The Origin and Nature of the Green Cells of Convoluta roscoffensis.

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With Plates 13 and 14.

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SECTION I. INTRODUCTION.

Xanthellæ and Chlorellæ are widely distributed among
the members of the basal groups of the animal kingdom. In
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some animals they are present constantly, in others they occur
sporadically.

Green, yellow, or brown cells have been described in
representatives of every division of free-living Protozoa, in
certain sponges, in most anthozoan Coelenterates, in a few
Hydrozoa and Scyphozoa, and in acephalous and rhabdocoelous
Turbellaria. Their occurrence in the higher groups is rare—
e.g. Zoobothrium (Polyzoa), Elysia (Mollusca), and Echino-
cardium (Echinoidea).

The association is obligate in Convoluta paradoxa,
C. roscoffensis, and in Hydra viridis; it is facultative
in the Protozoa, Anthozoa, and rhabdoceel Turbellaria.

In the former cases every individual of a species possesses
green or yellow or brown cells; in the latter cases only
certain individuals contain them.

Facultative association may exhibit itself in another manner:
in one part of its range all the individuals of a species may
exhibit the association, in another part the coloured cells
may be absent from all the individuals. Thus Noctiluca is
colourless in the North Atlantic and green in the Indian
Ocean. British Alcyonium have no zoochlorellae, whereas the
closely allied A. ceylonicum possesses them (Pratt, 1905).
It seems probable, indeed, that the maximum development
of these associations occurs in the warmer seas. According
as the association is facultative or obligate, it gives rise to a
less or greater modification in the behaviour and in the
structure of the animal.

Convoluta roscoffensis exhibits in a striking degree
such modifications (Gamble and Keeble, 1903). It lives
gregariously at mid-tide level on the beach and exposes
itself to the bright light of mid-day. Its colourless young
are as strongly phototropic as the green adults. With the
advent and multiplication of the green cells it ceases to ingest
solid food. Structural changes described in the body of this
paper also follow consequent upon the association of animal
and green cell.

Similar phenomena are exhibited by other animals. Thus
Cassiopeia, a medusa of warm seas, has adopted, as a consequence of the association with itself of green cells, a posture the reverse of that maintained by its allies. On certain reefs it may be seen fixed by its aboral surface, exposing its oral arms, and so the coloured cells contained therein to the light.

Various reef corals, richly provided with zoochlorellæ, show marked decrease in the size of their tentacles; in this respect, and also in the number and degree of development of their digestive filaments, they present a sharp contrast with their more freely feeding allies (Pratt, 1905). Facts such as these lead naturally to the hypothesis that the association of animal and green cell results in a supply of nutriment to the former from the latter.

Admitting, as it is reasonable to admit, this trophic hypothesis, it still remains to inquire—as we do in the case of Convoluta roscoffensis in the following sections—what food-substances are transferred from green cell to animal and how this transference is effected.

It is important to know how this hypothesis stands with respect to such cases of voracious feeders as anemones, Hydra, Convoluta paradoxa, and many green rhabdocells. Here it is clear that the animal is not subdued to the working of the “plant” within it.

It may also be asked how this at present vague hypothesis meets the cases of facultative association. One of the two main purposes of this paper is to put forward a supplementary hypothesis (Section VI, page 205), which we think throws new light on the significance of chlorellæ and xanthellæ in animals. The other purpose of this paper is to provide absolute proof of the source of origin of the green cells in Convoluta roscoffensis.

The view generally held, that the green or brown corpuscles of animals are of algal nature, is based rather on probability than on certain evidence.

The usual arguments in favour of this view are as follows: The corpuscles in question have been shown in certain cases to be capable of photosynthesis. In the light, gases
containing a high percentage (15–50) of oxygen may be evolved from the animals. Starch may be present in the corpuscles. They have been shown to contain chlorophyll, patent or masked by other pigment. Structurally the chlorellae resemble certain algal cells. A wall of cellulosic or pectose substance, absent in some cases, may be present. The corpuscles may contain a nucleus, a pyrenoid, and occasionally an eye-spot. This circumstantial evidence is strong, but cannot be said to amount to proof.

Evidence of the origin of the green or brown cells to be final must be of a like nature to that demanded by pathologists in the case of a micro-organism suspected of pathogenic properties; the organism must be isolated in pure culture and the infection-test applied.

So in the cases of animals infected with green cells; these cells must be isolated, and, by introduction into the body of an animal previously free from them, be shown to give rise to the normal green animal. In other words, the final proof of the algal nature of the green cells can be provided only by the synthesis of the green organism from its algal and animal components. This synthesis has not as yet been effected in an indisputable manner in any single case. The green or brown cells of Turbellaria, Coelenterates, or sponges have not as yet been isolated and cultivated.

Beijerinck (1890) and Entz (1881–1882), it is true, made cultures of green hydra, but owing to the proneness of the cultures to infection from within and from without both the authors and their critics have regarded the results with suspicion, especially in view of the fact that attempts at synthesis were unsuccessful.

Among green protozoa two or three cases of alleged isolation of the corpuscles have been recorded. By macerating the bodies of Stentor, Paramecium, and Frontonia, Faminzin (1889–1891), Dantec (1892), and Dangeard (1900) have obtained colonies of algae. The results are, however, somewhat discrepant, for whereas the first two authors regard the alga of Paramecium as a true chlorella, the third considers it
to be a chlamydomonas. Schewiakoff (1891) successfully fed colourless Frontonia on macerated green specimens, and states briefly that multiplication of the green cells within the host occurred. Dantec obtained a like result with Paramecium.

Brandt (1883), after depriving sea anemones of their yellow cells by long confinement in the dark, caused the reappearance of these cells by placing the animal in fresh sea-water in the light. But from experiments made by ourselves, we venture to express some doubt as to whether the apparently colourless animal had lost absolutely all its yellow cells.

Haberlandt (1891), whose admirable work on *Convoluta roscoffensis* is referred to in the text of this paper, was unsuccessful in his attempts to cultivate the green cells of this animal. Pending more exact information it seems to us that Lankester (1882 and 1890) has done valuable service by his championship of the opposed view, that of the intrinsic nature of the corpuscles under discussion. For his view compels those who hold the "algal" theory to investigate each case separately and to vindicate their view by the synthesis of the green animal.

When this has been done in such cases as those in which cell-wall and nucleus are present in the corpuscles, there will remain others—e.g. Hydra and Spongilla, whose green cells are devoid of definite nuclei, and after these, such puzzling instances as *Vorticella campanula* (Engelmann, 1883), with its diffuse chlorophyll, as well as *Pelomyxa viridis* (Bourne, 1891), the green corpuscles of which seem difficult of explanation except on Lankester's hypothesis.

Influenced by the forementioned considerations, we have investigated the origin of the green cells of *Convoluta roscoffensis*. The results of this investigation are set forth in Sections II—V.

The experimental work recorded in this paper has been carried on in the laboratory at Trégastel, Côtes-du-Nord,
France; in the Zoological Department, Victoria University, Manchester, and in the Botanical Laboratory, University College, Reading.

We acknowledge with gratitude the assistance we have derived from a grant made by Section D of the British Association for the purposes of this investigation.

SECTION II. PROOF OF THE ORIGIN OF THE GREEN CELLS BY INFECTION.

The dark spinach-green colour of Convoluta roscoffensis is due, as is well known from the descriptions of von Graff (1905–1906), Geddes (1879, 1879 A, 1882), Haberlandt (1891), and ourselves (1903), to dense layers of green cells. These green cells are distributed with great uniformity in the body, and extend from just below the epidermis into the deeper tissues. Only in the anterior end of the body, in front of the otocyst and rudimentary eyes, are the green cells so few in numbers as to reveal the whitish colour of the animal.

The general appearance of an adult Convoluta, when examined microscopically, is not unlike that of the mesophyll of a green leaf. In surface view the green cells are flat, somewhat variously shaped bodies, now of rounded outline, now drawn out at one end into long tail-like extensions which appear to connect cell with cell.

The individual cells, described by Haberlandt (1891) in his important contribution to our knowledge of the green cells of Convoluta, are naked protoplasts, the larger part of each of which consists of a more or less cup-shaped chloroplast containing a large polygonal or irregular pyrenoid. The small remaining part of the protoplast is colourless, and lies either in the hollow cup-like invagination of the chloroplast or, when the shape of this latter is irregular, excentrically.

Though often free, or almost free, from starch, the green cells may, under certain conditions, contain considerable quantities both of pyrenoid starch—that is, starch distributed
in the layer surrounding the pyrenoid (the starch sheath)—
and also of minute rods or granules scattered irregularly
throughout the substance of the chloroplast.

Two views as to the nature of the green cells have been
expressed—one, that they are algae which have been ingested,
and which have developed within the animal body, the other,
that they are integral parts of the animal tissue.

The observations of Georgevitsch (1899) that the just
hatched young are colourless lend support to the former of
these views, but do not suffice to establish it. For, since the
animals raised by Georgevitsch—hatched in filtered water—
only survived for three days, it is conceivable that ante-
cedents of green cells were present in a colourless form, but
that, owing to the short period during which the animals
lived, these colourless antecedents failed to give rise to the
green cells.

Our own observations (Section V) that the earliest recog-
nisable stages of the future green cells may be colourless
justify this reservation, lending no more support to the infec-
tion-hypothesis than to the suggestion of Haberlandt that the
green cells may be derived from colourless plastids, the per-
sisting remnants of once independent algae now transmitted,
as are the plastids of green plants, by the egg-cell.

In order to determine finally the origin of the green cells
of Convoluta, it is necessary, in the first place, to maintain
newly hatched animals under such conditions that infection
—if infection there be—cannot occur, and, in the second
place, if the result of the foregoing is the production of
colourless Convolutas, to expose the animals to infection
and to determine whether this exposure brings about the
development of green cells.

As we state in the Introduction to this paper, this rigorous
proof of the intrusive nature of the green or yellow cells of
animals has not hitherto been obtained in any single case,
we therefore describe in detail experiments which fulfil these
conditions, and which have led to a decisive result in the
instance of Convoluta roscoffensis.
In our previous work (1903), where strong, but not decisive, evidence in favour of the infection-hypothesis was given, we showed that it was possible to maintain young Convoluta alive and colourless for several weeks. The freshly laid egg-capsules containing the groups of fertilised eggs were—in these experiments—removed from the neighbourhood of adult animals and placed in sea-water which had been filtered by means of a Pasteur-Chamberland filter. The animals hatched out in the course of two or three days as colourless larvac and remained colourless for two or even three weeks (Table II, Columns A and D). Samples of these colourless animals taken at any time during this period and placed in fresh, unfiltered sea-water became green in three or four days.

So far the result of this experiment pointed most definitely to the environment as the source of the green cells; but this conclusion was rendered less certain by the subsequent behaviour of the animals reared in filtered sea-water. Certain among these, sometimes few, sometimes many, ultimately became green, and on microscopic examination were found to be possessed of normal green cells (Table II, Columns B and C). The sporadic and tardy appearance of green individuals among the Convoluta hatched in filtered sea-water could be accounted for best on the supposition that small numbers of the infecting organism had been introduced with the egg-capsules into the filtered water, and that the three or four weeks which elapsed before infection took place were required for the multiplication of this organism. Microscopic examination of the egg-capsules showed the high probability of this supposition, for they were found to be infested, even a day or so after they had been laid, and before the young had escaped from them, with numberless green and colourless cells of various kinds.

To eliminate this source of error it was necessary therefore to obtain capsules as clean as possible, and to take the precaution of isolating the young from this source of infection. This we succeeded in doing by the following somewhat laborious process.
Batches of Convoluta were collected in watch-glasses from the shore, care being taken to exclude particles of sand and other foreign matter. The animals so obtained were washed a number of times in filtered sea-water, and were then allowed to descend into a large glass dish containing filtered sea-water. This dish was illuminated unilaterally, and as soon as Convoluta had taken up its markedly positive phototropic position the dish was tilted, the water drained away, and the bottom of the dish cleaned. A fresh lot of filtered sea-water was added, and the dish so turned that the animals were impelled to crawl across to the opposite side. As soon as they had taken up their new light-position the tilting and cleaning were repeated, filtered sea-water was again added, and the vessel turned once more. In some experiments these processes were repeated as many as eight times. When the Convolutas in the dish began to lay, the egg-capsules were picked out daily and placed in glass vessels containing filtered sea-water. As a result of these precautions the capsules were much freer from those organisms which, when no precautions are taken, habitually infest them; and larger numbers of Convolutas hatched out under these conditions remained colourless. Nevertheless, even with these precautionary measures some green animals ultimately appeared among a great majority of colourless Convolutas. Hence an additional safeguard from possibility of infection had to be employed—namely, to separate the young as nearly as possible at the moment of hatching from the egg-capsules. This, though a tedious, was not a difficult, operation owing to the fact that when on the point of hatching, three or four days after the capsules have been laid, it suffices, in order to release the larvae, to take up the capsule in a fine pipette and to eject it with some slight force into the water. Numbers of animals were obtained in this manner, free or almost free from capsule-remnants. Absolute freedom it is almost impossible to obtain, owing to the extreme tenuity and transparency of the capsules at the time of hatching.
As Table III shows, we have, by adopting this procedure, succeeded in obtaining batches of *Convoluta* which remained absolutely colourless and uninfected.

The several columns of this table give the results obtained in the cases of:

1. Animals left in association with their capsule-remnants.
2. Animals removed from their capsule-remnants.
3. Animals which, having been so removed, were subsequently submitted to the risk of infection by placing them in fresh unfiltered sea-water.
4. Animals hatched without any precautions in ordinary sea-water.

The animals hatched in unfiltered sea-water became uniformly green after two or three days. Among those left with capsule-remnants infection occurred less rapidly and more sparingly. Thus, as Columns 3, 4, 5 of Table III show, eleven individuals out of fifty-nine became infected during the eight days which followed after general infection had declared itself among the animals reared in unaltered sea-water. After seventeen days, infection had become general in all these cases (Columns 3, 4, 5).

The contrast between this result and that set forth in Columns 1 and 2 of the same table is emphatic and conclusive. In those cases (Columns 1, 2, Table III) in which the animals had been separated at the time of hatching from their capsule-remnants the total numbers of animals showing infection were, in the one experiment (Column 1), five out of forty-four animals examined, and in the other none out of forty-seven examined. We conclude, therefore, that the green cells of *Convoluta* are of intrusive origin, or, to use the terms employed already, that they arise as the result of an infection from the water of the sea. From a single infecting cell, or at most from two or three, are produced the vast numbers of the green cells of the adult *Convoluta*: infection taking place normally during the first three days after hatching.

Experiments made in the course of this inquiry enable us to answer two other questions—viz. May the green cells of an
infected Convoluta escape from the body, live freely again, and again infect a young animal? and what is the origin of the cells which in the preceding experiments developed on the capsule and subsequently infected the larval Convolutas? are they derived from the interior of the body of the egg-laying parent or from the environment?

With respect to the first question, we have failed, as Haberlandt failed before us, to cultivate in nutrient media green cells liberated from the body of the adult. But more convincing than these negative results are those set forth in Table I (Column A) and Table III (column headed "Filtered sea-water with adults"). In these places are recorded the results of experiments in rearing young animals in association with large numbers of adults which had been washed many times in filtered water. Under these circumstances the larvae, though surrounded by great numbers of adults, many of which, having ruptured in the course of egg-laying, had discharged large numbers of green cells, failed for twenty days to show sign of infection. It thus appears certain that the ordinary green cells, as they exist in the body of one animal, are incapable on their discharge from that animal of infecting another. Indeed, under no conditions known to us do the green cells of a Convoluta ever escape alive from the body. Each Convoluta leaves the body of the mother as a colourless, uninfected animal; as such it hatches out from its capsule, and the cells which then infect it are derived from the environment, neither they nor their direct ancestors ever having been before within the body of a Convoluta. We return to this matter again in Section V, where we give an account of the histological changes which the green cells undergo in the course of their development in the body of Convoluta, and show that these changes account for the at first sight extraordinary facts just described.

There remains to be considered the second question—as to the origin of the green cells which make their appearance on the capsules and which served in various of the foregoing experiments as sources of infection to larval Convolutas.
These cells must have been derived from one or other or both of two sources—viz. from the body of Convolutas disintegrated during egg-laying or from the environment. In the former case they must be special cells which, unlike the great majority of the green cells of the adult body, have undergone no degenerative changes, and so retain the capacity for development.

The evidence points to the environment as the place of origin of the green cells of the capsules. In the first place, such cells are by no means uniformly present on the capsules, and in the second place they are present less often on capsules laid by animals which have been freed in some measure by repeated washings from their associated flora and fauna. The effect of this repeated washing is not to render the surfaces of the animals free from extraneous organisms, but to reduce their numbers and to confine them to such as attach themselves most tenaciously to the slime which covers the surface of Convoluta. Chief among these most intimate associates are the infecting alga, certain other minute unicellular algae, and various diatoms. Repeated washings, then, reduce the number of competitors for place on the capsules, and though some of the infecting organisms may themselves be swept away those which remain find their tenancy when they succeed in reaching a capsule less disputed than is the case under more natural conditions.

In the third place, as we show (Section IV), the infecting alga does not depend on chance for its association either with the surface of the animal or with the egg-capsule. It is attracted chemotactically thereto, and hence, though the numbers of infecting algae in the water should be but few, they will inevitably distribute themselves upon some of the capsules.

Summary of Section II.

(1) Convoluta roscoffensis commences life as a colourless (non-green) animal.
(2) At this stage it has no germ of infection within its body.
(3) Infection occurs normally within three days of "birth."
(4) Water taken from the shore—not necessarily in the
neighbourhood of Convoluta-patches—contains the infecting
organism in such numbers as to induce wholesale infection in
large batches of larvae.
(5) The infecting alga habitually settles down and de-
velops on the egg-capsules, which therefore serve as sources
of infection (see also Keeble and Gamble, 1905).
(6) Infection does not take place directly or indirectly from
the body of the parent.
(7) The green cells of an adult Convoluta are incapable of
life apart from the body of the animal.
(8) Consequently the association must be regarded, not as
a symbiosis, but as a case of parasitism, the host being the
green cells and the parasite Convoluta (see also Section VI).

Section III. The Isolation of the Infecting Organism
and the Synthesis of the Green Convoluta.

It is natural that, in seeking to isolate the infecting
organism of Convoluta, effort should be directed first toward
the cultivation of green cells obtained from the body of the
green animal. Haberlandt (1891) was the first to attempt
this. His efforts were unsuccessful. We have attempted it
again and again, but have failed. Miss Harriette Chick, who
has had much experience in such work, was good enough to
make in the laboratory at Trégastel in 1905 a large number
of culture experiments, using a great variety of nutrient
substances in liquid and solid media. The experiments gave
no positive result. These failures make it in a very high
degree probable that the task is impossible, and so lend some
support to the conclusion arrived at in the preceding section
that the green cells of Convoluta, once developed within
the body of that animal, are no longer capable of separate
existence.
It became necessary, therefore, if the search for the
infecting organism was to have a successful termination, to begin at the other end, namely, to seek for the organism before its entrance into the body of Convoluta.

Our observation that infection may take place, not only from fresh sea-water, but also from the remnants of egg-capsules laid in filtered sea-water suggested a mode whereby this search might be prosecuted with some hope of success: the mode being the isolation and observation of egg-capsules from which the larvae had escaped. The isolation was necessary because, if left with the young animals, the capsules disappeared, either being torn to fine shreds by the frequent entrances and exits of the larvae or, perhaps, being devoured by these larvae. Accordingly, numbers of egg-capsules obtained from well-washed animals were put into filtered sea-water, and as soon as the young had emerged from them the transparent, gelatinous remains of the capsules were removed to another vessel of filtered sea-water. There they were kept under daily observation. After seventeen days (Table III, columns 3 and 5) several green spherical bodies of about the size of the egg-capsules made their appearance. Microscopic examination showed that these spherical bodies were composed each of a pure culture of vast numbers of a unicellular green organism. During the examination the slight pressure of the cover-glass sufficed to burst the delicate membrane of the green spherule and a swarm of active, flagellated cells emerged, leaving behind the recognisable remains of an egg-capsule (figs. 1, 2, 3, 4, Pl. 13). These flagellated cells, a detailed description of which is given in Section IV, presented so many features in common with the green cells of Convoluta roscoffensis as to leave but little doubt that they represented a free stage of these cells: the cup-shaped chromatophore containing a polygonal pyrenoid, the colourless part of the protoplast occupying, as is sometimes the case in the green cells of the animal, the narrow cavity of the cup, the red, lateral-lying eye spot, also to be met with in the green cells of recently infected young Convolutas, all pointed to this conclusion.
THE GREEN CELLS OF CONVOLUTA ROSCOFFENSIS.

It only remained, therefore, to apply the inoculation test—that is, to submit colourless, uninfected animals reared in filtered water to the chance of infection by these flagellated cells. A batch of such animals was divided accordingly into three groups. One, serving as a control, was maintained in filtered water; another, also a control, was placed in unfiltered sea-water in order that its capacity for infection might be tested; the third was put into filtered sea-water to which numbers of the flagellated organism had been added. The result proved conclusively that these flagellated cells are a stage in the life-history of the infecting organism. Group 1 in filtered sea-water remained uninfected; Group 2 in unfiltered sea-water showed the susceptibility of the animals to infection: they became green in the course of three days; Group 3, exposed to infection by the flagellated cells, were observed to ingest these cells, to tolerate their active division, and to become in consequence normal green Convolutas. Subsequently, when we had perfected our procedure so as to be able to obtain fairly constant supplies of the flagellated cells, we repeated the experiment frequently, and in all cases with the same result. Thus Convoluta has been synthesised from its elements the colourless animal and the green, flagellated cell.

Summary of Section III.

(1) We have isolated and cultivated outside the body of Convoluta its infecting organism.

(2) It is not from the green cells of the body of Convoluta that the infecting organism may be isolated, but it may be obtained readily from the remnants of the egg-capsules.

(3) The infecting organism which occurs sporadically on the egg-capsules is derived, not from the green cells of the body of the parent, but from free cells frequenting the surface of the body at the time of egg-laying.

(4) If to filtered sea-water containing colourless Convoluta the infecting alga is added the synthesis of the green animal results.
SECTION IV. THE LIFE-HISTORY OF THE INFECTING ORGANISM.

The green spherules from which the swarms of flagellated cells issued as described in the preceding section served as a starting-point for the cultivation of the green alga which, as just shown, is the source of the green cells of Convoluta. The securing of material for this purpose was rendered comparatively easy owing to the well-marked positive phototropism of the alga in its motile stage. Issued from the egg-capule, the flagellated cells swarm toward the more brightly illuminated side of the vessel in which they are contained; there they settle down sooner or later, either singly or in pairs, along and just above the water-line. Thus the position of the algae is marked by a visible green patch. This patch consists of numbers of flagellated cells, and also of many which, having withdrawn their flagella, have surrounded themselves with a well marked and often stratified wall. A sample from such a patch was transferred by means of a platinum loop to a vessel containing filtered sea-water, to which a little potassium nitrate had been added, and in which had been placed a number of empty egg-capules. After some days a green streak along the water-line made its appearance on the brighter side of the vessel. The vessel was taken from Trégastel to England (Reading) in September, 1905, placed in the light in a cool incubator, and kept under observation. The green scum gradually disappeared, and it was feared that the organisms had died. Toward the end of May, 1906, the vessel was placed on a bench in the laboratory in a good light, and within a fortnight a green scum reappeared on the illuminated side of the vessel. Microscopic examination showed the identity of the organisms constituting this scum, with those added to the water the previous year. Beside the green layer on the side of the vessel loose masses of pale green mucilage, floated up to the surface by reason of included gas-bubbles, made their appearance. Imbedded in these mucilaginous masses were numbers of quiescent green cells, lying singly, in pairs, or in groups.
Subcultures in various media were made from this material. Some of these were taken back again to Trégastel in the summer of 1906, and proved sufficient for the infection of colourless young Convolutas. The cultures have also served to demonstrate that the alga which infects Convoluta roscoffensis has a very varied life-history. In the first place, the active flagellated cells are dimorphic. The macrocytes, 16 µ in length (figs. 4, Pl. 13 and 12 A, Pl. 14), are nearly double the size of the microcytes (4 : 2.5) (figs. 3, Pl. 13 and 12 B, Pl. 14). Except in point of size the large and small cells are similar in their histological details.

Both the large and small cells have four equal flagella arising from the anterior colourless part of the protoplast. The flagella in both bear the same relation to the length of the body (2 : 1). In both the chloroplast is cup-shaped, the pyrenoid single, the eye-spot lateral and situated in the anterior half of the cell. In both the wall is extremely delicate and gives no cellulose reaction—e.g. with sulphuric acid and iodine or with Schultze's solution or calcium chloride-iodine; but with zinc chloride-iodine it gives a faint rose-colour (chitin reaction).

The nucleus—a description of which is given in Section V, where a comparison between the free and imprisoned cells is drawn—lies, in both large and small cells, in the colourless part of the protoplast which fills the hollow of the cup-shaped chloroplast. It suffices to say here that the nucleus is suspended in a layer of the protoplast from which run strands, two downwards, serving as slings for the pyrenoid, and others outward through the chloroplast at regular intervals to meet the thin, colourless layer of protoplasm which forms a pellicle around the exterior of the chloroplast.

Large and small cells are alike equally phototropic, and both settle down after a period of activity, withdrawing their flagella and surrounding themselves with a more or less thick mucilaginous wall. The wall may form with great rapidity, so that encysting macrocytes are often to be met with whose flagella may be seen in undulating movement within the enclosing wall.
The resting-cells (Pl. 14, figs. 11 and 16) vary considerably in form and in behaviour. Thus, single flagellated cells may come to rest, withdraw their flagella and, without forming a thick wall, undergo longitudinal division into two or four cells contained within the wall of the mother-cell. These daughter-cells, at first without a distinct wall, organise flagella, form a delicate cell-wall, and escape from the deliquescent mother-wall as active flagellated cells. Again, in the case of the macrocytes, the active cell comes to rest, surrounds itself with an extremely thick stratified wall, takes on a spherical shape, and becomes green throughout as though the whole cell were filled with small, polygonal, green granules. This appearance is due to the colourless protoplasm, which in the active stage is in large measure confined to the cup-like hollow of the chloroplast, now radiating out in all directions through the chloroplast to the outer wall and so demarcating the green chloroplast into polygonal areas. In these rounded resting-cells the pyrenoid is at first recognisable, but later breaks up into a number of pieces, and finally into many granules. The eye-spot may take the form of a circular plate or ring lying near the periphery of the cell, or it may disappear altogether.

These rounded thick-walled cells (Pl. 14, fig. 11), after a period of rest, may organise four daughter-cells of oval shape and having all the characters of the active cells except that no pyrenoid is visible at first and no flagella are as yet developed. A third form of resting-cell resembles that just described, except for the fact that the green colour disappears. This colourless resting-cell appears, in surface view, to be divided up into small, regular, polygonal areas, and in this cell pyrenoid and eye-spot may be indistinguishable.

Yet, again, paired resting-cells are met with. These consist of two thick-walled cells, whose broad apposed surfaces are flattened as by mutual pressure (Pl. 14, fig. 16 a). Such paired cells are either green or colourless. Finally, yet another form of resting stage occurs. In this the resting-cells are paired, but one of the two cells is smaller than the
other; in short, a series of stages are met with—pairs of equal 
sized cells, pairs in which one cell is of normal and the other 
of reduced size, half normal, less than half, and even to a mere 
speck (Pl. 14, figs. 16 A, B, C).

This phenomenon we refer to again when dealing, at the 
end of the section, with the systematic position of the infect-
ing organism. The flagellated cells which settle down, with-
draw their flagella and surround themselves with a thick 
mucilaginous wall do not necessarily pass through a period 
of rest, nor do they necessarily divide up subsequently to 
two or four green or colourless cells as described previously; 
but the life-history is liable to be short-circuited in the 
following manner: a green cell may encyst itself temporarily 
and then cast off the cyst and escape once again as an active 
cell. The organism under consideration exists also in a 
colonical form (Palmella condition). The cells in the colony 
form plates imbedded in masses of mucilage produced by 
the coalescence of the outer layers of the walls of the indi-
vidual cells. The cells in the colonial state are rounded, 
uniformly green, and possessed of a pyrenoid less refractive 
than that of the active cell (figs. 14 and 15, Pl. 14). The 
cells constituting the colony undergo rapid division, being 
rather budded off from the parent-cell than produced by 
equal division of that cell. Here and there among the mass 
mature cells occur. In these an eye-spot is visible, and the 
protoplast is differentiated into green chloroplast and colour-
less neck as in the active cells. The mature cell may be seen 
moving inside its wall and every now and then escaping from 
the wall as an active flagellated macrocyte. Hence, scattered 
among the green cells of the colony are empty cysts consist-
ing of the walls left behind by escaped active cells (figs. 14 c 
and 15, Pl. 14). The green spherules from which we first 
obtained the infecting alga consist of such colonial stages 
which have developed within, and come to fill completely, the 
egg-capsole of Convoluta. Under other conditions the 
colonical form is made up of cells which show the reticular 
type of structure already described as occurring in resting-
cells. It is probable, therefore, that the constituents of a colony may, under certain conditions, all pass into the resting stage.

Another curious colonial form also occurs. Oval green cells showing the characters of the active cells, differentiated chloroplast, eye-spot, and pyrenoid, are attached to one another by sleeve-like branching columns of stratified mucilage (fig. 8, PI. 13).

One of the most striking points in the life-history of this alga is the occurrence in approximately equal numbers of green and colourless resting-cells. These cells may be—as already stated—isolated or in pairs. The single green cells undergo, after a period of rest, division into four, green, oval cells which resemble active cells except that their walls are extremely thin and that flagella are lacking. The four daughter-cells are extruded together from the envelope of the mother-cell and undergo further division or become at once active cells.

Similarly the green paired cells divide to eight oval cells. The colourless cells behave in a precisely similar way, giving rise to four or eight daughter-cells which are extruded through a circular opening in the wall of the mother-cell. The colourless daughter-cells consist each of a highly vacuolated foam-like protoplast, including a large refractive mass which appears to correspond with pyrenoid and chloroplast of the green cell. These cells may either undergo further division or assume gradually and without further division a green colour, becoming first faintly yellow, then quite yellow, and finally green.

The simultaneous formation by the alga of green and colourless cells is certainly a curious phenomenon.

It is well known that the zygotes of various algae lose their chlorophyll, but we know of no case where resting-cells are alternatively colourless or green. The division of the green cell into a green group of daughter-cells and the division of the colourless cell into a corresponding group of colourless cells appears to indicate that a true dimorphism exists.
Among the Euglenæ and also among Diatoms it is known that green (or brown) cells may lose their pigments, and, assuming a colourless state, exchange a holophytic for a saprophytic existence.

From the fact that when infection of Convoluta occurs normally from sea-water the earliest recognisable stage of the infecting organism in the body of the animal is a colourless or faintly yellow cell it would appear that the colourless phase of the alga described above represents a perfectly normal stage of its life-history.

The suggestion may be hazarded that the formation of colourless cells capable of a saprophytic mode of life is an adjustment which widens enormously the range of distribution of the alga. The green, active cells swarm toward the light, and so have their distribution determined by the light factor of their environment. The colourless forms, living saprophytically, increase by division even in darkness. Thus the alga may be enabled to live, not only in the surface waters, but also below the surface of the sand wherever organic débris provides material for its support. Such a divided habit would undoubtedly be of the utmost service to the alga, for, attached to the mucilaginous film which surrounds the body of Convoluta or to the gelatinous egg-capsule, it undergoes immersion deep in the sand at every tide. As each tide arrives at the patch of sand covered by the vast colonies of Convoluta these latter descend, as we have described in an earlier paper, out of reach of the disturbance caused by the moving water. The eggs of Convoluta, moreover are laid, not on, but beneath the surface of the sand, so that an organism which adjusted its habits so as to become an associate of Convoluta would be compelled to pass many hours of the day, and not infrequently, when attached to the egg-capsules, many days, in darkness. This it could support only if it were capable of a saprophytic habit.

That the infecting alga is capable of such a habit cannot be doubted, for, apart from the colourless stage being regarded as evidence, the active green cells themselves give corrobora-
ative testimony, since, in cultures containing disintegrating Convolutas, they take up positions among the breaking-down green cells of the animal and increase greatly in numbers. Again, the rapid increase of the alga which follows after infection is strong evidence of saprophytic powers of nutrition. Finally, we have proved by comparative cultures that the infecting alga increases by vegetative division quicker when supplied with organic nitrogen than when the nitrogen is in the form of nitrates. The details of these experiments are given in Table IV, which summarises the results of a series of cultures of the alga in sterile sea-water, to which nitrogen in different combinations was added. The most rapid increase took place in the culture containing uric acid, next in that containing urea, the increase in sea-water containing potassium nitrate being less than in either of these culture-fluids. The infecting organism, then, has even in its green-celled stage marked saprophytic tendencies—tendencies which appear to find full expression in the colourless stage and which offer the key to the origin of the remarkable association of green cell and Convoluta.

Two questions remain—(1) Is there any special adaptation in the habits of the infecting organism which brings it into close association with Convoluta, or is its prevalence in the surface slime of the animal and in the empty egg-capsules a matter of chance? (2) To what systematic position is this organism to be assigned?

To the first question the following experiments provide a decisive answer. If to a bulk of filtered sea-water containing egg-capsules which have been laid under the cleanest possible conditions a number of the flagellated cells are transferred by means of a platinum loop, then within a few hours one or more of these cells will be found to have settled down on each capsule. Yet more striking results are obtained if to a hanging-drop containing an egg-capsule with its group of developing eggs a number of the flagellated cells are added. In such a preparation the mobile green cells are seen to approach the capsule, to
swarm about it, to press in close ranks into the soft gelatinous wall and so to imbed themselves in the envelope (figs. 6, 7; Pl. 13).

We conclude that the egg-capsule exercises a chemotactic influence on the active cells, and that the constant presence of one or more green cells of the infecting alga on egg-capsules contained in water to which a platinum loopful of the active cells has been added is thus accounted for. The behaviour of the cells which settle on the capsule proves that they find this a favourable medium for growth. Within a few hours each cell, having withdrawn its flagella, increases considerably in size, and whilst retaining its green colour takes on a granular appearance; the eye-spot and pyrenoid become fainter and the cell undergoes division. This division, like that of the temporary resting-cells already described, is in most cases longitudinal, but occasionally instances occur in which the division is transverse.

The mode of division is possibly determined by the state of symmetry of the cell; as long as the colourless protoplasmic plug occupies the cavity of the cup-shaped chromatophore the division will be longitudinal, but when the protoplasm becomes distributed radially and uniformly transverse division may occur, and this in turn may give place to the budding of the spherical units of the palmella stage.

The daughter-cells produced by the division of the green cells settled on the egg-capsule undergo a continuous series of divisions, and so give rise to a loose colony, which colony constitutes the green spherules, the discovery of which formed the starting-point for this description.

The second question, that of the systematic position of the infecting organism, remains to be considered. The assemblage of characters presented by this alga—its firm wall, its single cup-shaped chromatophore containing in its hollow the colourless protoplasm and nucleus, its pyrenoid and lateral eye-spot far removed from the bases of the flagella, its power of starch-formation—indicate that it must be assigned to a position in that primitive group of green algae the Chlamydo-
monadæ; and the possession by the cells of four flagella would indicate the genus Carteria.

Nevertheless we would wish to assign it only provisionally to this genus for the following reasons:

First, the infecting alga never has contractile vacuoles, two of which are described in the known species of Carteria.

Second, though like some species of Carteria—e.g. C. multifilis (Fresen)—it possesses cells of two sizes (West, 1904); yet, whereas in C. multifilis the small cells are gametes and the large, vegetative cells, neither the large nor the small cells of our organism appear to be obligate gametes. Numerous experiments which we have made of bringing together microcytes from different cultures, microcytes and macrocytes, and macrocytes from different cultures have given but meagre and extremely rare evidence for any sexual fusion. Leaving aside as unexplained the phenomenon already described in which one of a pair of resting-cells gradually decreases in size and finally disappears, we have only once obtained evidence of what may be a gametic union. Pl. 14, fig. 11 v, shows the case in point. Here two motile cells have come together, their walls have united, and the cells appear in process of fusion.

If this be a true case of sexual fusion, it presents features of great interest. For, as Blackman (1900) has pointed out, the Chlamydomonadæ exhibit a remarkable series of modes of gametic fusion. In some forms the walls are thrown off whilst the gametes are approaching one another, in others at the moment of meeting. In Chlamydomonas multifilis, a four-ciliate form, partial fusion takes place first, so that the walls are left fused at one spot. Blackman adds that the series might be completed at its lower end by a form which fused without losing its walls. The figure just referred to appears to indicate that this primitive form of sexual union is exhibited by the infecting alga of Convoluta.

A third character which seems to separate it from Carteria is the peculiar branching habit which it sometimes presents. In this condition (Pl. 13, fig. 8) inverted green cells are
borne at the ends of branching gelatinous stratified stalks. This habit occurs, according to Oltmanns (1904), in other genera of the Chlamydomonadeae, viz. Chlorangium (Stein), and Physocyntium (Borzi). The nearest approach, however, to this habit of the infecting alga is exhibited by species of the genus Prasinoclados of the family Chlorodendraceae, which stands, according to Oltmanns, between the Chlamydomonadeae and the Polyblepharidae.

We conclude that the infecting organism is a true alga and a primitive member of a primitive group, the Chlamydomonadeae; and that whilst presenting many features characteristic of the genus Carteria, its possession of certain other characters, facultative gametes, branching as well as platelike colonial form and its lack of contractile vacuoles, makes its assignment to that genus doubtful.

Summary of Section IV.

The infecting organism of Convoluta is an alga belonging to the Chlamydomonadeae. In its free stage it bears four equal flagella and possesses the general characters of members of this family.

The active cells are of two sizes, but neither large nor small cells appear to be obligate gametes.

The organism is capable of a saprophytic as well as of a holophytic existence; in the former state it may be colourless.

The active cells are attracted chemotactically to egg-capsules of Convoluta. They settle down and undergo active vegetative division in the capsules, and are finally liberated as a swarm of four-flagellated active cells.

Section V. The Normal Course of Infection.

We have demonstrated in Section III that the active flagellated cells of the infecting alga may be ingested by Convoluta and, dividing in the body of the animal, give rise to the green-celled “tissue” characteristic of the adult.
When, however, infection takes place from ordinary sea-water, when, for example, young Convolutas raised under sterile conditions are placed in a few cubic centimetres of sea-water brought fresh from the shore, it is not the green flagellated cell which constitutes the first stage of infection. The first sign of the presence of the infecting alga under these normal conditions is afforded by a larger or smaller colourless body lying in the central vacuole of the animal. The large body consists of a pair of closely apposed resting-cells, such as have been described already (Section IV). The small body is a single resting-cell of the infecting alga. Soon after ingestion the mucilaginous wall which surrounds the resting-cell swells considerably and the contents divide, in the case of the smaller cell into four, in that of the larger cell into as many as eight, colourless daughter-cells. These colourless cells are 15-16 μ in length—that is, of about the same dimensions as those of the macrocytes. They escape or are discharged from the central vacuole, and take up fairly definite stations in the body of Convoluta, two right and left of and a little behind the otocyst, and two on either side about the middle of the body. These cells, which are to form the starting-points from which the green tissue of the adult animal will be developed, lie each, like the mother-cell which gave rise to them, in a clear vacuole. The colourless cell at this stage has very granular contents, and a pyrenoid which is large and somewhat oily-looking. Even in this stage the subsequent differentiation of the protoplasm into chloroplast and colourless protoplasm may be indicated by a plug or core of clearer protoplasm lying in the hollow of a more granular chloroplast. The cell now develops rapidly, an eye-spot makes its appearance, and a yellow tinge becomes visible in the leucoplast; at first extremely faint, the yellow becomes more marked, and is succeeded by a green colour which pervades the whole chloroplast. The cell is bounded by no well-marked wall, the limiting layer does not give a cellulose reaction, and is so delicate that the shape of the cell changes with the movements of the animal.
The infecting organism, though without flagella, now resembles in other respects the active flagellated free stage. It is, in fact, as both its histological features and its development show, a daughter-cell produced by the division of a colourless resting-cell. Such resting-cells on resuming activity undergo division into four (the paired cells into as many as eight) daughter-cells, which, as described in Section IV, are discharged together or severally through a circular opening in the thick mucilaginous wall of the cyst. Subsequently these cells surround themselves, in the free state, each with a thin wall. In the body of Convoluta the formation of a definite wall is suppressed.

The small colourless or faintly yellow cell, which is often the first recognisable sign of infection, is a daughter-cell which has been produced outside the body of the animal by the division of such a resting-cell as that just described. Convoluta is then infected normally either by a resting-cell or by non-motile daughter-cells produced by the division of a resting-cell, though, as already shown, the organism in its flagellated phase may be taken up and give rise to normal infection.

We have now traced the infecting organism to its place in the body of Convoluta. At certain, fairly constant, stations of the body, several naked green cells lie, each in a clear vacuole. At about this stage, or after their further division, the green cells lose their regular oval outline, and the colourless part of the protoplast becomes more or less excentrically placed with respect to the chloroplast. It is easy to cause free active cells to undergo similar changes. All that is necessary to effect this is to transfer them from sea-water to a fluid—e.g. diluted sea-water—of lower osmotic pressure.

Hence we infer that the peculiar and variable shapes of the green cells of adult Convolutas are due to the osmotic pressure of the vacuolar fluid in which they lie being lower than that of sea-water. Incidentally, this observation serves to clear up another point. Haberlandt makes the suggestion that the colourless, excentrically-lying part of the green cell is in
reality an animal cell or part of an animal cell which has associated itself with the green cell, and that the nucleus lying in this colourless part belongs to this animal cell. From the preceding it follows that this suggestion cannot be maintained; for the colourless part of the green cell is nothing other than the plug or core of colourless protoplasm which in the free, active cell occupies the cavity of the cup-shaped chromatophore, and which in a fluid of osmotic pressure lower than that of sea-water becomes displaced excentrically. The question of nucleus we reserve (see p. 196). We turn now to consider the further development of the green cells which, as the result of infection, are planted about in the body.

The cells increase, sometimes very considerably in size, and undergo division. The process of this division in cells which have not yet become distorted is as follows: The colourless "neck" of protoplasm elongates, extending toward the base of the cell, where come to be placed pyrenoid and eye-spot. A vertical fold appears in the pyrenoid which later cleaves into two. The eye-spot degenerates during the division of the cell, taking on the form of a broken hoop of dull red colour. The eye-spot does not reappear in the daughter-cells which arise by longitudinal division. In the case of the larger originally colourless cells the colourless protoplasm occupies the middle of the cell. When about to divide a pyrenoid makes its appearance toward either end of the cell, and what appears to be a transverse division occurs.

The daughter-cells in either case separate from one another and, lying each in a clear space, undergo further divisions. These subsequent divisions are, like those which take place in the palmella state, more of the nature of budding than of equal division; hence are produced rows of cells of gradually decreasing size, which run from the periphery into the deeper tissues of the body (figs. 9 and 10, Pl. 13). The resemblance between the green tissue of loosely associated cells which occupies the body of Convoluta and the palmella stage of the alga is noteworthy.

The appearance presented by an abnormal Convoluta—
unique among the many thousands examined—lends support
to the view which we put forward that the "Convoluta stage"
of the alga is to be regarded as consisting of an hypertrophied,
senescent palmella.

The animal in question presented an appearance under the
low power of the microscope very like that of a fern-pro-
thallus. Its body was lobed posteriorly, so as to present a
conventionally heart-shaped form, and its green cells, so
closely packed as to obscure all other elements of the body,
were rounded and uniformly green, quite like those of the
typical palmella-stage, which stage at the time of these
observations was unknown to us.

To return to our consideration of the green cells of the
normal animal. The rapid division of the green cells in
recently infected Convoluta is accompanied by significant
changes in the nucleus, changes which since they render
intelligible the loss of power of independent existence on the
part of the green cells of the adult animal we now describe in
some detail.

The nucleus of the flagellated cell (fig. 12, Pl. 14) lies in the
colourless part of the protoplast, about the middle of its depth,
equidistant from the bases of the flagella and the pyrenoid.
Its general appearance is that of a spherical uniformly staining
body, from the periphery of which radiating branches run,
two upward towards the points of insertion of the cilia and
two downward toward the pyrenoid. These branches, though
mainly cytoplasmic, show by their staining reactions with
nuclear stains (e.g, Benda's iron haematoxylin) that they also
contain nuclear material.

In other flagellated cells the nucleus presents the appear-
ance described by Dangeard (1899) as occurring occasionally
in the Chlamydomonadaceae, of a clear spherical area, in the
centre of which lies a deeply staining "nucleolus." Again, in
other specimens the nuclear substance consists of three fairly
large granules lying either side by side or else pyramid-wise;
the granules may be spherical or elongated, of equal or
unequal size. Occasionally specimens show two vertical rows,
each of three granules. So far we have met with no indications of mitotic division except this separation of the nucleus into rows of granules.

In the division stages of the non-motile cells, groups of rods and granules may be made out and appear to represent phases of nuclear division.

The cells of the colonial stage (Fig. 15, pl. 14) present two types of nucleus. The resting nucleus, lying slung in cytoplasm in the centre of the cell, consists of a homogeneous, deeply-staining central body, surrounded by a clear area, the limits of which cannot be sharply distinguished from those of the cytoplasmic envelope containing it. The dividing nucleus consists of a diffuse group of fine granules, occupying a circular or oval area of the cytoplasm. The granules sometimes form two groups at opposed ends of a cell.

Thus the nuclei of the flagellated stage and of the actively developing palmella stage are, though small, distinct and readily recognisable. So, too, is the nucleus of the green or colourless cell, as it lies in the body of a larval Convoluta immediately after ingestion. After the first division in the body the nucleus of each daughter-cell has the appearance of that just described as characteristic of the actively dividing cells of the colonial stage—namely of an oval area occupied by diffuse granules (Fig. 13, pl. 14). As division of the infecting cells proceeds the presence of nuclear granules in the resultant cells is made out with increasing difficulty, till finally, in the adult animal, whose body is densely packed with green cells, it is often difficult to pick out any in which remnants of nuclear granules remain. The great majority of the green cells of Convoluta are not complete cells, but cells which show all stages of diminishing nuclear substance (Figs. 9 and 10, pl. 13). Thus the conclusion to which our physiological investigations led us that the body of Convoluta is the grave of the green cell receives histological confirmation.

If this degeneration of the nucleus begins almost immediately after the ingestion of the infecting cells some light is perhaps thrown on the fact to which attention has frequently been
drawn—that the green cells of Convoluta are without cell-walls, for it is well known that the nucleus of the plant-cell presides over cell-wall formation. An enucleated fragment of a plant-cell, though capable of performing certain vital processes—e.g. photosynthesis—is unable to form a new wall.

We may therefore regard the suppression of cell-wall as the first result of a progressive nuclear degeneration. Enough, however, of the nucleus is left to preside over cell-division, which proceeds rapidly after infection; as this division goes on less and less nuclear material remains, till finally partial cells, incapable of independent life and probably also incapable of further division, alone are left.

It is noteworthy that we have come across no evidence of mitosis in the dividing green cells of the animal.

A significant phenomenon is revealed by a reference to Figs. 9 and 10, pl. 13, and to the description thereof. Here, as at (Nu., gc.), are isolated green cells, in each of which a nucleus is recognisable; in other parts of the section are ingrowing rows of cells budded off from the outermost cell, and of these rows only the outermost cells contain a distinct nucleus; others contain only deep-staining granules; and others, again, no nuclear substance whatever (Fig. 9, pl. 13).

A parallel suggests itself between the green cells of Convoluta and the red blood-cells of the higher vertebrates. As the red discs are enucleate partial cells budded off from the nucleated red cells, so may the green cells be regarded as enucleate partial cells budded off from the outermost row of nucleated green cells, and as the red discs are of limited life and specialised (respiratory) function, so are the green cells of limited life and of specialised (photosynthetic) function.

The green cell devoid of a nucleus of its own would not, however, appear to be shut off from all nuclear influences. For the enucleate green cell may be connected by a finely drawn process with another green cell still possessed of nuclear substance (Pl. 13, fig. 9). Moreover, such green cells as are without nuclear material are accompanied each by a large attendant nucleus of animal origin; this close
association of "attendant nucleus" and green cell is shown in Pl. 13, fig. 9, *Nu. Mes.* Further investigation of this phenomenon is required; but such observations as we have made point to the belief that these attendant nuclei are those of wandering cells which lie in wait, as it were, for enucleate green cells and at a subsequent stage bring about their destruction by digesting them.

The foregoing interpretation of the series of facts described offers some support in favour of the hardy suggestion, due originally to Schimper,\(^1\) that the higher green plants are an association of two organisms—one a colourless organism, the other originally a green alga but now represented by the chloroplasts and by them alone.

For an adult Convoluta is a complex of two organisms—one the colourless animal, the other the chloroplast remainders of the original green alga; but in this case, unlike that imagined for the green plant, the synthesis is not a permanent one. It endures but for the lifetime of the animal and has to be recommenced in every larval Convoluta.

**Summary of Section V.**

1. The colourless phase of the infecting alga normally supplies the cells which develop in the body of Convoluta into the green-coloured tissue, which tissue is comparable with the palmella-stage of the alga.
2. But infection may also result from the ingestion of the green active or green temporarily resting-cell.
3. Ingestion is followed by the active division of these cells which develop transient eye-spots, etc., but which do not form a cell-wall.
4. The distorted shapes of the green cells in the animal are due to osmotic conditions.
5. The nucleus of the green cell dividing in the animal undergoes progressive degeneration and finally disappear; leaving the photosynthetic machinery intact.

\(^1\) *Bot. Zeit.*, 1883, p. 112, footnote.
(6) The green partial cells which result are incapable of independent existence.

SECTION VI. THE SIGNIFICANCE AND THE CONSEQUENCES OF THE ASSOCIATION OF ANIMAL AND GREEN CELL.

The relation between Convoluta and its green cells has sometimes been regarded as an example of parasitism, the parasite being the green cell and the host the animal. That this is an erroneous view we have already demonstrated.

More usually it is assumed that both partners in the association receive advantage therefrom, and consequently the relation is considered to be symbiotic.

It is obviously important, if such a term as "symbiosis" is to retain any value whatever, that a detailed examination of each case which appears to fall into this category should be made before it is relegated thereto. This is the purpose of the present section. In it we show that the relation between Convoluta and its green cells is both intricate and variable in the sense that the nature of the relation changes as the association becomes more intimate.

We show that it is only by taking the widest view of the case that it can be brought within the category of symbiotic phenomena.

The inquiry as to what is the significance and what are the consequences of the association of green cells and Convoluta resolves itself in large measure, but not entirely, into an examination of the modes of nutrition of infecting alga and of animal. The mode of nutrition of the infecting alga in its free state must be first described.

The fact that we have succeeded in obtaining luxuriant cultures of the alga in artificial sea-water to which traces of potassium nitrate were added proves that it is capable of a typical plant-like (holophytic) mode of nutrition. The alga under these conditions photosynthesises carbohydrates—storing the surplus as starch—and utilises the nitrogen of the nitrates in the formation of its proteids.
But, as we have already shown in Section IV, the alga in its free state grows more actively when enabled to obtain its nitrogen from organic compounds (uric acid and urea) than when "inorganic nitrogen" (nitrate) is supplied.

Next, as to the mode of nutrition of the alga in its "Convoluta stage."

Geddes (1879–1881) was the first to demonstrate that Convoluta gives off oxygen when exposed to light. He also proved the presence of starch in the green cells, stating that this substance is present always in small quantities. Our own experiments complete the proof that the green cells of Convoluta photosynthesise carbohydrates. Thus, we have shown that the green pigment of these cells is true chlorophyll (op. cit., p. 377), and that starch, present in the green cells, disappears in darkness and reappears when the animals are brought into the light. The photosynthetic activity of the green cells having been demonstrated, the questions remain, Does the animal receive a share of the elaborated carbohydrate? and, if so, How is the transference of this substance from green cell to animal tissue effected?

Geddes has stated that Convoluta survives only a few days' exposure to darkness, the implication being that when photosynthesis is arrested death from starvation ensues. But according to our experiments (op. cit. p. 375), if care is taken with respect to the water supply Convoluta may live in darkness for several weeks.

Again, Geddes' statement that starch occurs in Convoluta only in small quantities might be interpreted as meaning that the product of photosynthesis is passed on to the animal as soon as it is formed, and that only a small residue is accumulated in the green cell. The observation, however, is incorrect, for the green cells of Convoluta at certain times contain so much starch as to give to the body when stained with iodine a deep blue or blue-black colour.

We have endeavoured to ascertain to what conditions the variable amount of starch in the green cells is due.

For this, samples of animals were collected twice daily
during a lunar month from a certain patch of Convoluta. The samples were taken just after the tide had left the patch—i.e. immediately after the sojourn of the animals in darkness below the surface, and again after their spell of isolation, just as the tide was about to cover them.

The animals were fixed on glass slides, decolorised, stained with potassium iodide-iodine, and the amount of starch estimated by the resulting coloration.

The records obtained (Table V) during August, 1905, exhibit a bi-monthly periodicity in the amount of starch present in the animal. The amount is large during the spring tides and falls off during the slack tides. The result is perhaps susceptible of a simple explanation. For during the spring tides low water, and consequently exposure of Convoluta to light, occurs about the middle of the day (and night), whereas during the neap tides the exposure to light takes place during the early morning and late afternoon. Consequently, though the number of hours of daylight to which Convoluta is exposed may be actually greater during the slack tides than during the spring tides, the animals are exposed to a higher light intensity (of the mid-day) during the spring tides than during the neaps. Hence it seems reasonable to suppose that photosynthesis will be more active during the spring tides, and that this greater activity will be recorded by the larger deposit of starch. It is worth noting—though aside from the question under consideration—that the periodicity of egg-laying by Convoluta receives some explanation from the foregoing; for the egg-laying periods, occurring soon after the spring tides, follow closely on periods of abundant nutrition. Returning to the question, Does the animal receive a share of the carbohydrate elaborated by the green cells? we have just seen that on this hypothesis periodicity of egg-laying receives a simple explanation. But conclusive evidence is derived from observation of the rates of growth of recently infected Convoluta. The rapid increase in the numbers of green cells in the body—from one or two to thousands—is accompanied, not by any diminution of the
animal's tissues, but by the marked growth of the animal. Whilst uninfected Convoluta, as described presently, remain diminutive, those which become infected resume growth and increase rapidly in size.

It appears probable on several grounds that the animal cells are unable to lay hold of the starch contained in the green cells. Thus, though young Convolutas fed on starch-grains ingest them readily, they digest them not at all. Again, when adult Convolutas are kept in darkness the starch of the green cells disappears with extreme slowness—even after eight days some starch may still be present. This slowness of disappearance would seem to indicate that it takes place according to the requirements of the green cell, and not according to those of the animal. It is therefore likely that the animal cells obtain the product of photosynthetic activity directly as sugar. Just as from the green cells of a plant exposed to light there is a constant osmotic streaming of photosynthesised sugar to the colourless cells, so from the green cells of Convoluta sugar passes to the colourless animal cells. In general, the demand in adult Convolutas for food material is so great that but little starch accumulates in the green cells. It is only during the mid-day exposure to high light-intensity at the spring tides that photosynthetic activity considerably exceeds that of translocation. At all other periods during the adult life of the animal the photosynthesised carbohydrate passes away as fast as it is formed from the green cell to the animal. The sugar thus obtained is stored ultimately in the animal tissues or in the eggs in the form of fat.

We have next to consider the system, green cell and animal, with respect to nitrogen metabolism. To do this requires a review of the facts known with respect to the nutrition of the constituents of the system.

Von Graff (1905–1906) was the first to draw attention to the absence of any vestiges of solid food from the body of Convoluta. He concluded that this animal does not take up solid food. Geddes' observations, which, however, as we
have shown, require considerable modification, led him to conclude that the animal relies on the green cells for its food supply.

Georgevitsch (1899), finding that colourless larvae die, if uninfected, within two or three days, seemed thereby to establish the existence of a yet closer dependence of animal on green cell.

We have shown in a former paper that von Graff's statement is too absolute, and that of Georgevitsch inaccurate.

We may summarise our own observations as to the ingestion of food by Convoluta.

A well-marked mouth—already described by von Graff in adult animals—is present in just hatched animals. This mouth, capable of a wide gape, is situated on the under surface, about the middle of the body. When open the mouth is connected by a short, ciliated, ectodermal invagination with an axial mass of highly vacuolated tissue which constitutes a rudimentary gut.

By means of this mouth a young Convoluta takes up almost any objects which it can encompass; diatoms and unicellular green algae form the staple diet in the open, while in the laboratory starch-grains, litmus, lamp-black, and sand-grains may be swallowed.

If colourless uninfected Convolutas are cultivated in water devoid of the infecting alga but rich in other organisms, such as diatoms, they continue to feed actively for some time—for a week or more. But after this period they become increasingly inert and ultimately cease altogether to take up food.

In this state they await, as it were, the infecting alga. If the latter is supplied they ingest it, and, as it develops, the animals resume their activity. If, on the other hand, the infecting organism is still withheld, the lethargic condition becomes more pronounced, and undergoing gradual diminution in size, Convoluta finally dies though lying in the midst of food of a kind which, at an earlier stage, it took up and digested readily enough, and on which young infected Convolutas are still feeding. It is to this remarkable behaviour
that we attribute the failure of all our attempts to raise a colourless race of Convolutas.

The infected young animals continue during the period of development of their green cells to ingest solid food. But when this period is complete and the reproductive organs are mature all ingestion of solid food ceases.

The only food materials now available are such substances synthesised by the green cells as may pass in solution from green cell to animal cell.

This mode of nutrition of the animal is of short duration. It is soon succeeded, or rather overlapped, by another which consists in the digestion by the animal cells of whole groups of green cells. During the adult life of Convoluta large or small aggregates of green cells in all stages of disintegration are to be met with, lying in vacuoles in the axial tissue which constitutes the gut of the animal.

From this stage onward the ultimate significance of the relation between animal and green cells stands more and more clearly revealed. The animal is now and henceforth parasitic on its green cells. As a result of this habit old animals are not infrequently to be met with in laboratory cultures whose bodies are half green and half colourless.

If in order to summarise these conclusions we employ the terms used by plant physiologists in describing the principal modes of nutrition, and if we consider for this purpose a green Convoluta as an organism, then we may distinguish the following styles of nutrition:

(1) In the pre-infection stage the animal feeds heterotrophically—i.e. typically animal-wise.

(2) Young infected stage; the green animal is nourished mixotrophically, viz. by (solid) ingested food, and by photosynthesised food-substances.

(3) Mature green Convoluta; the mode of nutrition is holophytic—typically plant-like. The green cells are the photosynthetic agents, the animal's colourless cells receiving, just as do the colourless cells of plants, the products of photosynthetic activity.
(4) Old green Convoluta; the only term that can be applied is autotrophic; the green animal behaves to its green cells just as any animal or plant may behave to any reserves of food-material; it digests them.

If we consider the same facts from the point of view of the animal Convoluta; then stages two, three, and four represent the progressive parasitism of the animal on its contained green cells.

We have next to consider the sources of the supplies of nitrogen compounds to the green cells and to the animal.

The facts already described, proving that the infecting alga thrives better on organic than on inorganic nitrogen compounds suggest that the habit of this alga of settling down on the egg-capsules of Convoluta originated in obedience to these nitrogen-requirements.

The following considerations and observations lend support to this view. Whereas most Turbellarians have a well-developed excretory system of flame-cells, *Convoluta roscoffensis* has none. Nor do we find in this species those granular accumulations, often appearing as localised refractive bands or patches, which are constant features in allied species and which von Graff regards as being of an excretory nature. The fruitlessness of our prolonged search for flame-cells justifies us in holding that none exist in *Convoluta roscoffensis*. Moreover, green Convolutas show no signs of any excretory substances. The green cells we know are capable of utilising such excretory nitrogen compounds as uric acid and urea. It seems, therefore, probable in a high degree that the green cells of Convoluta are its excretory system.

The following observations lend a certain measure of support to this hypothesis. If larval Convolutas are protected from infection and kept without food, their large stores of reserves gradually disappear, and vacuoles charged with long acicular crystalline bodies make their appearance. The numbers of these vacuoles and of their contained crystals increase till they form one of the most striking features of the
body. We have not yet succeeded in determining the chemical nature of these crystals; they disappear when the "vacuole" containing them is destroyed, as it is by every fixative or reagent we have used. Nevertheless it cannot be doubted that these crystals are products of metabolism, and it seems likely that they are of the nature of excretory nitrogenous substances. Now, these acicular crystals do not occur in the infected green animal.

Admitting the argument here set forth, the conclusion follows that the green cells, on gaining entrance to the body, take over the business of disposing of the waste products of nitrogenous metabolism. We have learned already that the life of the animal depends on the occurrence of infection, that failing infection the animal ceases to feed and dies, possibly as the result of auto-intoxication by the accumulated waste products. Thus the closeness of the relation between animal and green cells is such that they together constitute, in the green Convoluta roscoffensis, one organism.

The conclusions concerning the rôle of the green cells in utilising the nitrogenous waste of the animal as material for proteid synthesis appear to us to throw light on the last phase of the nutrition of the animal. That phase is characterised by the digestion of the green cells. In the early stages of its development Convoluta offers to the green cells ample supplies of waste nitrogen compounds, the products of its proteid metabolism the materials for which were derived from the reserves in the egg and from the products of digestion of solid food. On these excretory nitrogen compounds, and on the product of its own photosynthetic activity, the green cell flourishes, and in turn furnishes soluble carbohydrates to the animal. But when ingestion of solid food ceases there follows a shortage of nitrogenous waste substance. The green cell, adjusted to utilise organic nitrogen, now has only available for proteid synthesis the soluble nitrogen compounds of the sea-water. A dearth of nitrogen compounds available for anabolic processes subvenes. This dearth falls earliest and acutest on the animal. Urged by its
nitrogen hunger, it turns upon the green cells and raids such stores as they contain by digesting them. These stores are but limited, and when exhausted, death, both of the animal and of its green cells, inevitably follows.

The election by the infecting alga of the egg-capsules and the surface of the animal as a normal habitat may also be regarded as a symptom of nitrogen hunger; so, too, is the ultimate digestion of the green cells by the animal.

Taking the broadest view of the whole relationship, including therein, not only the green cells inhabiting the body of Convoluta, but also the free green cells living on soluble organic waste contained in the egg-capsules, we may classify it as a symbiotic relationship, for it is now only by reason of its infection by the green cells that Convoluta roscoffensis, the species, persists, and though the green cells which enter the animal never escape alive, this is, as it were, but the price which the species has to pay for its lodging.

But if we confine our attention to a green Convoluta, not looking beyond the association of green cells and animal, then the relation constitutes a case of parasitism, the host being the green cells and the parasite the free-living animal.

The extraordinary restriction of the range of the species C. roscoffensis we may regard as the result of this peculiar and economically unsound attempt on its part to solve the "nitrogen question."

Summary of Section VI.

(1) Convoluta exhibits four phases of nutrition, passing from the typically animal to the completely parasitic.

(2) The infecting alga shows specialisation in the direction of saprophytism.

(3) This habit enables the green cells to utilise the products of the animal's nitrogenous metabolism, and so to develop rapidly within the body, where they serve as an excretory system to the animal.

(4) The habit of the infecting alga, in its free state, of fre-
quenting the egg-capsules and also the surface slime of the body of Convoluta is to be ascribed to its nitrogen requirements; this habit developed originally with no reference to Convoluta (being shared with various Chlamydomonadae; e.g. Carteria subcordiformis, Wille n. sp. (1903)), was nevertheless an essential preliminary to the association of animal and green cell.

(5) The association entails ultimately the death of the green cell and of Convoluta; but whereas the former dies without issue the latter first produces one or more batches of eggs.

(6) The consequences of the association are: To the green cell hypertrophy, nuclear degeneration, premature senescence, and death. To the animal: suppression of excretory system, cessation of feeding, resignation of power of existence apart from the green cells—i.e. obligate parasitism; adaptations facilitating the photosynthetic activities of the green cell—e.g. marked positive phototropism identical with that displayed by the infecting alga in its free state.

SECTION VII. GENERAL SUMMARY.

Convoluta roscoffensis hatches out from its egg-capsule as a colourless animal whose body contains neither green cells nor antecedents of green cells.

Infection takes place neither directly nor indirectly from the body of the parent, but from the sea-water or from the egg-capsules to which the infecting organism is chemotactically attracted and on which it habitually settles down and develops.

Experiment shows that the green cells of adult Convoluta are incapable of life apart from the body of the animal; histological examination, proving that the development of the green cell within the body is accompanied by degeneration of its nucleus, supplies the explanation.

The infecting organism has been isolated, and, by the addi-
tion of it to colourless Convoluta, the green animal has been synthesised.

In its free stage the infecting organism shows the essential characters of the Chlamydomonadaceae; its four equal flagella point to its inclusion in the genus Carteria; certain peculiarities suggest that the assignment of the infecting organism to this genus should be only provisional. The infecting organism is capable of a saprophytic as well as of a holophytic mode of life and occurs in both colourless and green forms. Its saprophytic habit leads to its association with the egg-capsules and constitutes a first physiological step toward its association with the body of Convoluta.

The green cells serve as an excretory system to the animal.

The relation between green cell and animal changes with their development, passing from a symbiotic relation to one in which the animal is parasitic on the algal cells.

The association leads to marked changes of habit on the part of the animal—e.g. to its ceasing from the ingestion of food—and is best interpreted as an economically unsound attempt on the part of both green cell and animal to solve the "nitrogen problem."
**TABLE I.—Infection Experiments.**

**Convoluta roscoffensis,** Trégastel, 1903. Cultures in sterile and in ordinary sea-water.

**Method.**—Some thousands of mature Convolutas placed in each of three large glass vessels (A, B, C), eggs laid, and larvae hatched and left with adults during time of experiment. Sea-water sterilised by filtering through Pasteur-Chamberland filter.

<table>
<thead>
<tr>
<th>Time of records</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aug. 31</strong></td>
<td>Capsules laid</td>
<td>Capsules laid</td>
<td>Capsules laid</td>
</tr>
<tr>
<td><strong>Sept. 3</strong></td>
<td>Larvae hatched (colourless)</td>
<td>Larvae hatched (colourless)</td>
<td>Larvae hatched (colourless)</td>
</tr>
<tr>
<td><strong>Samples, 12 from each of A, B, C, Sept. 7, examined microscopically</strong></td>
<td>Larvae colourless</td>
<td>10 colourless; 2 with colourless vacuoles in gut</td>
<td>5 colourless; 7 with colourless or yellow cells in vacuoles</td>
</tr>
<tr>
<td><strong>Ditto, Sept. 9</strong></td>
<td>Larvae colourless; in 6, 5, or fewer, colourless vacuoles scattered over the body</td>
<td></td>
<td>1 containing a typical green cell; 11 others with colourless or yellow cells in vacuoles</td>
</tr>
<tr>
<td><strong>Ditto, Sept. 11</strong></td>
<td>Larvae colourless; vacuoles persistent and empty</td>
<td></td>
<td>All infected; in some 6 or more green cells</td>
</tr>
<tr>
<td><strong>Ditto, Sept. 13</strong></td>
<td>Larvae colourless; vacuoles persistent; some full of long spicules; = no infection</td>
<td></td>
<td>10 larvae, many green cells; 2 larvae, no infection; = infection</td>
</tr>
<tr>
<td><strong>Ditto, Sept. 20</strong></td>
<td></td>
<td></td>
<td>All colourless; vacuoles empty.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>All colourless; = no general infection.</td>
</tr>
</tbody>
</table>
TABLE II.—Infection Experiments.

Convoluta roscoffensis, Tregastel, 1904. Cultures in sterile and ordinary sea-water. Procedure as in Table I, but eggs picked out as laid (August 9th) and washed in filtered sea-water, then put in a series of vessels containing:

<table>
<thead>
<tr>
<th>Date</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh unfiltered sea-water</td>
<td>Filtered sea-water</td>
<td>Filtered sea-water</td>
<td>Filtered sea-water</td>
<td>Filtered sea-water</td>
</tr>
<tr>
<td>Aug. 13</td>
<td>10 uninfected</td>
<td>10 uninfected</td>
<td>9 uninfected</td>
<td>10 uninfected</td>
<td>2 uninfected</td>
</tr>
<tr>
<td></td>
<td>0 infected</td>
<td>1 infected</td>
<td>1 infected</td>
<td>0 infected</td>
<td>8 infected</td>
</tr>
<tr>
<td>Aug. 28</td>
<td>3 uninfected</td>
<td>9 uninfected</td>
<td>3 uninfected</td>
<td>1 uninfected</td>
<td>None remain</td>
</tr>
<tr>
<td>Sept. 16</td>
<td>3 uninfected</td>
<td>1 infected</td>
<td>9 infected</td>
<td>1 infected</td>
<td></td>
</tr>
</tbody>
</table>
TABLE III.—Infection Experiments.

Convolvula roscoffensis, Trégastel, 1905. Cultures of larvae in sterile and in fresh sea-water, with and without capsule-remnants, and with and without adults.

**Method; July 19th.**—Large numbers washed 3 times in filtered sea-water. Egg-capsules transferred when laid to filtered sea-water in covered watch-glasses—3 eggs per watch-glass. When hatched the young placed under the conditions indicated:

The fractions represent the ratios: \[
\frac{\text{infected}}{\text{uninfected}}
\]

<table>
<thead>
<tr>
<th>Method</th>
<th>July 19th</th>
<th>Aug. 2 and 3</th>
<th>4-6</th>
<th>8-11</th>
<th>11-15</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtered sea-water without capsules.</td>
<td>2/8</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Filtered sea-water without capsules.</td>
<td>0/0</td>
<td>0/4</td>
<td>3/2</td>
<td>1/10</td>
<td>0</td>
<td>10/0</td>
</tr>
<tr>
<td>Filtered sea-water with capsule remnants.</td>
<td>0/10</td>
<td>10/6</td>
<td>3/2</td>
<td>0</td>
<td>0</td>
<td>20/0</td>
</tr>
<tr>
<td>Filtered sea-water with capsules.</td>
<td>0/0</td>
<td>0/10</td>
<td>3/1</td>
<td>10/10</td>
<td>0</td>
<td>10/75</td>
</tr>
<tr>
<td>Filtered sea-water with adults.</td>
<td>3/2</td>
<td>10/0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fresh sea-water without capsules.</td>
<td>0/0</td>
<td>0/0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fresh sea-water with capsules.</td>
<td>3/4</td>
<td>1/10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fresh sea-water with adults.</td>
<td>3/35</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Young form a visibly green cloud

Visibly green colonies formed

Flagellate-cell colonies formed

Filtering with adult infection

General infection
Table IV.—Comparison of the Effects of various Compounds of Nitrogen when added to Cultures of the Infecting Organism of *Convoluta roscoffensis* in Filtered Sea-water.

Trégastel, August 31st, 1906.—Samples of active flagellated stage planted out by platinum loop in test-tubes containing sea-water + the following:

<table>
<thead>
<tr>
<th></th>
<th>A (Asparagin)</th>
<th>B (Urea)</th>
<th>C (Uric acid)</th>
<th>D (Potassium nitrate)</th>
<th>E (Ammonium chloride)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept. 8</td>
<td>No growth</td>
<td>A little growth</td>
<td>Considerable growth</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>Sept. 20</td>
<td>&quot;</td>
<td>Fair growth</td>
<td>Much growth, the alga forming a visible green scum</td>
<td>A little growth</td>
<td>&quot;</td>
</tr>
</tbody>
</table>
TABLE V.—Daily Estimate of Starch-content of Green Cells of C. roscoffensis during Twenty-eight days. Trégastel, 1905.

<table>
<thead>
<tr>
<th>Date</th>
<th>Tide</th>
<th>Recorded day-period during which Convoluta zone was uncovered</th>
<th>Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug. 18</td>
<td>82 decimetres</td>
<td>11—3.30</td>
<td>Much.</td>
</tr>
<tr>
<td>, 19</td>
<td>80</td>
<td>11.20—4.15</td>
<td></td>
</tr>
<tr>
<td>, 20</td>
<td>78</td>
<td>11.30—4.15</td>
<td></td>
</tr>
<tr>
<td>, 21</td>
<td>75</td>
<td>11.45—4.50</td>
<td></td>
</tr>
<tr>
<td>, 22</td>
<td>72</td>
<td>12.30—6</td>
<td></td>
</tr>
<tr>
<td>, 23</td>
<td>68</td>
<td>1—6.15</td>
<td></td>
</tr>
<tr>
<td>, 24</td>
<td>67</td>
<td>1.50—6.40</td>
<td>Little.</td>
</tr>
<tr>
<td>, 25</td>
<td>65</td>
<td>3.30—7</td>
<td></td>
</tr>
<tr>
<td>, 26</td>
<td>70</td>
<td>4.30—dusk</td>
<td></td>
</tr>
<tr>
<td>, 27</td>
<td>74</td>
<td>daylight—9.30</td>
<td></td>
</tr>
<tr>
<td>, 28</td>
<td>79</td>
<td>7—11.30</td>
<td></td>
</tr>
<tr>
<td>, 29</td>
<td>83</td>
<td>5—11.30</td>
<td></td>
</tr>
<tr>
<td>, 30</td>
<td>85</td>
<td>6.50—12.50</td>
<td>Much.</td>
</tr>
<tr>
<td>, 31</td>
<td>90</td>
<td>7.50—1.30</td>
<td></td>
</tr>
<tr>
<td>Sept. 1</td>
<td>91</td>
<td>8.30—2.30</td>
<td></td>
</tr>
<tr>
<td>, 2</td>
<td>90</td>
<td>9—3.15</td>
<td></td>
</tr>
<tr>
<td>, 3</td>
<td>88</td>
<td>10.15—4.15</td>
<td></td>
</tr>
<tr>
<td>, 4</td>
<td>84</td>
<td>11—4.40</td>
<td></td>
</tr>
<tr>
<td>, 5</td>
<td>79</td>
<td>.12—6</td>
<td>Less.</td>
</tr>
<tr>
<td>, 6</td>
<td>73</td>
<td>1—6.40</td>
<td></td>
</tr>
<tr>
<td>, 7</td>
<td>71</td>
<td>.10—7</td>
<td></td>
</tr>
<tr>
<td>, 8</td>
<td>71</td>
<td>2—7.30</td>
<td></td>
</tr>
<tr>
<td>, 9</td>
<td>73</td>
<td>—8.15</td>
<td></td>
</tr>
<tr>
<td>, 10</td>
<td>76</td>
<td>3—8.30</td>
<td></td>
</tr>
<tr>
<td>, 11</td>
<td>79</td>
<td>—9</td>
<td>More but still little.</td>
</tr>
<tr>
<td>, 12</td>
<td>82</td>
<td>4—9.30</td>
<td></td>
</tr>
<tr>
<td>, 13</td>
<td>84</td>
<td>—10</td>
<td>Little.</td>
</tr>
<tr>
<td>, 14</td>
<td>84</td>
<td>6.50—12 noon</td>
<td></td>
</tr>
<tr>
<td>, 15</td>
<td>84</td>
<td>6.30—12 night</td>
<td></td>
</tr>
<tr>
<td>, 16</td>
<td>84</td>
<td>7—1.10</td>
<td>Much.</td>
</tr>
<tr>
<td>, 17</td>
<td>84</td>
<td>8—1.50</td>
<td></td>
</tr>
<tr>
<td>, 18</td>
<td>84</td>
<td>8.30—2</td>
<td></td>
</tr>
<tr>
<td>, 19</td>
<td>84</td>
<td>10.30—3</td>
<td>—</td>
</tr>
</tbody>
</table>
TABLE VI.—Diagrammatic Reproduction of Table V, showing Coincidence of Periodicity of Starch-formation in the Green Cells of *Convoluta roscoffensis* with Tidal Periodicity.

Dotted curve = heights of day tides.

Thick line = estimated starch (daily record).
LITERATURE REFERRED TO IN THE TEXT.

THE GREEN CELLS OF CONVOLUTA ROSCOFFENSIS. 217


22. 1904. WEST, G.—"The British Fresh-water Algae," Cambridge, p. 188.


EXPLANATION OF PLATES 13 & 14,
Illustrating the paper by Dr. Keeble and Dr. Gamble on "The Green Cells of Convoluta roscoffensis."

REFERENCE LETTERS.

**Bact.** Bacteria on the caps. (egg-capsule). **B. M.** Basement membrane. **C.** Empty cyst left by an escaped flagellated cell from the palmella state. **CHL.** Chloroplast. **Cil.** Cilia. **C. Pl.** Cytoplasmic plug forming the "neck" of the green cell. **Ep.** Epidermal layer. **G. c.** Green cells. **Mes. C.** Mesenchyme (probably phagocytic) cells of Convoluta. **Mu.** Mucilage of wall of green cell. **Nu. G. C.** Nucleus of green cell. **Nu. Mes.** Nucleus of mesenchyme cell associated with the green cell. **Ov.** Eggs of Convoluta in the common capsule. **Pyr.** Pyrenoid. **St.** Stigma (eye-spot).
PLATE 13.

FIG. 1.—Egg-capsule of Convoluta filled with a dense mass of flagellated green cells forming a pure culture of the infecting organism. ×35.

FIG. 2.—The same capsule under slight pressure. The flagellated cells are swarming actively and escaping through the ruptured wall.

FIG. 3.—The smaller green cells (microcytes) of the infecting organism in the active phase, showing the delicate wall, four flagella, chloroplast, stigma and clear “neck” of cytoplasm in which the nucleus is lodged. (For histology see also Fig. 12 b, Pl. 14.)

FIG. 4.—The larger green cells (macrocyes). (No contractile vacuoles in either form of green cell.) Cf. Fig. 12 a.

FIG. 5.—A portion of the egg-capsule; the green cells have come to rest and lie in pairs.

FIG. 6.—The infection of an egg-capsule by the green cells chemotactically attracted thereto (see pp. 188, 189 of text).

FIG. 7.—Infected capsule, more highly magnified. Green cells (and bacteria) seen on the outer edge of the capsule. Other green cells have made their way through the capsule to the egg (ov.).

FIG. 8.—Peculiar colonial form of the green-celled infecting organism (for regular palmella stage see Figs. 14 and 15, Pl. 14). Non-flagellated green cells in inverted position borne at the ends of branching columns of stratified mucilage.

FIG. 9.—Dorsal portion of transverse section through the body of an adult Convoluta roseoffensis showing the structure, arrangement, and relations of the green cells. The majority of the green cells have no nuclei, some mere granular traces. The green cells are arranged in rows running inward from the periphery; the outermost green cell of a row has a nucleus (red). Indications of continuity between green cells are seen and the close relation between them and the mesenchymal cells of Convoluta is shown. × 750.

FIG. 10.—Another more highly magnified section through the body of Convoluta (fixed with Fleming, stained with safranin). Four green cells suspended by slender processes from the basement membrane and separated by mesenchymatous cells. The green cell on the left hand has a nucleus (Nu. ge.), the others contain only granules which stain deeply with safranin; similar deep-staining granules are also plentiful in the subjacent mesenchyma (Cam. luc., oc. 12, obj. 19).
PLATE 14.

Fig. 11.—Life-history of the green-celled infecting organism in the free state. A, Encysted colourless macrocytes, resting and dividing into colourless daughter-cells. B, Microocyte dividing. C, Encysted green macrocytes, resting and dividing into green daughter-cells. D, Apparent fusion of two macrocytes (see text, p. 190). E, Apparent fusion of two unequal colourless cells.

Fig. 12.—A, A typical free macroocyte. B, A typical free microocyte. The nucleus is homogeneous, and in A sends processes towards the bases of the flagella and also towards the pyrenoid. The reticulate surface-cytoplasm is shown, the more internal chloroplast being drawn in optical section.

Fig. 13.—Green cells from the body of just-infected larval Convolutas. The large nucleus of the recently ingested green cell contrasts markedly with the degenerate nucleus of the daughter green cell budded off later from such a mother-cell. (Cam. luc., oc. 12, obj. 1⁄12 Zeiss.)

Figs. 14 and 15.—The infecting organism in the palmella (colonial) stage. A, B show green cells of the palmella organising active cells. C, The empty cyst left after the escape of a flagellated cell. Fig. 15 illustrates the origin of new cells of the palmella by budding.

Fig. 16.—A, B, C, the gradual disappearance of one of a pair of apposed resting-cells (see text, p. 184). D, E, Colourless and green resting-cells. F, G, Formation of colourless daughter-cells within a colourless mother-cell. H, I, Formation and discharge of green daughter-cells.
THE GREEN CELLS OF CONVOLUTA.