The Collar-cells of Heterocœla.

By

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With Plate 2.

Summary.

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

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

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

Prefatory Remarks.

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

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

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

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

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

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

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

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

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

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

Mesocyte. A parenchym cell.

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

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

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

General Structure of the Living Collar-cells.

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

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

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

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

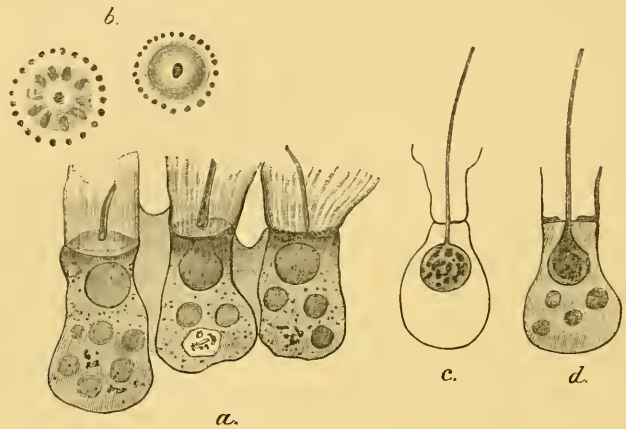
Collar.

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

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

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

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



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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Flagellum.

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

Intra-choanal Area.

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

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

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

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

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

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

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

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

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

Nucleus.

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

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

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

acid alcohol, shows more often a fine, stained, tapering point, forming a distal prolongation to the nucleus, issuing through the pupil of the iris as the flagellum (cf. cut, *d*). In the nuclei of a preparation treated with ½ per cent. osmic acid, stained in bulk with borax-carmine, and on the slide with hæmatoxylin, the two forms are also seen: where the nucleus is spherical the flagellar radix is seen as a faintly-stained thread piercing the dark nuclear membrane (cf. cut, *c*); where the nucleus is pointed, the point—that is, the radix of the flagellum—can often be seen to be a protrusion of the nuclear membrane. In either case the nuclear membrane is interrupted, so that in profile the outline shows a clear break opposite the flagellum.

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

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

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

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

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

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

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

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

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

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

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

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

Interstitial Substance.

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

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

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

Pathological Changes.

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

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

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

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

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

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

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

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

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

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

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

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

Apparent migration of collar-cells into the parenchym

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

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

Nutrition.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Distortion of Cells in Preservation.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

There are, therefore, two principal phenomena due to the

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

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

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

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

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

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

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

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

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

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

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

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

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

Methods.

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

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

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

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EXPLANATION OF PLATE 2,

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Fig. 12.—See fig. 10.

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

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

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

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

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

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

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

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

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

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

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

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

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

Scale × 1000 10 5 0 10 20 30 40 50 thousandths of a millimetre

Fig. 1.

Fig. 2.

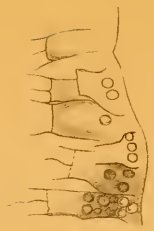


Fig. 3.

(× 2500)

Fig. 4.

Fig. 6.

Fig. 5.

Fig. 7.

Fig. 10.

Fig. 11.a

Fig. 11.b

Fig. 9.

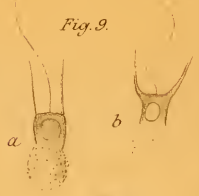
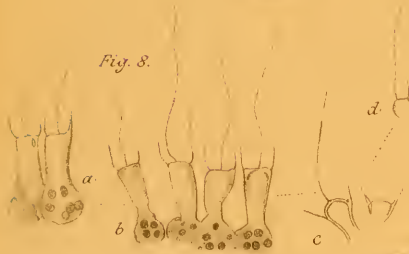


Fig. 8.



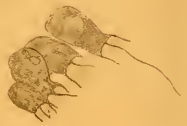


Fig. 11.c

Fig. 12.

Fig. 13.

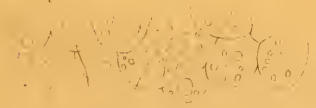


Fig. 15.

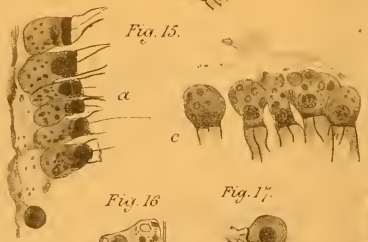


Fig. 20.

Fig. 13.a

Fig. 14.

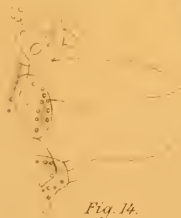


Fig. 16

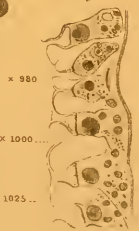


Fig. 17.

Fig. 18.

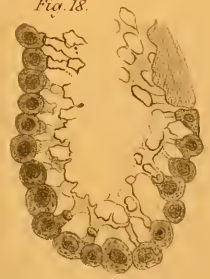


Fig. 19.

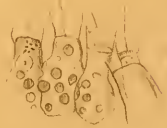


Fig. 22.

Fig. 21.