognisable trace of indigo-carmine.

b. Bugula avicularia.

The blue colour is taken up by the leucocytes as in the last species. These cells may consist of a comparatively small number of large vacuoles (figs. 20, 21), or of a much larger number of small vacuoles, or both kinds of vacuole may occur in the same cell. In any case the pigment is found diffusely colouring the vacuoles.

The alimentary canal, unlike that of B. neritina, takes up large quantities of indigo-carmine (fig. 16). The pigment observed in the walls of the alimentary tract is probably derived from pigment which has been actually swallowed, as those polypides which are still so young that they do not communicate with the exterior have no deposition of pigment in their gut-walls.

The absorption of indigo-carmine takes place in the walls of the stomach, cæcum, and rectum (fig. 16). No pigment appears in the clear area on the inner side of the base of the cæcum, nor in the wall of the pharynx or of the intestine. The indigo-carmine is deposited in granules, which, as may easily be seen, are identical with the normal yellowish-brown granules; and the admixture of these two pigments naturally produces, in most cases, a greenish colour. In young polypides in which the granules are less strongly pigmented, and in the rectum of all the polypides, the bright blue colour of the indigo-carmine is not materially altered by the presence of any other pigment.

c. Flustra papyrea.

Indigo-carmine is taken up by leucocytes which are similar to those of Bugula. These cells become intensely blue, the pigment being diffused through the cell; darker blue granules, associated in some cases with the orange granules already noticed in the normal zoœcium, occur here and there in the diffusely coloured portions of the cell (figs. 23 and 24). In many cases large complexes of these cells occur in various parts of the zoœcium (fig. 23). The absorption of the indigo-carmine is independent of the functional activity of the alimentary canal. The general funicular tissue remains uncoloured.

In many of the zoœcia occur structures which are figured by Haddon[1] in Pl. XXXVIII, fig. 12 (*l. c.*). These structures occur near the posterior wall of the zoœcium, either on one or on both sides. They are not found in all the zoœcia, and are not recognisably present in those which are near the growing edges of the colony. They do not appear to have any special connection with the exterior or with the rest of the funicular tissue. They consist externally of a membrana propria, outside which are a few orange granules, the interior of the cord being finely fibrillated.

Freese[2] mentions the finely granular character of these cords in Membranipora. Nitsche[3] had previously termed them "funiculi laterales" or "lateral cords," and had stated that they passed from one rosette-plate to another. Their contents are sometimes granular according to Nitsche.[4]

When still incompletely developed the lateral cords are not affected by indigo-carmine; but in their older stages they invariably take up a large quantity of this pigment, which appears to be contained in the membrana propria, outside which is a layer of cells containing the orange-yellow granules (Pl. II, fig. 7).

Indigo-carmine is taken up by the granules of the functional alimentary canals only. The first part of the stomach and

[1] 'Quart. Journ. Micr. Sci.,' vol. xxiii, 1883.

[2] W. Freese, "Anatom.-histol. Unt. von Membranipora pilosa," &c., 'Arch. f. Naturg.,' Jahrg. 54, Bd. i, 1888, p. 16.

[3] H. Nitsche, "Ueb. d. Anat. u. Entw. von Flustra [Membranipora] membranacea," 'Zeits. f. wiss. Zool.,' Bd. xxi, 1870, pl. xxxv, fig. 1; pl. xxxvi, fig. 9.

[4] Loc. cit., p. 425, pl. xxxvii, figs. 19, 20.

the whole of the cæcum of old polypides consequently appear deep blue-green. In younger (functional) polypides, which are normally but slightly pigmented, the blue colour of the indigo-carmine is more obvious.

B. Carminate of Ammonia.

The colonies were placed for about two days in sea-water containing this substance in solution, and were then transferred to a tank containing ordinary sea-water.

a. Bugula neritina.

The pigment is taken up by the granules contained in the walls of the alimentary canal of those polypides only which have actually swallowed solid (precipitated) carmine particles.[1] The pigment is most copiously deposited in the blind end of the cæcum, gradually shading off thence on to the rest of the stomach. The wall of the rectum and the inner borders of the cells of the pharynx also contain carmine deposits. The clear patch on the cæcum never contains the pigment.

In some of the zoœcia carmine is taken up in granules by cells belonging to the funicular tissue. These cells are distinct both from the network of pigmented cells and from the leucocytes, and belong to the type shown for the next species in fig. 22. The growing-points are usually brightly coloured with red pigment.

b. Bugula avicularia.

The pigment is taken up by the same parts of the alimentary canal and of the funicular tissue as in B. neritina, being most copiously present in the latter position in the growing-points. The leucocytes contain no trace of the pigment. About twelve days after the commencement of the experiment it was noticed that the tips of many of the branches were

[1] C. Vogt ("Sur le Loxosome des Phascolosomes," 'Arch. de Zool. Exp. et Gén.,' v, 1876, p. 320) has shown that carmine given as food is deposited in the yellow "hepatic cells" of the stomach of Loxosoma phascolosomatum, but in no other part of the alimentary canal.

growing out into abnormally long and slender growing-points, in the funicular tissue of which carmine-particles derived from those originally taken up by the growing-points were present in the form of sharply circumscribed spherules, which contained (normal) yellowish granules in addition to the red pigment (fig. 22).

c. Flustra papyrea.

None of the tissues of this animal were shown to take up carminate of ammonia, the experiments with which, in the case of this species, were not altogether successful.

C. Bismarck-Brown.

A weak solution, sufficient to give the sea-water a yellow tinge, was found to give the best results.

a. Bugula neritina.

No part of the alimentary canal could be shown to take up Bismarck-brown; but as the granules of the alimentary canal are normally very dark in this species it was not possible to be quite sure that none of the pigment had been absorbed by them. All the other structures which are naturally pigmented, i. e. the funicular tissue, the pigmented part of the tentacles, the strongly pigmented region round each aperture, and the pigmented parts of the growing-points, take up the Bismarck-brown freely. The fact that the alimentary canal in this species takes up neither indigo-carmine nor Bismarck-brown points to a physiological difference between it and that of allied species. It may be suggested at once that this difference is associated with the pigmentation of the funicular tissue. If it is assumed that the pigment-granules of the alimentary canal are in part of excretory nature—a question which will be further considered below—the difference between Bugula neritina and other species may be expressed by saying that some of the excretory functions normally possessed by the alimentary canal are here performed by the funicular tissue, which is hence pigmented.

The Bismarck-brown is deposited only in the granules of the funicular tissue. Thus the finer processes of that tissue, which are devoid of granules, remain quite colourless after the action of the pigment. The nucleus thus becomes visible, in most cases, as a clear area, unstained by the Bismarck-brown (fig. 18, *A*).

b. Bugula avicularia.

The Bismarck-brown is taken up in intensely brown-red granules by the tentacles and by the proventriculus, cæcum, stomach, and rectum of the functional polypides, the cæcum being the part which becomes most deeply pigmented. The inner borders of the cells of the pharynx become distinctly brown; but the pigment is here diffuse, and not in the form of a granular deposit. No pigment is deposited in the œsophagus (region between pharynx and proventriculus), nor in the clear area on the base of the cæcum, nor in the intestine. These parts may, however, acquire a faint yellow tinge. In "brown bodies" which are in process of formation the remains of the stomach, &c., take up the pigment, none of which is absorbed by the fully-formed "brown bodies," nor by the polypide-buds which do not yet communicate with the exterior.

Most of the funicular tissue remains uncoloured, but in some cases groups of intensely brown spherules are observed here and there in the body-cavity. This is especially the case in the region surrounding a "brown body." The leucocytes become hardly tinged with yellow.

The Bismarck-brown is taken up freely by young, growing cells. The growing-points and the young avicularia hence become strongly pigmented. Most of the pigment is deposited in these regions in the funicular tissue; but some of it appears to be in the ectoderm, which can also be traced in some of the older zoœcia as a series of small cells, separated from one another by considerable intervals, and stained brown by the action of the pigment. The tactile bodies of the old avicularia and the surrounding regions also become pigmented.

A remarkable form of cell, shown in Pl. III, fig. 20, was constantly noticed in this species, but in none of the others examined by me. Each cell consisted of a group of large vacuoles, some of which contained small granules or concretions, while others were completely filled with a number of minute granules, which always exhibited an active Brownian movement.

The larger concretions in the former kind of vacuole are normally colourless, but in colonies treated with a mixture of Bismarck-brown and indigo-carmine most of them became a pale brown colour, while some of them had taken up a small quantity of indigo-carmine (fig. 20).

These cells always occur in a special zone, in the growing-points, at the junction of the comparatively solid distal funicular tissue, and of the space in which the young tentacle sheath lies. They are perhaps in some way concerned in the excavation of this space out of the more solid funicular tissue of the younger part of the zoœcium. They are also found, here and there, in the cavities of the older zoœcia.

They have been noticed in the same species by Claparède,[1] who describes them as finely granular, brown structures, occurring only in the cavities of the buds, and only found in this particular species.

c. Flustra papyrea.

The granular parts of the stomach and cæcum become intensely coloured by the Bismarck brown, which also appears in smaller quantities in the rectum. The intestine, the inner borders of the pharyngeal cells, and the tentacles may become diffusely coloured. Small quantities of the pigment may appear, in spherules, in cells belonging to the funicular tissue (never in cells of the type shown in fig. 14).

D. Carmine Particles in Suspension in Sea-Water.

The carmine particles are readily swallowed by the polypides, whose alimentary canals become filled with dense masses of

[1] 'Zeits. f. wiss. Zool.,' xxi, pp. 141–2, pl. viii, fig. 1.

solid carmine. In some of these cases a small quantity of carmine becomes deposited in the granules of the cæcum and stomach in B. neritina. In B. avicularia a small amount of carmine was deposited in the rectum and proventriculus as well; while the funicular tissue of the growing-points took up a little of the pigment. This was no doubt derived from the small quantity of carmine which dissolved in the sea-water, the colour of which was distinctly red.

III. The Formation of the "Brown Body" and the Further History of the Absorbed Pigments.

Flustra papyrea.

The formation of the "brown body" in the normal zoœcium takes place as follows.[1]

The tentacles lose their distinctness and shorten themselves, then forming a mass situated at the proximal end of the tentacle-sheath, which is also degenerating. The alimentary canal at the same time begins to degenerate, its granules collecting into two masses, one placed at the apex of the cæcum, and the other at the opposite end of the stomach.[2] The tentacle-sheath loses its connection with the aperture, and the three masses formed respectively by the tentacles (with some part of the remains of the alimentary canal) and the two sets of granules fuse into a small rounded mass, which ultimately acquires a bright red colour. The granules of the alimentary canal can be distinguished for some time as a darker mass in the "brown body."

The new polypide-buds have a distinctly bilateral origin, which, so far as I know, has not been noticed by any previous observer. The inner layer of the bud contains orange granules, which give it a distinct colour; this layer is derived from the two angles of the operculum. At each angle a rounded

[1] The account which follows, as well as that given of the origin of the new polypide-bud, is almost identical with that given by Haddon ('Quart. Journ. Micr. Sci.,' xxiii, 1883) for the same species (Haddon's F. carbasea).

[2] Cf. Haddon's fig. 13 (pl. xxxviii).

swelling, doubtless formed from ectoderm, grows out towards the middle line, immediately beneath the ectocyst (fig. 10). The two swellings unite (figs. 11 and 12) and form a single rounded mass, which remains connected with the angles of the operculum by a tract of modified ectoderm, which soon disappears, on each side. The outer layer of the bud is colourless, and is formed from ordinary funicular tissue.

In some cases the end of each bud-rudiment is considerably swollen before fusion takes place (fig. 11); in other cases no swelling appears until after fusion. In the former case the zoœcium appears to possess two buds placed side by side.

This mode of origin of the buds is no doubt the cause of an abnormality, frequently noticed, in which two polypide buds occurred side by side in the same zoœcium.[1] There was no doubt that in these cases each half of the polypide-bud had developed into a complete polypide, the normal fusion of the two halves having been, for some reason, prevented. Double polypides were observed in various stages of development, from the small ovoid stage, when the two halves should normally fuse, to the condition in which the tentacles of the two polypides are well developed. Each polypide then usually possessed a distinct tentacle-sheath, although in one case observed the two tentacle-sheaths fused distally, although separate at an early period of their development, when the double bud was first noticed.

In a particular experiment, all the colonies which had been treated with indigo-carmine were observed 313 hours later to be remarkable for possessing a considerable number of zoœcia with twin polypides; as many as six of these zoœcia having been noticed in one small area of a colony. This probably indicated that the indigo-carmine abnormally present had acted

[1] This abnormality is recorded by Haddon (loc. cit., p. 520), as well as by Ostroumoff ('Arch. Slaves de Biol.,' t. ii, 1886, p. 341). Ostroumoff found that the two alimentary canals were always united by their stomachs; which he explains by assuming that there were at first two buds, which later became united through the intermediation of a common "brown body." I am unable to say whether a union of this kind would have been effected later in the cases of twin polypides which I observed in their immature condition.

on all the polypide buds which were in their critical state immediately preceding fusion in such a way as to prevent fusion, and so to cause each half to develop into a complete bud.

In a few cases which appeared to be abnormal the young bud made its first appearance in close contact with the "brown body." Buds developed in this position differed from the normal buds in being more spherical at a stage when the tentacles were first seen, and in some other respects. The differences subsequently became less marked, and the bud appeared to develop into a normal polypide, although my time was not sufficient to allow me to watch the last stages in this process.

At the growing edge of the colony the polypide-buds are formed at the extreme proximal edge of the zoœcia.

The development of the young bud into a complete polypide may be considered in connexion with the indigo-carmine experiments, the behaviour of the developing buds being, in those experiments, exactly similar to that of the bud developing under normal circumstances.

The zoœcia are found, after the action of indigo-carmine, to have a brilliant blue colour owing to the absorption of the pigment by the leucocytes, as already described. Some of the zoœcia may be dead, and are then readily recognised by the fact that they are stained intensely, but diffusely, blue, the operculum being usually open. In these zoœcia, Infusoria and Nematodes soon make their appearance.

In zoœcia which are still alive the pigment is confined to the cells already described. The leucocytes after several days tend to group themselves in masses, and some of them may be seen closely surrounding the "brown bodies" (fig. 1).

About 114 hours after the commencement of the experiment which turned out most successfully, all the functional polypides of the colony were noticed to be degenerating, the evidence of which was given by the fact that the fæces had ceased to rotate in the intestine. At the 165th hour the indigo-carmine taken up by the two groups of granules in the wall of the alimentary canal was, in many cases, becoming aggregated into two round masses, one situated at the apex of the cæcum, and the other at the opposite end of the stomach. This is exactly what happens in the degeneration of the normal alimentary canal.[1]

At 238 hours from the first immersion in indigo-carmine new polypide-buds were commencing to appear in a good many of the zoœcia, many of which, at the 264th hour, had the appearance shown in Pl. II, fig. 1. The degenerating tentacles can be distinctly made out, as well as the two masses of indigo-carmine (mixed with the normal pigment of the stomach, and so of a green colour) derived from the wall of the alimentary canal. The leucocytes containing the absorbed pigment are grouped in a dense mass round the "brown body," and are enveloped in funicular tissue, strands of which radiate out to various parts of the body-wall. Some of these strands contain blue leucocytes, intermingled with which were seen, in some cases, granules of the brown or orange pigment found in the normal zoœcia (Pl. III, figs. 23, 24). The funicular tissue and the muscles shown in the sketch are perfectly colourless. The whole of the indigo-carmine taken up by the animal is contained in the "brown body" and leucocytes.

One of the strands of funicular tissue passing from the "brown body" is thicker than the rest. It is directed towards the operculum, and the polypide-bud is attached to it, having shifted its original position, and slipped down the strand of funicular tissue in the direction of the "brown body." The polypide-bud is pointed at the end turned towards the operculum, and its walls are here thinner than elsewhere. This portion will become the tentacle-sheath of the new polypide.

Somewhat later (286th hour) many of the polypide-buds had developed tentacles. The tentacle-sheath had become very thin, and was sharply differentiated from the proximal end of the bud. The tentacles were present as a bilateral incomplete ring of small tubercles developed on the side of the bud turned towards the opercular surface of the zoœcium, the ring being open on the side nearest the operculum. The intestine and

[1] Haddon, loc. cit., fig. 13 (pl. xxxviii).

stomach were growing out from the other (posterior) side of the bud in the manner described by Haddon.[1]

The tentacles soon become arranged in the form shown in fig. 2, the bilateral series bending away from the opercular side of the zoœcium on each side as far as the emargination which marks the point from which the stomach and intestine have been evaginated. The retractor-muscles of the polypide are by this time developed as a group of fibres converging from the polypide to the "brown body."

The tentacles next rotate completely, so as to point towards the aperture, and their bases now form a simple circle, the plane of which is transverse to the long axis of the zoœcium, and at right angles to its opercular surface (fig. 3). The tentacle-sheath has become prolonged along the strand of tissue connecting it with the operculum, and has met a semicircular thickening lying beneath the operculum, and destined to give rise to the new aperture.

The apex of the evagination which forms the rectum, intestine, and stomach corresponds to the apex of the cæcum. It is attached to the strong funiculus already described. Fig. 3 (305 hours from the beginning of the experiment) shows the commencement of the pharynx and œsophagus as a new outgrowth from the front surface of the bud. The "brown body" is more spherical than before, and has retreated to the proximal end of the zoœcium, a position which it normally occupies at this stage. The tentacle-sheath has met the developing aperture.

At the 353rd hour the two green parts of the "brown body" of the individual shown in fig. 3 were commencing to fuse. The end of the cæcum was of a lighter colour than the rest of the alimentary canal, and had passed, guided by the funiculus, very nearly as far as the "brown body."

At the 376th hour (fig. 4) this part of the cæcum had met the "brown body," in which the two green masses (seen through the "brown body") were in the act of fusing; the "brown body" was commencing to be retracted away from the

[1] Loc. cit.

proximal end of the zoœcium. The first part of the stomach was being developed as an outgrowth of the cæcum, passing towards the pharynx. The tentacles were obviously increasing greatly in length, although their number had been for some time complete.

At a later period (401st hour) the rectum was distinctly differentiated from the rudiment which gives rise to the stomach, intestine, &c., and contained the so-called "meconium." The tentacles were individually contractile, and the stomach had met the pharynx, but without yet fusing with it.

At the 424th hour (same individual) the "brown body" was further covered by the cæcum, and the intestine was becoming bent over to one side of the zoœcium. The funiculus was present as before, passing along the back of the bud to the distal end of the zoœcium; it has been omitted from most of the figures for the sake of clearness.

At the 448th hour (fig. 5) the cæcum had grown over about half of the surface of the "brown body," which had by this time retreated still further from the proximal end of the zoœcium. The tentacles had increased greatly in length, and the intestine was still further bent over to one side. The intermediate tube, connecting the rectum with the tentacle-sheath, is clearly seen in fig. 5. This tube, which is a normal feature of the polypide, was first noticed in this individual at the 353rd hour.

At the 496th hour the curvature of the intestine to one side was becoming still more pronounced (fig. 6), and the "brown body" was by this time not far from the middle of the zoœcium. A projection of its substance into the lumen of the cæcum indicated its approaching dissociation into fragments destined to pass into the alimentary canal. It had become so dark in colour that the mass of indigo-carmine contained in its interior could no longer be seen, the "brown bodies" being, in individuals of this colony, much darker than the brown-red colour so characteristic of the normal "brown body."

At the 520th hour the "brown body" projected still further into the cavity of the alimentary canal, but there were no other changes of importance.

It may be here expressly pointed out that the history of this experiment, as traced from the 286th hour, refers specially to a single polypide, which was carefully examined and drawn at intervals of about twenty-four hours, so that the amount of time taken by the development was actually determined. Numerous other zoœcia in the same colony completely confirmed the observations made on this particular individual.

My own observations unfortunately came to an end at the 520th hour, owing to my departure from Naples at that time.

In the particular individual whose history has been traced to the 520th hour after the commencement of the experiment no special change had taken place in the leucocytes, which still contained the bright blue pigment. In some other individuals of the colony, however, a much larger quantity of indigo-carmine had been taken up; so much so, indeed, that it did not at first appear possible that the zoœcia could recover. After a time, as shown in fig. 9 (476th hour), the pigment was deposited in dense masses, no longer contained, as it seemed, in the leucocytes, but probably deposited by these cells in a more or less insoluble form. These masses were found in various positions in the zoœcium, usually in the angles formed by the intersection of neighbouring sides, but sometimes running across the body-cavity. It appeared probable that in fig. 9 the "lateral cords" had formed a kind of focus, into which the pigment had been deposited from the neighbouring leucocytes.

In the same individual (which was certainly alive, and in which the young polypide-bud may be seen in the figure) a mass of indigo-carmine had been deposited by leucocytes at the proximal end of the "brown body," while a similar process seemed to be taking place all round the "brown body," though to a less marked extent than at the proximal end. It was further noticed that, in many individuals, the aggregation of the blue leucocytes round the "brown body" was much more dense than in the specimens figured on Plate II; and other instances were noticed in which indigo-carmine had almost certainly passed from these leucocytes into the periphery of the "brown body." I at first expected to find that the leucocytes which had taken up indigo-carmine would, in some manner or other, discharge their pigment into the "brown body," with which it would be removed from the zoœcium through the agency of the young polypide. My experiments were, unfortunately, incomplete when I had to leave Naples, so that I cannot speak with any certainty on this point. I am inclined to believe that the greater part of the indigo-carmine taken up by the leucocytes is deposited, in an insoluble form, in masses situated in various parts of the zoœcium (as shown by fig. 9 and, to a less extent, by fig. 5), but that some may pass into the "brown body," as indicated in fig. 9, and so leave the zoœcium. The unusually dark colour of the "brown bodies" in the individuals of the colony treated with indigo-carmine was possibly due to an admixture of indigo-carmine derived from the leucocytes with the normal red pigment of the "brown body."

It has already been pointed out that indigo-carmine is freely taken up by the "lateral cords." During the formation of the "brown body" these cords shrivel, still containing the pigment, and sometimes becoming divided into two or more pieces (fig. 8). The fragments which are left at the distal end of the zoœcium appear to deposit their pigment as a densely blue mass on some part of the inner surface of the wall of the zoœcium. Other parts of the lateral cords may become closely connected with the "brown body," with which they may, perhaps, ultimately pass to the exterior.

The colony on which these observations were principally made did not belong to the experiment previously described. Very little indigo-carmine had been taken up, and nearly all of it was in the lateral cords and in the granules of the alimentary canal; very few of the leucocytes having taken up any blue colour.

I am very much indebted to my friend Mr. A. H. L. Newstead, of Christ's College, Cambridge, for having made and communicated to me, after my departure from Naples, some further observations on the history of the experiment which I had commenced.

The following statements, referring to F. papyrea, are given on the authority of Mr. Newstead, to whom I express here my very best thanks.

At the 622nd hour the disintegration of the "brown body" had already progressed some distance, and dark masses derived from it were observed in the rectum.

In the following days the disintegration of the "brown body" continued, fragments being broken off from it and passing into the lumen of the alimentary canal. The fragmentation of the "brown bodies" was taking place actively at about the 743rd hour; in some of the individuals the greater part of the "brown body" was by this time accumulated in the rectum.

At the 959th hour the rectum had, in some cases, emptied itself, although a small portion of the "brown body" was still left at the apex of the cæcum (as in Haddon's pl. xxxvii, fig. 11).

During the process of fragmentation of the "brown body," Mr. Newstead was able to observe most conclusively that the indigo-carmine which was contained in the "brown body" passed into the cavity of the new alimentary canal, where it was seen as distinct blue masses in the intestine, rectum, &c. This is especially alluded to in Mr. Newstead's notes referring to the 790th—808th hours.

At the 880th hour these blue fragments had entirely disappeared in some of the individuals, and there can be no doubt that they had left the alimentary canal, with other parts of the "brown body," by a process of defæcation.

In other cases blue fragments were still observed in the intestine and rectum as late as the 1744th hour, the last observation recorded.

During the fragmentation of the "brown bodies" which contained indigo-carmine, Mr. Newstead was unable to observe that the walls of the alimentary canals took up any of the blue pigment contained in their cavities, with the doubtful exception of the part of the cæcum immediately overlapping the "brown body." This shows that the pigment taken up by the first generation of alimentary canals is really excreted, and that there is no (or at least very little) secondary absorption of the pigment by the new polypides.

I may remark incidentally that my observations, and Mr. Newstead's continuation of them, give some information with regard to the amount of time necessary for the development of a "brown body" and the complete formation of a new polypide in this species of Flustra.

One of the effects of the indigo-carmine on the colony was to induce the degeneration of all the polypides. This process was observed to be commencing rather more than four days after the immersion of the colony in the solution of the pigment. The further history of the experiment is explained by the following table.

Colony treated with a Solution of Indigo-carmine.

5th day (114 hours) .	Polypides commencing to degenerate.
7th „ (165 „) .	Aggregation of granules of stomach and cæcum into two masses.
10th „ (238 „) .	Appearance of young polypide buds.
12th „ (286 „) .	Tentacles developed, the lophophore having become circular in the oldest individuals.
13th „ (305 „) .	Stage shown in fig. 3.
15th „ (353 „) .	Fusion of two masses of granules of stomach, &c.
16th „ (376 „) .	Tip of the cæcum becoming connected with "brown body" (fig. 4).
19th „ (448 „) .	Fig. 5.
21st „ (496 „) .	"Brown body" preparing to break up (fig. 6).
26th „ (622 „) .	"Brown body" broken up into numerous fragments, contained in the alimentary canal of the new polypide.
40th „ (959 „) .	"Brown body" almost completely absorbed.

The process of absorption of the "brown bodies" was not, however, completed in all the individuals at the seventy-third day (1744 hours), the last observation recorded. It is, however, obvious that the formation of new polypide-buds commences (and therefore ends) at different periods in the several zoœcia.

The account which has just been given of the growth of the end of the cæcum round the "brown body," and of the manner in which the latter passes into the lumen of the alimentary canal, is supported by the fact that precisely similar processes were noticed in fresh zoœcia which had not been exposed to the abnormal condition of being placed in indigo-carmine.[1]

Mr. Newstead did not find that any of the indigo-carmine contained in the leucocytes passed with the "brown body" into the new alimentary canal; and at the end of the series of observations (seventy-third day) all the living zoœcia still contained blue-coloured leucocytes. Further observations will be necessary to show what is the ultimate fate of these leucocytes. It is not impossible that the pigment contained in them may be excreted in small quantities with successive generations of "brown bodies;" but it appears to be more probable that the pigment is simply left behind in the zoœcia.

Bugula neritina and B. avicularia.

The "brown body" in these species has a very different history from that which has just been recorded in Flustra, inasmuch as it is not taken up by the alimentary canal of the newly formed polypide.

In B. avicularia the formation of a new polypide in an old zoœcium was, in my specimens, comparatively rare. The older parts of the colony merely contained "brown bodies," smaller masses of pigment, leucocytes, and connective-tissue cells of various kinds; and polypides were usually only found in the neighbourhood of the growing-points.[2]

In B. neritina new polypides are freely regenerated in the old zoœcia. They are developed in the middle of a dense network of funicular tissue situated between the front wall of the zoœcium (i. e. the surface which bears the aperture) and the

[1] See also Haddon's account ('Quart. Journ. Micr. Sci.,' xxiii, 1883).

[2] See Hincks, 'Brit. Marine Polyzoa,' vol. i, 1880, p. 52.

remains of the old polypide. The cæcum of the young polypide soon projects beyond the proximal side of the "brown body," which is ultimately left near the distal end of the zoœcium, and at its posterior side (fig. 17).

Nearer the base of the colony the zoœcia usually contain at least two "brown bodies,"[1] sometimes three, which always lie at the back of the zoœcium, and which have probably been formed by the disintegration of as many successive polypides. The older zoœcia also contain numerous brown spherules, which have resulted from the disintegration within the zoœcium either of the older "brown bodies" or of the pigmented funicular tissue.[2] The leucocytes, in normal old zoœcia, retain their ordinary, slightly yellowish, clear colour, and the zoœcium further contains a certain quantity of ordinary funicular tissue.

No change of importance was noticed in the blue-coloured leucocytes at any period of the experiments in either of these species of Bugula. To the end of the observations they retained their bright blue colour, and were scattered in the meshes of the funicular tissue. In some cases they became more or less aggregated into masses situated near the "brown body" (see fig. 17). In other cases some of the pigment appeared to pass from the leucocytes into the "brown body," apparently by immigration of the entire leucocyte into the latter; but it was not quite certain that this was a normal process.

The effect of immersion in indigo-carmine on B. neritina was, in some cases, to induce the degeneration of most of the polypides. This was noticed, for instance, in one set of colonies at the beginning of the fourth day (seventy-three hours) after the first treatment with indigo-carmine solution. New polypide buds were being formed in most of the zoœcia at the fifth day (114 hours), and tentacles had appeared at the seventh day (148 hours).

[1] 'Brit. Marine Polyzoa,' vol. i, 1880, p. 53.

[2] Claparède, 'Zeits. f. wiss. Zool.,' xxi, p. 154; pl. ix, fig. 1 C; pl. x, fig. 2.

The amount of pigment in the funicular tissue had considerably diminished by the eighth day (170 hours), probably indicating that it had been used for the nourishment of the young polypide, a view which is confirmed by the facts that the bud is invariably surrounded by a dense network of funicular tissue, and that the tissue in question is very largely developed at the growing-points. By the end of the same day the cæcum of the young polypide projected considerably beyond the proximal side of the "brown body," and one or two of the blue leucocytes had passed into the ring-canals of the young polypides.

At the eleventh day (243 hours) the polypides had reached the stage shown for Flustra in fig. 4 (sixteenth day). The "brown body" was fully formed and the aperture was in process of development. The funicular tissue appeared to be appreciably diminished in amount, and had lost most of its granules. Although some of these granules are probably used for the nutrition of the young polypide, others appear to give rise to the spherules of brown matter seen in various parts of the body-cavity. These spherules may be regarded as effete portions of the funicular tissue which are deposited, with the "brown body" itself, in the body-cavity.

At the end of the fourteenth day (288 hours) the funicular tissue was regaining its purple colour, which had been lost during the occurrence of the processes already described. I am inclined to regard this purple pigment as being of nutritive rather than of excretory value.

Fig. 17 shows the condition of the polypides regenerated since the beginning of the experiment at the fourteenth day (316 hours). The polypide had by this time become very irritable, but was apparently not yet capable of protrusion. No essential change had taken place in the leucocytes, which were still bright blue. In the individual shown in the figure a large mass of these leucocytes occurred at the distal end of the zoœcium. Twenty-four hours later food was circulating in the intestine, and the aperture appeared to be completely formed.

In Bugula avicularia the "brown bodies" are usually situated in a similar position, at the distal end of the zoœcium, and the leucocytes are in most cases aggregated around them. There was not much reason to suppose that the pigment taken up by the leucocytes passed into the "brown bodies."

In this species, parts of the wall of the alimentary canal took up indigo-carmine. In one case observed, the fæces circulating in the intestine of one of these polypides contained bright blue particles, indicating that some of the pigment taken up by the walls of the cavity may be excreted with the fæces. Most of the indigo-carmine, however, does not escape in this way; and the "brown body," when formed, is very obviously blue-green in colour, owing to the admixture of the indigo-carmine with the natural pigments of the stomach.

At 261 hours after the commencement of the experiment it was noticed that many of the old zoœcia had developed new growing-points at their tips; and some of these already possessed polypides. Blue-coloured leucocytes had in these cases passed in an apparently unaltered state from the old zoœcia into the young growing-points. No obvious alteration of the indigo-carmine contained in these leucocytes took place during the whole of this experiment, which lasted about 350 hours.

Carminate of Ammonia.

B. neritina.—The pigment taken up by the walls of the alimentary canals is left in the "brown bodies" formed by their degeneration. The part of the "brown body" formed from the tip of the cæcum is at first very brilliantly coloured by the pigment. By the 286th hour the colonies contained numerous compact "brown bodies," which were most distinctly carmine-coloured. Many of the polypides whose development had commenced since the beginning of the experiment were nearly mature. At the time of the last observation made (478th hour) no change of importance had taken place. The zoœcia were left with "brown bodies" containing carmine, and probably destined to remain permanently in the zoœcia, while the body-cavity contained small quantities of vesicles or spherules containing carmine, probably derived from the small quantities of that substance taken up by the funicular tissue; or, in some cases, formed by the fragmentation of the "brown body" within the zoœcium.

Precisely similar results were obtained with B. avicularia. The "brown bodies" formed by the degeneration of polypides whose alimentary canals had absorbed the carmine into their walls were of a bright red colour.

At the 480th hour—the end of this series of observations—these red "brown bodies," together with spherules containing carmine (as shown in fig. 22) were left behind in the body-cavities of the zoœcia.

Bismarck-Brown.

Bugula neritina.—It has already been pointed out that the pigmented funicular tissue of this species takes up Bismarck-brown very freely. The leucocytes are at first unaffected by this pigment; but 120 hours after the beginning of one of the experiments they had become bright orange-yellow in colour, presumably by the absorption of Bismarck-brown from other tissues, since they had remained quite uncoloured by the Bismarck-brown for some hours at least after being transferred to pure sea water. The vacuoles of the leucocytes contained, in some cases, a few deposited granules of the pigment.

Thus, although apparently not adapted to take up Bismarck-brown directly, the leucocytes do their best, at a later period, to remove portions of this pigment which have been absorbed by other tissues; and this is an additional argument in favour of the view that these cells possess excretory functions.

In one of the experiments the action of the Bismarck-brown had been so strong that the processes of the cells of the funicular tissue had been to a large extent retracted; and it at first appeared that the zoœcia had been killed, the movements of nearly all the polypides having completely ceased.

Subsequent observation showed that the animals were not really dead. At 48 hours from the beginning of the experiment, the pigment taken up by the funicular tissue was commencing to be deposited in intensely brown (almost black) spheroidal masses of granules in various parts of the funicular tissue. This process continued as time went on, and fig. 18 represents the appearance of the tissue at the 143rd hour. It will be observed that the leucocytes have by this time taken up the pigment, but that in three of those which are drawn the pigment is not contained in all the vacuoles of the cell; so that the cell consists partly of coloured and partly of uncoloured vacuoles. The Bismarck-brown taken up by the funicular tissue is obviously, for the most part, segregated into dense deposits of granules, while the remainder of the tissue has become absolutely hyaline and colourless, containing, however, occasional granules of Bismarck-brown. The parts of the tissue which are now hyaline are, in their normal condition, filled with coloured granules, the absence of which clearly shows that the granules, all of which originally took up the Bismarck-brown, have accumulated in the dense brown masses seen at the nodal points, and have thus succeeded in ridding most of the tissue of the obnoxious foreign substance.

At the same period some of the zoœcia contained minute polypide-buds.

At a later period (238th hour) the leucocytes were still bright yellow, most of them having deposited a few granules of Bismarck-brown in their vacuoles. The funicular tissue retained its previous appearance, the deposited pigment usually tending to accumulate round the "brown bodies," in the form of a dense brown mass at the back of the zoœcium. The tissues which were in course of regeneration, i.e. the polypide-buds, the new muscles, and the greater part of the funicular tissue, were quite transparent and colourless.

At the 312th hour the leucocytes were still more brightly coloured, and contained deposited granules of Bismarck-brown, indicating a continued effort on their part to remove the deleterious pigment from other tissues. The funicular tissue had deposited nearly all the pigment taken up by it in deep brown masses of granules.

Finally, at the 378th hour, the funicular tissue, having rid itself of the granules which had taken up Bismarck-brown, was commencing to redevelop fresh granules, which had an appearance perfectly similar to those of the normal zoœcium.

During the actual excretion of the Bismarck-brown by the funicular tissue, the deposited granules are contained in vacuoles like those which have already been noticed as concerned in taking up carminate of ammonia. At a later period the vacuoles disappear, and the pigment is in the form of the dense masses already described.

The zoœcia to which the above statements refer were obviously living. Some of the zoœcia were, however, dead, and in these cases the ectocyst and the tissues generally were stained deeply, but diffusely, by the Bismarck-brown. A similar fact was noted with regard to the action of indigo-carmine on dead zoœcia of Flustra papyrea.

In B. avicularia the leucocytes are at first uncoloured by the Bismarck-brown. At a later period, as was the case in B. neritina, they may become coloured by that pigment. An analogous phenomenon was observed with regard to indigo-carmine, the blue colour of the leucocytes increasing in intensity after the first absorption of the pigment. This probably implies that a certain amount of the pigment occurred at first dissolved in other parts of the zoœcium, and that the leucocytes had charged themselves with the office of removing all the indigo-carmine with which the other tissues were infiltrated.

The alimentary canal and tentacles in this species take up large quantities of Bismarck-brown. In some cases such polypides simply degenerate into "brown bodies" which have an intensely orange-brown colour, in consequence of the artificial pigment contained in them.

In other cases, however, the alimentary canal undoubtedly excretes the pigment into its lumen. This process takes place by the separation of small round vesicles from some part of the wall of the alimentary canal, and probably from the cæcum.[1] These vesicles contain granules of Bismarck-brown, and may be seen in the stomach, intestine, or rectum, where they are no doubt on their way to the exterior. The effect of the continuation of this process was that, whereas the alimentary canals were brightly coloured with Bismarck-brown shortly after their immersion in that fluid, many of the polypides, at the 143rd hour, possessed brightly coloured tentacles, whilst the alimentary canals had become almost colourless, or at least were much less brightly coloured than at an earlier period.

At the 192nd hour the alimentary canals of all those polypides which were functional at the beginning of the experiment, and which had not since degenerated to "brown bodies," had got rid of almost every trace of Bismarck-brown, while the tentacles still remained brilliantly coloured by that pigment. At the 212th hour the alimentary canals of some of these polypides with coloured tentacles were quite colourless, and it was obvious that the polypides had been obliged to discharge all the granules normally contained in the wall of the stomach, &c., in order to excrete the Bismarck-brown. The pigment taken up by other parts of the colony (growing-points, &c.) showed no appreciable diminution in quantity. In those zoœcia in which the "brown bodies" were stained, the body-cavity contained a considerable quantity of irregular masses of granules coloured by Bismarck-brown. This fact further supports the view which has been already suggested, that the "brown bodies" may undergo a certain amount of fragmentation in the body-cavity.

At the 378th hour, polypides with tentacles stained by Bismarck-brown were still left, although the colonies had been kept, since their original immersion in Bismarck-brown, in pure sea-water. The leucocytes had got rid of nearly all their pigment, having probably excreted it into the masses of brown granules seen in various parts of the body-cavity.

[1] The vesicles were frequently noticed in the normal animal, and in one case in B. neritina.

Flustra papyrea.

Some days after the beginning of the experiment, the leucocytes, as in other species, are found to be coloured by Bismarck-brown. In a colony which had been treated first with indigo-carmine and afterwards with Bismarck-brown, the leucocytes, at the 188th hour, were either yellow or green (in the latter case obviously containing the two pigments), and contained, within their vacuoles, granules coloured by Bismarck-brown or by indigo-carmine, or by both substances.

In one experiment it was noticed that the "lateral cords" of nearly all the individuals had the appearance shown in fig. 15, 213 hours after their first immersion in Bismarck-brown. The contents of the lateral cords appeared granular, but enclosed a number of round vacuoles of a yellow colour. This is probably to be interpreted as an excretory process.

IV. Summary and General Conclusions.

The experiments made with various artificial pigments showed conclusively that the tissues did not all react alike to these pigments, the absorption of which was, in each species, limited to certain definite tissues. As Kowalevsky has pointed out for other Invertebrates, the action was precisely the same if the animals were immersed in a solution of two pigments mixed. Thus, when indigo-carmine mixed with Bismarck-brown was used, the leucocytes were at first blue (although they subsequently absorbed Bismarck-brown from other tissues), just as if the former pigment had been used by itself.

The results on the absorption of the pigments may be summarised as follows:

Leucocytes.—These cells, in all the species examined, readily absorb indigo-carmine. Although they do not appear to take up Bismarck-brown directly, they abstract it from other tissues at a later period. They are not in the least affected by carmine in suspension, nor by carminate of ammonia.

Alimentary Canal.—The pigmented granules of the stomach and cæcum of B. avicularia readily take up indigo-carmine, carminate of ammonia, or Bismarck-brown, and become less obviously red when the animals are fed with carmine in suspension.

In B. neritina the same parts of the alimentary canal take up carminate of ammonia or carmine, but appear to be quite unaffected by indigo-carmine or Bismarck-brown. In F. papyrea the granules take up indigo-carmine or Bismarck-brown. The experiments made with carminate of ammonia and with carmine were unsuccessful.

Funicular Tissue.—This tissue is deeply pigmented in B. neritina, and readily takes up Bismarck-brown. In other species investigated the funicular tissue was for the most part colourless, and did not take up Bismarck-brown in the manner characteristic of B. neritina. Small quantities of this pigment and of carminate of ammonia might, however, appear in spherules apparently contained in cells of a specialised type, which may be regarded as belonging to the funicular tissue; these spherules are often pigmented in their normal condition.[1]

Young, slightly Differentiated Tissues of the Growing-points.—These tissues readily took up considerable quantities of carminate of ammonia and of Bismarck-brown.

It now remains to consider how far the experiments already described throw any light on the normal excretory processes of the marine Polyzoa.

It can hardly be doubted that the pigments experimented with were actually excreted. This was clearly seen in the action of Bismarck-brown on the funicular tissue of B. neritina. The pigment was taken up so freely that it at first appeared extremely improbable that the animals could recover; but at a later period it was deposited in an apparently insoluble form in various parts of the funicular tissue, leaving the remaining parts of that tissue quite free from it. In order to bring about this result the granules normally present in the funicular tissue had to be deposited with the Bismarck-brown

[1] For a more complete account of the absorption, by various tissues, of the pigments experimented with, see the earlier part of the paper.

they had taken up, and fresh granules had to be developed later.

Similarly the alimentary canal of B. avicularia was able to rid itself of Bismarck-brown, a process involving the loss of its normal granules, by excreting it into the lumen of the alimentary canal, enclosed in spherules noticed as of normal occurrence in this species; these spherules are probably concerned in the excretion of some of the normal pigments of the alimentary canal, although their function may be in part a digestive one.

Again, the pigments taken up by the alimentary canal, whether carmine, indigo-carmine, or Bismarck-brown, passed for the most part into the "brown bodies" at the time of the degeneration of the polypides. In F. papyrea it was definitely proved that indigo-carmine contained in the "brown bodies" left the zoœcia by way of the alimentary canals of the new polypides. In the two species of Bugula the "brown bodies," coloured by pigments taken up at an earlier period, were left behind in the zoœcia.

The leucocytes charged with indigo-carmine did not appear to take any further active part in the life of the zoœcium; but they certainly play the important part of retaining the greater quantity of the pigment which originally soaked into the tissues. The other tissues thus remain without the slightest trace of a blue coloration; and it can hardly fail to be admitted that the leucocytes are performing an excretory function, so far as they are exercising a selective absorption of an abnormal substance like indigo-carmine from the other tissues. In dead zoœcia all the tissues become at once diffusely stained with indigo-carmine. So long as the zoœcium remains alive the leucocytes retain this pigment, and shield other tissues from its action.

On the regeneration of a polypide the growing tissues are invariably quite free from the artificially introduced pigments. The exposure to the action of these bodies may in some cases result in the degeneration of the polypide or of the funicular tissue; but the young polypide-bud and its muscles, as well as the freshly formed funicular tissue, do not develop with their cells impregnated with the pigment in question. It will probably be admitted that the pigment is deleterious, and that certain tissues capable of taking it up retain it in order to allow the newly formed tissues to escape its injurious action.

It may thus be taken for granted that certain tissues of the animals investigated have an excretory function, so far as certain pigments which are abnormally introduced into the animal are concerned. Can it be further shown that these tissues, or any of them, are to be regarded as normal excretory tissues?

In the first place it may be noticed that the marine Polyzoa are not known to possess specific excretory organs. The "intertentacular organ" described by Farre,[1] Hincks,[2] Prouho,[3] and others, has not been shown to be of sufficiently general occurrence in marine Polyzoa to allow it to be regarded as the principal excretory organ. Hincks has, indeed, stated that he has "repeatedly seen a mass of excrementitious matter pass into the organ from below," and that it is "at last ejected, as a pellet, through the terminal orifice."[4] This observation seems to show that, in those Polyzoa which possess this organ, it may really serve for the excretion of the effete products of the metabolism of various tissues, and this, together with the discharge of the spermatozoa, is the function ascribed to it by Hincks. But in spite of the known occurrence of this organ in a few species, and of structures like the semilunate pore of Microporella Malusii, stated by Pergens[5] to open directly into the body-cavity, it is obviously necessary to look for other excretory arrangements in the vast majority of marine Polyzoa in which no special structures of this kind have been detected.

It may next be pointed out that one or two of the pigments used in these observations are, in other animals, specially

[1] 'Phil. Trans.,' 1837, pp. 408 and 412.
[2] 'Brit. Marine Polyzoa,' vol. i, p. lxxxix.
[3] 'Comptes rendus,' t. cix, 1889, p. 197.
[4] Loc. cit., p. xc.
[5] 'Zoolog. Anzeiger,' Jahrg. xii, 1889, p. 507.

selected by organs which are known on other grounds to have an excretory function.

Kowalevsky,[1] starting from the well-known facts with regard to the excretion of indigo-carmine and carminate of ammonia in Vertebrates,[2] has shown that both these substances are removed from the body, in many Invertebrates, by organs which are undoubtedly excretory in nature. Indigo-carmine injected into the body is excreted, for instance, by the Malpighian vessels of Insects, by the tubules of the green gland of the Crayfish (but not by the end-sac), by the organs of Bojanus in Pecten and in other Lamellibranchs, by the kidney in Gasteropods, by the brown tubes of Phacolosoma, &c., all of them organs which are admitted to have an excretory function. Carminate of ammonia was excreted by the end-sac of the green gland of the crayfish, by the nephridia of Nereis, by the excretory tubes of Tænia, &c. That the removal of indigo-carmine is really a process of excretion is shown, for instance, by the experiment recorded with regard to Paludina. On injecting this animal with a mixture of indigo-carmine and carminate of ammonia, the tissues were at first violet; after one or two days the blue colour was entirely taken up by the kidney, and the animal became red, and the red colour itself disappeared after a further interval.

Additional proof that the removal of these pigments is a process analogous to the normal excretory processes is afforded by Kowalevsky's observations that the crystals of (excreted) indigo-carmine appear, in the organ of Bojanus of Pecten, in the very vacuoles which actually contain normal excretory concretions, and that in Phallusia and in Molgula crystals of this substance make their appearance in a similar relation to the concretions which are normally present.

Kowalevsky's observations further show that these two pigments may be excreted by different parts of the same excretory

[1] 'Biolog. Centralblatt,' Bd. ix, p. 33, &c.

[2] See L. Hermann's 'Handbuch der Physiologie,' Bd. v, Theil i; 'Absonderungsvorgänge,' by R. Heidenhain, Leipzig, 1883, p. 345, and the references there given.

organ, as in the case of the green gland of the crayfish, where the end-sac excretes carminate of ammonia and the tubules excrete indigo-carmine, or as in the well-known case of the Vertebrate kidney. Similarly, the facts recorded above with regard to the Polyzoa show that the function of taking up these and other pigments is by no means restricted to one set of cells,—the leucocytes, for instance, taking up indigo-carmine, but being quite unaffected by carminate of ammonia.

Kowalevsky's results on Nereis are of special interest in connexion with my own results on the Polyzoa. In that animal Kowalevsky showed[1] that indigo-carmine is taken up principally by the blood-corpuscles, but also by certain segmentally arranged organs consisting of glandular cells lying on the dorsal side, and containing, in the normal animal, accumulations of brown or yellow bodies. This latter statement agrees closely with Eisig's results on the excretion of carmine in Capitella,[2] in which carmine taken up and digested by the alimentary canal is ultimately excreted into pigmented granules which normally occur in the skin. Eisig shows that there is considerable reason for regarding the cutaneous pigment of Capitella and of many other animals as an excretory product.[3]

The taking up of indigo-carmine by the cells which have been above described as "leucocytes" is analogous to its absorption by the blood-corpuscles of Nereis. This pigment as well as the others employed may, however, be taken up by certain normally pigmented cells occurring principally in the walls of the alimentary canal.

This fact recalls the observation of Chrzonszczewsky[4] that

[1] Loc. cit., p. 71.

[2] 'Fauna u. Flora G. v. Neapel,' Monographie xvi ("Capitelliden"), 1887.

[3] The interesting results of H. E. Durham ("The Emigration of Amœboid Corpuscles in the Starfish," 'Proc. Roy. Soc.,' vol. xliii, 1888) have not much bearing on my own observations, inasmuch as Mr. Durham investigated the ingestion of granules of precipitated pigments by leucocytes, whereas my observations concerned the excretion of solutions of pigments permeating the tissues.

[4] N. Chrzonszczewsky, "Zur Anat. u. Physiol. d. Leber," 'Virchow's Archiv,' xxxv, 1866, p. 157.

indigo-carmine is excreted in large quantities by the liver of Vertebrates, as well as by the kidney; the bile, like the urine, becoming blue soon after the injection of indigo-carmine,—suggesting a new analogy between the so-called "liver-cells" of Polyzoa and the liver of Vertebrates. Without going into the question of the excretory value of the processes which take place in the Vertebrate liver—a question I am not competent to discuss—I may express my conviction that the appearance of pigments like indigo-carmine, carminate of ammonia, and Bismarck-brown in the granules of the walls of the alimentary canal in Polyzoa, taken in conjunction with the normal appearance, in the same place, of a natural pigment, and the ultimate passage of much of that pigment into the "brown body," is to be regarded as—in part at least—a process of excretion. As has been already pointed out, Ostroumoff[1] has definitely formulated the view that the occurrence of "brown bodies" is correlated with the absence of nephridia, and indications of a similar manner of regarding these bodies are not wanting in the writings of other observers.

It is certainly a significant fact that, while the young polypide-bud is, in most cases, at first quite colourless, brown pigment soon appears in the wall of the stomach, &c.; and that when the polypide degenerates, the most conspicuous feature of the "brown body" is the pigment whose presence has suggested that name for the degenerated polypide. In some Ectoprocta the "brown body" leaves the zoœcium by way of the alimentary canal of the new polypide; in others it is simply left behind in the zoœcium, just as, in many Tunicates, the excretory concretions are stored up in various parts of the body without ever finding a way to the exterior.

It has already been pointed out that in B. avicularia Bismarck-brown was excreted into the lumen of the alimentary canal, enclosed in spherules which were also noticed in the normal condition. This fact tends to show that the spherules in question are, in part at least, excretory, although their

[1] A. A. Ostroumoff, "Cont. à l'Ét. Zool. et Morphol. des Bryozoaires du Golfe de Sébastopol," 'Arch. Slaves de Biol.,' t. ii, 1886, p. 339.

formation may not be altogether unconnected with the process of digestion. Instances of excretory processes carried on by the walls of the alimentary canal are, however, by no means unknown in other animals.

In this connexion, too, Eisig's results on the Capitellidæ[1] must be further cited. Eisig gives a most elaborate discussion of the excretory value of many natural pigments, not only in the Capitellidæ, but also in various other animals.[2] The facts observed by myself with regard to the appearance of indigo-carmine and other pigments in cells which are normally pigmented is in complete accord with Eisig's results; tending to establish the conclusion that these normal pigments are to be interpreted as, to a considerable extent, excretory in nature. As instances of this may be mentioned the deposition of these pigments in the granules of the wall of the alimentary canal, and in the granules of the funicular tissue of B. neritina; the appearance of carminate of ammonia in cells of the type shown in Pl. III, fig. 22, these cells also containing natural brown pigments; and the association of indigo-carmine with natural pigments in the leucocytes of F. papyrea (figs. 23 and 24).

The general conclusion of the experiments described above is that excretion is performed, in the marine Ectoprocta, partly by the cells which have been described as "leucocytes," partly by the walls of the alimentary canal, and partly by the funicular tissue. The so-called "lateral cords" probably play some part in excretion, although I am by no means confident that this is their principal function.

A few remarks may be made with reference to Cuénot's recent paper[3]—the general conclusions of which had been

[1] Loc. cit.

[2] I have had the opportunity of looking through the manuscript of a paper which is to be shortly published by Mr. H. E. Durham, who has brought together a large series of observations tending to establish the same conclusion.

[3] L. Cuénot, "Études sur le sang et les glandes lymphatiques dans la série animale. 2e Partie: Invertébrés," 'Arch. Zool. Exp. et Gén.,' 2e sér., t. ix, 1891, pp. 12 and 365.

given in the preliminary note already referred to,—which has come into my hands since the above was written. Cuénot throws doubt on the value of the evidence given by the reaction of the several tissues to pigments such as indigo-carmine in elucidating the nature of the excretory processes; and supposes that the absorption of carmine by the pericardial tissue of Insects, for instance, is due to the affinity of that tissue for colouring matters (see p. 398). The enumeration given by Cuénot of the details of the absorption of various pigments by certain tissues to which an excretory function is usually not ascribed does not seem to me so convincing, in Cuénot's sense, as the demonstration by Kowalevsky and others that these pigments are selected by organs known to be excretory in nature in the opposite sense. Cuénot shows that the leucocytes of Polyzoa are quite similar to the "amibocytes" of other animals (see p. 407), the most important function of which would appear to be the transformation of the peptones thrown into the blood by the alimentary canal into non-dialysable albuminoids. The same author alludes to the formation of "pseudo-plasmodia" by the "amibocytes" of various animals (cf. the description given on p. 131 of a similar process in Flustra); and mentions more than once the occurrence, in various Invertebrates, of cells whose granules exhibit a Brownian movement, and which he usually regards as of respiratory nature ("hématies"). I have not, however, found in Cuénot's figures or text an account of any cells which can be exactly compared with the remarkable cells above described in Bugula avicularia (see p. 135).

EXPLANATION OF PLATES II and III,

Illustrating Mr. Sidney F. Harmer's paper "On the Nature of the Excretory Processes in Marine Polyzoa."

(All the figures were drawn from living animals.)

PLATE II.

Flustra papyrea (indigo-carmine experiments: all the figures were drawn with a camera lucida, under a Zeiss' C objective).

Fig. 1.—264th hour. Front view. The blue leucocytes are grouped round the "brown body," in which can be clearly distinguished the remains of the tentacles and two green masses derived from the granules of the alimentary canal, which had taken up indigo-carmine in addition to their normal pigment. A young polypide bud, slung in a cord of funicular tissue attaching it to the "brown body," is already present.

Fig. 2.—286th hour. Front view, showing the condition of the lophophore when half rotated (leucocytes not represented).

Figs. 3—6.—Successive stages of a single polypide.

Fig. 3. 305th hour. Front view. The "brown body" is more compact than in Fig. 1, and is situated at the extreme proximal end of the zoœcium. Its two green masses are approaching one another. The tentacle-sheath has met the new aperture, which is in process of formation.

Fig. 4. 376th hour. Front view. The "brown body" has become much darker, and its two green masses (which are not very distinctly visible, lying, as they do, at the other side of the "brown body") are beginning to fuse. The cæcum of the stomach has met the "brown body," and the proventriculus is growing out to meet the œsophagus.

Fig. 5. 448th hour. Back view. The cæcum has nearly half covered the "brown body." The intestine is bent over to one side, and the rectum contains the "meconium."

Fig. 6. 496th hour. Back view. The "brown body" is now much nearer the middle of the zoœcium than in earlier stages, and has a process projecting into the lumen of the alimentary canal, indicating its approaching absorption (leucocytes not represented).

For later stages in the absorption of the "brown body" in this species, see Haddon's figs. 9, 10, and 11 (this Journal, vol. xxiii, Pl. XXXVII).

Figs. 7 and 8.—From an experiment in which very little indigo-carmine had been taken up by leucocytes. 283rd hour. Back views. Polypide buds and other structures not represented. Notice the indigo-carmine contained in the two green masses in the "brown body."

Fig. 7. The zoœcium has a somewhat abnormal shape. The "lateral cords" contain a quantity of indigo-carmine, and are less uniform in calibre than in zoœcia with functional polypides.

Fig. 8. The lateral cords have broken up into several portions.

Fig. 9.—From an experiment in which the leucocytes, &c., had absorbed an unusually large quantity of indigo-carmine. 476th hour. Back view. A new polypide bud is developed. The "brown body" appears to be absorbing indigo-carmine from the leucocytes, which have, however, deposited the greater part of their pigment in continuous deep blue strands situated in the body-cavity.

PLATE III.

Figs. 10—12.—F. papyrea. Successive stages in the formation of the (ectodermic part of the) polypide bud (Zeiss, DD).

Fig. 13.—F. papyrea. Normal excretory (?) cell from endocyst, with natural pigment (Zeiss, $\frac{1}{12}$ oil immersion).

Fig. 14.—F. papyrea. Normal funicular tissue, with colourless refractive granules, giving a starch-like reaction with iodine (Zeiss, F).

Fig. 15.—F. papyrea. Bismarck-brown. 213th hour. Portion of one of the lateral cords (Zeiss, F).

Fig. 16.—Bugula avicularia. Indigo-carmine. 72nd hour. Side view. Rough sketch of the alimentary canal, to show the distribution of the granules which take up indigo-carmine. Parts of the stomach, &c., are green, as the natural blue of the indigo-carmine contained in the granules is masked by the normal pigment of these structures. In the rectum, in which the granules are practically colourless, the blue colour is not obscured (Zeiss, DD).

Fig. 17.—B. neritina. Indigo-carmine. 316th hour. Back view. The polypide, which has been completely developed since the beginning of the experiment, is not quite mature. The arrangement of the leucocytes in the meshes of the funicular tissue is well seen (Zeiss, C).

Fig. 18.—B. neritina. Bismarck-brown. 143rd hour. Portion of the funicular tissue of a colony in which a very large quantity of the pigment had been absorbed. At *A* is seen a cell whose granules have an almost normal appearance. The greater part of the pigment taken up by the funicular tissue has been deposited in the form of dense brown masses, leaving the rest of the tissue hyaline. Most of the vacuoles of the leucocytes contain Bismarck-brown in solution, but one or two of them still remain colourless (Zeiss, F).

FIG. 19.—B. neritina. Indigo-carmine. 64th hour. Three leucocytes (Zeiss, F).

FIGS. 20—22.—B. avicularia.

Fig. 20. Bismarck-brown and indigo-carmine (mixed). 40th hour. Three leucocytes containing indigo-carmine, and two of the cells which contain minute granules exhibiting a Brownian movement, or larger concretions. These cells appear to have taken up minute quantities of Bismarck-brown and of indigo-carmine (Zeiss, F).

Fig. 21. (Same treatment as Fig. 20.) Two leucocytes and one of the cells with colourless granules found in the body-cavity of this species (Zeiss, F).

Fig. 22. Carminate of ammonia. 330th hour. Two leucocytes, whose natural colour is unaltered, and several of the well-defined spherules, found especially near the growing-points, and containing carmine as well as natural brown pigments (Zeiss, F).

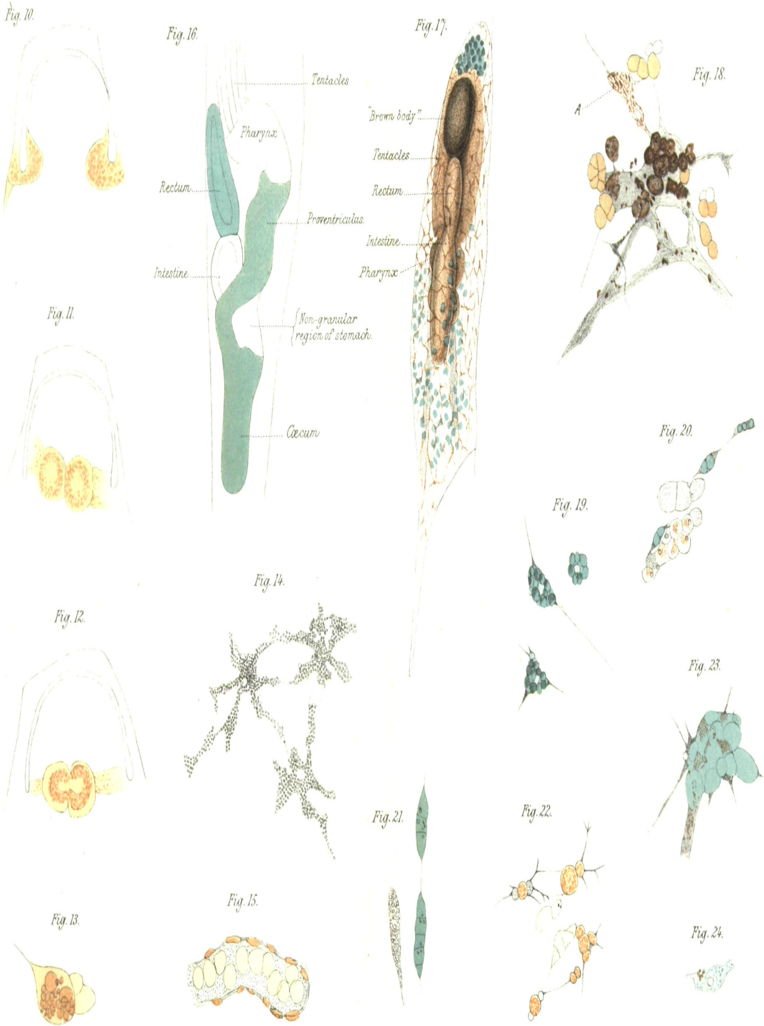
FIGS. 23 and 24.—F. carbasea.

Fig. 23. A complex of leucocytes which have taken up indigo-carmine, and which contain granules of deposited indigo-carmine as well as natural pigments (Zeiss, F).

Fig. 24. An individual leucocyte (Zeiss, F).

F. Harmer del.

F. Huth, Lith.r Edin.r



S.F. Harmer del.

F. Huth, Lithr. Edinr.