

The Yellow-Brown Cells of *Convoluta paradoxa*.

By

Frederick Keeble, Sc.D.,

Dean of the Faculty of Science and Professor of Botany in University
College, Reading.

With Plates 26—28, 3 Text-figures, and 2 Tables.

CONTENTS.

	PAGE
SECTION I.—INTRODUCTION	431
SECTION II.—THE BIONOMICS OF <i>C. PARADOXA</i>	432
(<i>a</i>) The <i>Paradoxa</i> Zone	432
(<i>b</i>) Migrations—Tropisms	434
(<i>c</i>) Periodicity of Egg-laying	440
(<i>d</i>) The Eggs and Larvæ	442
(<i>e</i>) Ingestion of Solid Food	443
SECTION III.—THE YELLOW-BROWN CELLS	445
(<i>a</i>) Description	445
(<i>b</i>) Photosynthetic Activity	447
(<i>c</i>) Reserve Fat	449
(<i>d</i>) Fate	453
(<i>e</i>) Origin	456
(<i>f</i>) Nature	463
(<i>g</i>) Significance	464
SECTION IV.—GENERAL CONCLUSIONS	473
TABLES 1, 2	475—476
LITERATURE	477
EXPLANATION OF PLATES	478

SECTION I.—INTRODUCTION.

Convoluta paradoxa (= *C. convoluta*, Auct.) is a small brown acœlous Turbellarian which lives among the seaweeds of the shore. The characteristic colour of the animal

is due partly to orange-red glands occurring in the superficial tissues, but chiefly to numerous yellow-brown cells, which are distributed fairly regularly in the sub-epidermal and deeper tissues of the body.

Though excellent descriptions have been published by von Graff (1891) of the habits and structure of *C. paradoxa*, nothing appears to be known with respect to its yellow-brown cells. The origin, development, significance, and fate of these cells are alike obscure.

It is chiefly with these yellow-brown cells that the present paper deals.

The investigations on which the paper is based were begun by the writer in collaboration with Dr. Gamble.

Of the conclusions enumerated in the summary, those to which the letters "G and K" are appended are the result of our joint work. For the others the writer of the paper is alone responsible. The research has been conducted in the laboratory, Trégastel, Côtes du Nord, and at University College, Reading.

SECTION II.—THE BIONOMICS OF *C. PARADOXA*.

(a) *The Paradoxa Zone*.—*Convoluta paradoxa* has its habitat among the finer brown and red seaweeds which occur at some little distance below the low-water mark of all but the larger spring-tides (Pl. 26, fig. 1).

The animal is flattened dorsi-ventrally; its anterior end is somewhat blunt, whilst posteriorly the body is prolonged into a slender tapering tail (Pl. 26, fig. 5; Pl. 28, fig. 10). The lateral margins of the body are flexed ventrally, and form, with the ventral surface, a groove whereby the animal is fitted saddle-wise over the weed on which it glides. It progresses by a gliding motion, and whenever it meets with some minor obstacle the sides of the saddle-like flexure give way and adjust themselves once again to fit the surface over which it is passing. If the animal encounters a more serious obstacle it fixes itself by its tail-end, partly by a mucilaginous secre-

tion and partly by bristle-like pegs, which stand out from the delicate cilia with which the body is clothed, and which are more numerous in the posterior region. Thus fixed, the anterior end is reared up caterpillar-wise, and the ventral surface fitted again to the substratum, or, quitting the substratum, the animal may swim freely in the water.

Though by no means gregarious like *C. roscoffensis*, *C. paradoxa* may be taken in fair quantity by following down the big tides and washing the finer weeds which it chiefly affects into a white porcelain dish, or by collecting the weeds, bringing them into the laboratory, taking them piece by piece and holding them so that the water drains down from them into a white dish. The animals follow the water draining from the weed, and so collect in the dish below. A particularly good catch may result in the collection of a hundred or more specimens. It is advisable to follow the falling tide, since many of the animals desert the weed as the tide falls off it, whilst those that remain cling so obstinately to the weed that even vigorous shaking fails to dislodge them. A white dish is better for the purpose than a transparent glass vessel, since *C. paradoxa* is much more easily pipetted off from the former than from the latter.

The sequence of sea-weeds on the rocks at Trégastel proceeding toward the sea is:—*Pelvetia*: *Fucus*, with a fine yellow-brown epiphytic algal flora attached to the fronds of *Fucus* on the seaward side; *Ascophyllum*: *Himanthalia*, the long strands of which, only exposed at fairly low tides, are also clothed toward their extremities with finer brown and dull red weeds; and, in the deep water, rarely and then but partially exposed, *Pycnophycus*, whose rounded thallus is covered with delicate red weeds, the chief of which are species of *Ceramium* and *Rhodomela*.

The limits of the *Paradoxa* zone are, on the landward side, the lower edge of the *Fucus* zone, and, on the seaward side, a little before the line which marks the permanently submerged part of the *Pycnophycus* formation. Within this zone the distribution of *C. paradoxa* varies with the phase

of the tides. At the onset of the spring tides the animals, chiefly immature specimens, may be found among the fine yellow weed attached to *Fucus* on its seaward edge. During the succeeding tides *C. paradoxa* moves seaward, and must be sought among the delicate weeds attached to *Ascophyllum*, whilst still later in the same series of spring tides it is only to be found among the *Ceramium* and *Rhodomela* and similar red weeds which cover the cord-like thallus of *Pycnophycus*. The same weeds permanently submerged a few yards further from the shore yield no animals. At both upper and lower limit of its zone of distribution *C. paradoxa* is represented only by immature, minute forms.

(b) Migrations—Tropisms within this Zone of Distribution.—There is, during the large spring tides, a tidal migration of *Convoluta*. The animals follow the falling tides seaward, and the rising tides landward. A study of the behaviour of the animals in the laboratory provides the probable explanation of this “ebb and flow” movement.

As has been mentioned, *Convoluta* tends to let itself be carried downward by the water draining off the weed. An explanation of the tidal, seaward movement based on this fact, sufficient as it seems at first sight, does not account for the zonal distribution of the animals, nor for all the facts of their “ebb and flow” movement. Thus for a little while after the water has left the weeds of the *Paradoxa* zone, animals may still be found in fair numbers among them. Later *C. paradoxa* deserts the weeds, and may be caught in the act of doing so by catching the drip from them. Closer examination of the question shows that a number of other factors co-operate in determining these tidal migrations. These factors are—(1) response to contact with weed or other solid body; (2) behaviour in stillness and darkness; (3) background reaction; (4) phototactic reaction.

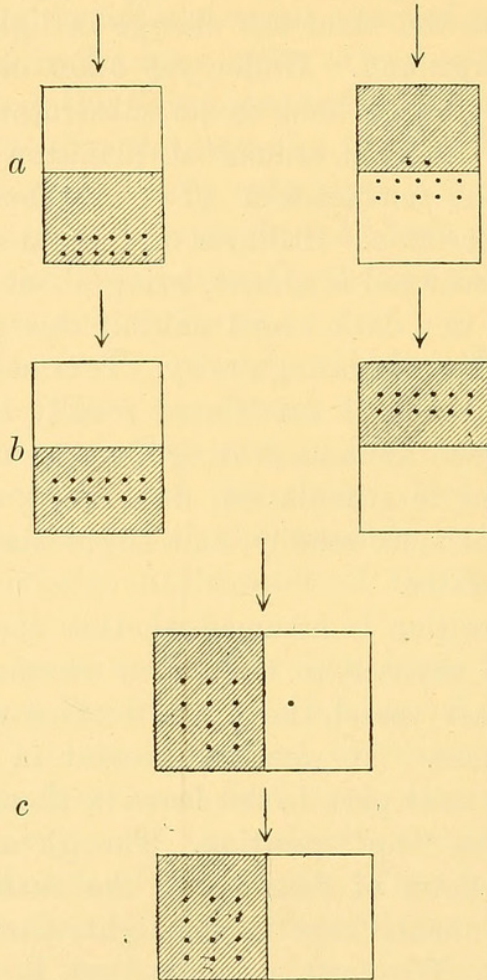
(1) and (2) Response to Contact with Weed, etc.—*Convoluta* exhibits the reaction of thigmotactism, that of clinging to a solid object, in a marked yet peculiar fashion.

The body, fitted to the weed in the manner already described, is dislodged from it with difficulty. Nevertheless there are conditions under which *C. paradoxa* relaxes its hold and becomes a swimming and no longer a gliding animal. Among these conditions the chief are change in light-intensity and change in background. Under yet other circumstances *C. paradoxa* relaxes its hold on its substratum, and becomes temporarily at least an animal of plankton habit, floating passively on the surface-film of water buoyed up by a mucilaginous secretion. Stillness of the water and darkness, particularly if seaweed is absent, bring about this behaviour. Animals placed in a dark vessel exhibit this phenomenon of "upness" with uniform regularity. It is not a pathological phenomenon, since it is manifested readily by fresh-caught animals, and since animals floating thus on the surface-film at once respond to stimulation, descending on exposure to light or, and yet more readily, on a slight disturbance of the surface of the water.

The "up" position is assumed whether the upper surface of the water is exposed to the air, or whether, contained in an inverted glass vessel, the upper surface of the water is against the glass. If weed is present in the vessel the animals for the most part do not leave it, though occasionally some assume the "up" position. Though more marked in darkness, this habit of floating on the surface-film is also exhibited by animals kept in the light, particularly if the light is feeble. We must conclude that, in the absence of any orientating stimulus whatever or of one of sufficient intensity, *C. paradoxa* leaves the "ground," rises to the surface-film, and floats there passively. Like all such movements, this at the present time is as inexplicable physiologically as ecologically it is obvious. Anticipating the evidence (p. 453) proving that *C. paradoxa* is dependent on light for its existence, we recognise that, borne out of its zone by currents or tides, it has, by virtue of this habit of "upness," a chance of regaining its home or of maintaining its existence in a new region; without it, condemned to hold

to the dim sea-bed beneath deep water, its light-requirements would fail to be satisfied, and it would perish.

"Upness," then, is a habit, and, like any other habit, it



TEXT-FIGURE 1.—Phototactism of *C. paradoxa*: the influence of background on phototactic response. The flat, porcelain troughs containing the animals are represented (in plan) by the oblongs. The bottom of each trough is half white and half black. In the diagram the white ground is indicated by the unshaded, the black ground by the shaded part of the oblong. The animals are represented by dots, and arrows show the direction of the light.

a. In bright light.

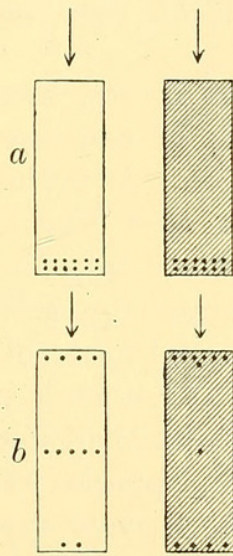
b. In weak light.

c. "Choice" of black ground in preference to white ground.

may manifest itself in opposition to weak stimuli, which, of themselves, tend to produce an opposing movement, or it

may be overridden by strong stimuli. Under normal conditions, whilst *C. paradoxa* keeps well within the region of a perpetual rain of powerful directive stimuli, "upness" will not manifest itself. Under abnormal conditions the habit will assert itself, and, on the average, to the benefit of the animal.

(3) Background Reaction.—*C. paradoxa* tends to stick to a black ground and to move freely over a white



TEXT-FIGURE 2.—Phototactism of *C. paradoxa*: the influence of light-intensity on phototactic response. *a*. Mode of response when the light-intensity is high. *b*. Mode of response when light-intensity is low. The glass troughs containing the animals are represented (in plan) by oblongs. The troughs standing on a black ground are represented by the shaded, those on a white ground by the clear oblongs. The animals are indicated by dots, and the arrows show the direction of the light.

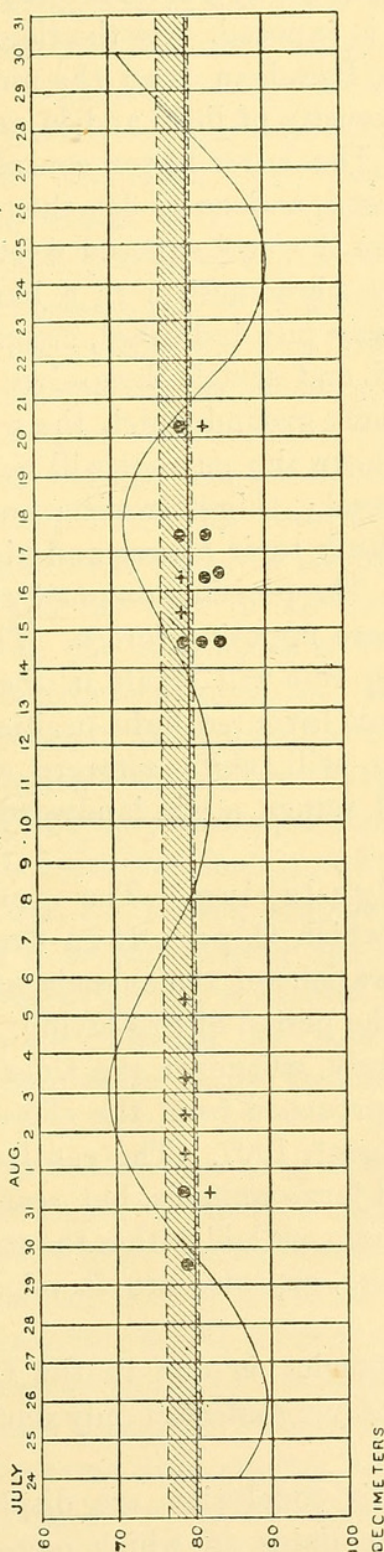
ground. It is probable that the sticking is due to light-perception by the orange-red "glands" of the body (Pl. 26, figs. 4 and 6), and particularly of the tail and to consequent reflex secretion of mucilage and activation of the stout bristle-like structures which, as described, project from the surface of the body. It is significant that the tail-like extremity of the animal is at once richer in pigment-containing glands and in bristles than is the rest of the body, and that it is also the sticking-organ.

When offered the alternative of white or black ground *C. paradoxa* takes up its position rapidly and permanently on the latter (text-fig. 1 c).

(4) Phototactic response.—*C. paradoxa* is negatively phototactic in light of fairly high intensity; for example, in the north light of the laboratory. This reaction is demonstrated readily by the usual methods (text-fig. 2). In light of low intensity the sign of the reaction changes, and the animals become either feebly positively phototactic or aphototactic (text-fig. 2 b).

The intensity of light is not, however, the only factor which modifies the mode of response of the animal to the directive influence of light. As is the case in so many other littoral animals (Gamble and Keeble [1903], Keeble and Gamble [1904]) back-ground may also modify phototactism. This is readily demonstrated by putting equal numbers of animals in each of two troughs the bottoms of which are half black and half white. When such vessels are placed in a good light, one with the white half toward the source of light, the other orientated in the opposite way, the animals exhibit their negative phototactism more rapidly if the movement involves a passage from white to black than if it requires a passage from black to white ground (text-fig. 1 a). Directive reaction masters back-ground reaction here though the latter produces an effect. When, however, the same experiment is carried out in dim light the tables are turned, and back-ground reaction dominates directive reaction (text-fig. 1 b). Thus in dim light the animals accumulate on the black ground no matter whether the black half of the dish is toward or away from the source of light.

Now to apply these facts to account for the zonal distribution of *C. paradoxa*, and to explain the "ebb and flow" tidal movements of the animals within the zone. Experiments described in Section III prove that *C. paradoxa* cannot flourish in darkness, and that it requires light of a certain intensity in order to carry on its nutritive processes. Now the zone in which *C. paradoxa* lives is characterised, speci-



TEXT-FIGURE 3.—Periodicity of egg-laying and hatching of *C. paradoxa*. The shaded band shows the position of the Paradoxa zone with respect to low water-marks of spring and neap tides. The undulating line, joining up the low water-marks of successive day-tides, is obtained by marking off, along the verticals indicating successive days, from a zero line above, the amount of vertical descent (in decimeters) of each day's tide.

On those days when the undulating line falls below the shaded band the Paradoxa zone is uncovered during low water; on those days when the low water-line lies above the shaded band the zone is continuously submerged. The dots represent egg-capsules, the crosses signify larvæ hatched; the positions of dots and crosses give the dates on which the capsules were laid and larvæ emerged.

ally during the large spring tides, by a periodic change in the amount of light to which it is exposed. As the tide is falling off the upper limit of the *Paradoxa* zone the animals are exposed to an increasing intensity of light and to an increasingly lighter background. The latter factor causes them to release their hold on the weed, and so to let themselves go with the water draining from the now exposed weed. Other animals turn their negative phototactism to a like account and, failing escape in the way just indicated, creep into the thickness of masses of weed and attach themselves firmly in obscure situations on the dark ground which the weeds provide. Those which follow down the current will be arrested by the weeds of a lower level; thigmotropism and darker background will tend to check their course and high light-intensity to drive them on. The position assumed will be the physiological resultant of these opposing forces. The return movement with the incoming tide will result in the animals once again taking up the most favoured light-position. This most favoured light-region will shift seaward with the increasing spring tides, and return again landward as these tides fall off.

(c) Periodicity of Egg-laying.—The periodically changing conditions under which *C. paradoxa* lives induce rhythm not only in the migrations of the animals within the *Paradoxa* zone, but also in the period of egg-laying.

Animals were collected daily, whenever the tides allowed the *Paradoxa* zone to be approached from the shore, during the months of July and August, 1907. The results showed many minute, mid-sized, and large animals, but scarcely any mature females. That the eggs are laid within the zone there is no doubt, since, occasionally, capsules are found attached to the weed of the zone.

The scarcity of mature females is due to the fact that maturity is reached and egg-laying effected only during certain tidal periods.

The results supporting this conclusion are displayed in text-fig. 3, which records the dates on which egg-capsules

were observed, and also the dates of hatching of the larvæ in the laboratory. The undulating line in the diagram represents the magnitude (in decimetres) of successive daily tides from July 24th to August 30th, and is based on the tide-almanac for Trégastel. The shaded band represents approximately the extent of the *Paradoxa* zone. The position of the zone is such that it is fully exposed by a fall of 80 decimetres, whereas a fall of 76 decimetres leaves it submerged.

Where the undulating line falls above the shaded band in the diagram the zone is submerged, viz. July 30th to August 8th, and August 14th to 21st; where the tide-line falls below the shaded band the zone is uncovered twice daily during low water. Each black dot records the date on which an egg-capsule was found either in the open attached to the weed or, and this was the case in the large majority of the records, was laid in the laboratory by *C. paradoxa* kept under as normal conditions as possible. Each cross marks the date on which a batch of larvæ hatched out in the laboratory.

The relation between time of egg-laying and tide is at once apparent, and may be stated thus:—Egg-laying occurs only at periods when the zone is submerged for six or seven successive days, i. e. during neap tides.

Hence it is that, during the spring tides, only immature animals are to be met with, and that supplies of egg-capsules can only be obtained by collecting the submerged weed during neap tides or by keeping the animals in the laboratory.

It must remain for the present an open question whether this phenomenon of periodic egg-laying is to be interpreted as a physiological consequence of the more active nutrition which obtains during exposure to the higher light-intensity of the preceding spring tides or whether it must take its place in the innumerable crowd of biological phenomena of adaptive significance (compare Keeble and Gamble, 1907). It is evident, of course, that during the slack tides the eggs, being permanently submerged, are protected from the risk of dessication, and to some extent from shock to which they would

be exposed if they were deposited during the springs when the zone is laid bare at each tide and receives the full force of the incoming waves.

Another noteworthy point is the rapidity with which the larvæ hatch. Within 24—48 hours after the capsules have been laid, the young emerge. Thus the majority of *C. paradoxa* are, during the first few days of their existence, continuously covered by water, and during this time also their habit of clinging to the weed is but ill-developed. Soon after the larvæ emerge the yellow-brown cells appear and multiply rapidly in the body and the animals develop or acquire those tropisms whereby they maintain themselves, despite the recurring changes of tide and consequently of illumination, within the narrow limits of the *Paradoxa* zone.

(d) The Eggs and Larvæ.—The eggs of *C. paradoxa* are laid in clutches contained in a common transparent capsule, each egg being likewise enveloped by a thin membrane (Pl. 26, figs. 1, 2, 3). The common capsule is less mucilaginous than that of *C. roscoffensis*, but sticks very firmly to any object on which it is deposited. The number of eggs in a capsule varies very considerably from as many as twenty-five to half or even less than half that number. By reason of the pigment of the eggs the capsules appear of an orange or pink-orange colour. The amount of pigment in the eggs varies very considerably. Its formation does not appear to be dependent on light, since eggs laid by animals kept for many days in darkness are as deeply pigmented as those produced by animals in normal situations. The pigment consists of small oval or dumb-bell shaped granules, each of which presents the appearance of a double-contoured band of orange pigment with a clear inner part. These pigment-bodies are probably the forerunners of the orange-pigmented glands of the adult animal. In addition to those granules which are distributed with fair uniformity, aggregations of finer granules of pigment may also occur in the egg. It was doubtless owing to the presence of the larger orange granules in the egg and larva that von Graff was led to the conclusion

that the yellow-brown cells of *C. paradoxa* are present in the egg.

Careful examination shows, however, that this view is erroneous, and that the orange granules of the egg have no relation whatever with the yellow-brown cells of the larva or adult. The pigment-bodies of the egg have none of the histological features of the yellow-brown cells of the larva or adult; the pigments are distinct in colour and react differently with reagents. Thus, whereas the orange granules of the egg and also the orange glands of the animal become colourless when treated with 90 per cent. alcohol, the yellow-brown cells of the animals become green. In other words, these cells contain two pigments, a yellow and a green, whilst the orange granules contain only one pigment. Further proof that the orange granules are in no way whatever related to the yellow-brown cells is given in Section III.

(e) Ingestion of solid food by *C. paradoxa*.—Immediately after hatching *C. paradoxa* like *C. roscoffensis* begins to ingest solid bodies (Pl. 26, fig. 4).

But whereas the latter species, soon after its infection by the green cells, ceases altogether from ingesting solid food, *C. paradoxa* continues throughout its life to be a voracious feeder. Its staples of diet are diatoms, many dozens of which may often be found in all stages of digestion lying in digestive vacuoles (Pl. 26, fig. 4). In addition to diatoms *C. paradoxa* takes up almost any small organisms it comes across. It also swallows and digests relatively enormous copepods; whilst later in the year its body may become so studded with the tetraspores of various algæ as to give to the animal a dark red colour. Failing suitable food-bodies *C. paradoxa* swallow greedily any inert, granular substance supplied to it. When supplied with congo red, masses of this pigment are ingested, and lie in colourless vacuoles in the solid gut. The congo red retains its colour in these vacuoles, indicating that, though ingestion does not require any chemical stimulus, the secretion of digestive juices into the vacuoles depends on such a stimulus. It is probable that the digestive secretion

acts in an acid medium, since, when under certain circumstances to be described presently the yellow-brown cells undergo digestion in vacuoles, the space between the wall of the vacuole and the cell undergoing digestion is occupied by a faintly pink fluid. The solid food is taken in through a mouth which is not recognisable when closed, but which is seen during the act of ingestion to be capable of an enormous gape (cf. Bronn's 'Tierreich,' iv, 68—71).

The solid gut which consists of parenchymatous tissue has no clearly defined outline; but its shape may be traced roughly by the positions which solid bodies fed to the animals take up in it. A convenient substance for this purpose is uric acid, the crystals of which are recognised readily, and which, unlike many inert substances, does not seem to provoke excretion. Not only may large quantities of this substance be ingested, but the crystals remain for a very long time in the digestive vacuoles. By such means it can be seen that a short broad gullet passing back from the mouth gives off two successive pairs of transverse processes, and then ends in a broad but gradually tapering mass of granular tissue, which reaches almost to the posterior end of the animal. The lateral processes are connected with prolongations which run one on either side of the body forward almost to the level of the statocyte and also a short distance backward. It is noteworthy that the two broad and prominent transverse bands of dark refractive material (concrement granules), Bronn (loc. cit.), which are so characteristic of *C. paradoxa* (Pl. 26, figs. 4 and 5), and which von Graff regards as being of the nature of nitrogenous excretory substance, lie over the two pairs of transverse processes which connect the gullet with the large laterally placed parts of the gut.

Mention may be made here of several attempts to infect larval *C. paradoxa*, whose bodies were free from yellow-brown cells, with the green, flagellated infecting organism of *C. roscoffensis*. The flagellated cells were ingested by *C. paradoxa* and transferred to large vacuoles, where they remained intact for several days with their eye spot and

pyrenoid sharply defined. After a further period, however, the green cells gradually disintegrated, and the attempt to effect what, if it had succeeded, would have been a synthesis of no small interest proved abortive.

SECTION III.—THE YELLOW-BROWN CELLS.

(a) Description.—In every specimen of *C. paradoxa* taken from the sea yellow-brown cells are present (Pl. 26, figs. 5 and 6; Pl. 27, figs. 7, 8, and 9). These cells are as constantly characteristic of *C. paradoxa* as are the green cells of *C. roscoffensis*. Though not so numerous as to give, when examined microscopically, the appearance of a continuous tissue as is the case with the green cells of *C. roscoffensis*, the yellow-brown cells of *C. paradoxa* are, nevertheless, present in the adult body in great numbers. They occur in groups or rows situated for the most part just beneath the epidermis, though they also occur in the deeper tissues. The number of yellow-brown cells increases with the size of the animal. Very minute young specimens contain but few, mid-sized animals more, and adults most. This increase is effected in part, at all events, by the division of the yellow-brown cells (Pl. 28, fig. 18). When examined in the living state, soon after the capture of the animal, each yellow-brown cell is seen to contain many irregularly oval or polygonal, yellow-brown discs which occupy the greater part of the cell. Lying about the margin are a number of more elongated, browner, somewhat boat-shaped bodies. The remainder of the cell consists of one or more clear, transparent, vacuolar areas; occasionally, colourless, rounded refractive bodies also occur (Pl. 27, fig. 7).

But if the microscopic examination is made immediately after the animals have been caught, the yellow-brown cells are seen to differ markedly from those of animals kept for an hour or so before examination. Most of the yellow-brown cells in the just-caught specimens have a large colourless, transparent, anterior region, which may be as much as half

the size of the whole cell ; in others, two or even three such clear vacuole-like structures may occur. Moreover, instead of the occasional refractive globules, the yellow-brown cells of animals examined immediately after capture are found to contain, in many instances, large numbers of such globules (Pl. 27, figs. 8 and 9). Not only are these globules to be observed in the yellow-brown cells, but they may be seen also lying in the animal tissue itself (Pl. 26, fig. 6).

The numerous yellow-brown discs which make up the bulk of the cell contain two pigments—a yellow pigment and a green pigment having the appearance of chlorophyll. The green is masked by the yellow pigment, but may be demonstrated by treating the animal with 90 per cent. alcohol. Thus acted on, the yellow pigment screening the chlorophyll dissolves rapidly and discs become green. Similarly, hot water destroys the yellow and reveals the green pigment. In this respect the yellow colouring-matter behaves like Phycophæin, the pigment of the brown algæ. When 90 per cent. alcohol is added to cells, the yellow pigment of which has been extracted by hot water, the green colour is intensified. The yellow pigment is only slightly soluble in dilute alcohol. Acted on by concentrated sulphuric acid the cells take on a beautiful emerald-green colour. These reactions serve to distinguish between the yellow-brown cells and the orange glands. The pigment of the latter is removed by 90 per cent. alcohol, but when so removed leaves no green colour behind. Treated with concentrated sulphuric acid the orange pigment of the glands undergoes no change.

There can be no doubt that the discs which make up so large a part of the yellow-brown cell and which give to it its distinctive colour are chloroplasts. The reason for the marked difference between the more central, polygonal, or oval, pale chloroplast, and the peripheral, elongated, boat-shaped, darker chloroplasts is not obvious. The contrast between the two kinds is so marked as to make it unlikely that the difference is merely optical—due to the latter presenting themselves in profile. It may be that the more peripheral

chloroplasts are somewhat affected by the presence of the cell in the animal tissue, and, shrunken somewhat, appear darker in colour (Pl. 27, fig. 8, and Pl. 28, figs. 11, 12, and 13), or it may be that the differences indicate a real dimorphism of the chloroplasts.

The wall of the yellow-brown cell is extremely delicate, and gives no staining reaction whereby its nature may be determined. It is not composed of cellulose. A central, spherical nucleus staining green with methyl-green acetic may be demonstrated in many of the cells. Where present it lies about the middle of the cell in a sheath of protoplasm from which radiating strands run toward the periphery (Pl. 28, fig. 18). In some preparations the nucleus, slung in the manner just described, appears to lie at the base of a colourless "neck" of protoplasm in the same position as that in which it occurs in a *chlamydomonas* cell. Occasionally two nuclei may be seen in a yellow-brown cell, at other times the cell appears to have divided, but division of the nucleus not to have taken place so that a nucleus occurs in one part, and not in the other (Pl. 28, fig. 18 *d*).

(*b*) The Photosynthetic Activity of the Yellow-brown Cells.—The structure of the yellow-brown cells of *C. paradoxa* points to the conclusion that these bodies are of the nature of algæ. Other evidence based on their origin and given in Section III confirms this conclusion.

Now if the yellow-brown cells are of the nature of algæ, and if, as shown, they divide and grow in the animal tissue, it is to be expected that they may exhibit photosynthetic activity. Unfortunately but little is known either of the nature of the substances photosynthesised by members of the different groups of the brown and yellow-brown algæ or of the form in which these substances are stored as reserves in the plant-cells.

According to Hansteen (1900) the reserve-substance formed from the product of photosynthesis in the Phæophyceæ (brown algæ) consists of refractive globules of a carbohydrate which he calls fucosan. Crato (1893) holds that the reserve-substance of the brown algæ is contained

in vacuole-like bodies termed physodes. The contents of the physodes blacken with osmic acid, and give the vanillin reaction for phloroglucin or tannin. According to Koch, (1896) who denies the presence of tannin, the physodes contain a colloidal substance formed from a polysaccharide and also a nitrogen-containing body.

In Dictyota, Hunger (1902) states that a carbohydrate occurs in the outer, and a glucoside in the inner, layers of cells, and that these substances disappear from the cells of dark-kept plants. The assimilate of the Diatomaceæ is stored generally outside the chloroplast in the form of oil, (Oltmanns, 1905). Dinobryon, a member of the Chryso-monadineæ, contains white balls of unknown nature termed by Klebs (1892) leukosin. In other members of this group Meyer (1897) describes a fatty oil as the reserve substance. The nature of the reserve-form of the assimilate of the Zooxanthellæ occurring in Radiolaria has been investigated by Haeckel, Müller, Brandt, and others, but the conclusions are not very convincing. According to Haeckel these yellow cells give a blue reaction with iodine (Cienkowski, 1871), whereas Müller obtains a brown reaction, deepening on the application of sulphuric acid. Brandt (1885), in his well-known monograph on the Radiolaria, states that the assimilate lies in the chloroplast (chlorophyll body), and is of two kinds. One form is starch, consisting of fine granules, which occur as a thin ring about a large vacuole. These bodies are not doubly refractive, and are described as hollow starch grains. The other form of assimilate consists of doubly refractive, fine, irregular, red-violet granules, which undergo no colour-change with iodine. They are not numerous in diffuse light, but increase in numbers when animals are exposed to bright light for half an hour.

With reference to these various observations several points may be noted. First, the iodine coloration, brown or blue, must not be taken to indicate the certain occurrence of starch. Yellow algal cells such as Zooxanthellæ are rich in carotin, which substance gives a green or blue-green colour

with iodine. Hence the colour-reaction obtained by Haeckel and Müller may well have been due to the presence of carotin, and not to the presence of starch.

Again, Brandt's hollow starch grains—fine granules surrounding a vacuole—suggest, though somewhat remotely, a degenerated pyrenoid with its starch sheath. Third, the red-violet granules described by Brandt cannot be regarded, merely because they increase in numbers in bright light, as of the nature of photosynthetic products. It is at least as likely that they owe their origin to a light-induced modification of carotin or some similar pigment.

In any case accurate information as to the nature of the assimilate of the *Zooxanthellæ* is lacking, and it is to be hoped that those engaged in researches on the *Radiolaria* will clear up this matter.

(c) The Reserve-fat of the Yellow-brown Cells.—The yellow-brown cells of *C. paradoxa* contain no starch; but reference has already been made to the occurrence, in the yellow-brown cells of animals examined immediately after capture, of refractive globules. These droplets (Pl. 27, fig. 8) lie apart from the chloroplasts in the colourless reticulum of the cell. The substance composing them is soluble in absolute alcohol, stains a grey or brownish colour with osmic acid, and gives the yellow-red fat-reaction with Sudan III. The osmic reaction is somewhat faint till after the preparation has been treated with alcohol, when the brown coloration of the globules gives place to black. This behaviour is, according to Bolles Lee ('Microtometist's Vade Mecum,' p. 36, 1900), characteristic of stearin and palmitin, and of the corresponding fatty acids. It may, therefore, be concluded that the refractive droplets of the yellow-brown cells consist of, or at least contain a fatty substance. That they are of the nature of reserves is suggested by the facts that they disappear gradually from the yellow-brown cells of animals kept under the somewhat abnormal conditions obtaining in the laboratory, and that this disappearance is more rapid in dark-kept animals.

For example, the yellow-brown cells of animals caught on July 24th, 1907, contained numerous large fat-globules. Animals from this catch were transferred to filtered sea-water, and kept, some in darkness, others in the light. After four days (July 28th) the dark-kept animals contained no fat, whilst those kept in the light still contained fat-globules in their yellow-brown cells.

It follows, therefore, that either the oil-globules are reserve substances formed from the products of the photosynthetic activity of the yellow-brown cells, or they are derived from the tissues of the animal.

So little is known of the modes of nutrition of the lower algæ that the *à priori* objection to the latter interpretation is valueless. For it is based implicitly on the hypothesis of autotrophic nutrition of the algæ in general.

Experiments designed to determine this question seem at first sight to support the conclusion that the fat is derived from the animal tissues. If animals, whose yellow-brown cells contain fat, are placed in darkness, some in filtered sea-water, others in ordinary sea-water with seaweed from the *Paradoxa* zone, it is found that the fat disappears more rapidly from the animals kept in filtered sea-water than from those supplied with food. From this it might be concluded that new supplies of fat reach the yellow-brown cells from the digested products of the food taken in by the animal. Against this, however, is the fact that the disappearance of the fat from the yellow-brown cells of animals kept in darkness and supplied with seaweed and hence with food is only a matter of time. Kept under such conditions, although the animals continue to feed, the fat disappears from the algal cells, and finally these cells undergo degeneration. The right inference to be drawn, therefore, from the slower disappearance of fat from the yellow-brown cells of dark-fed animals would seem to be that the food ingested by the animal acts in some measure as a protection to the yellow-brown cells hence the reserves of food-material accumulated in these cells are not drawn upon by the animal so rapidly as is the

case when no other food is available. What seems to be conclusive proof that the fat of the yellow-brown algal cells is a product of photosynthetic activity is the fact that when animals are placed in filtered water and so deprived of all food except that which reaches them from the algal cells and when the conditions for the nutrition of these cells are made as favourable as possible, e. g. by the addition of extra nitrogen in such form as potassium nitrate, then so long as the animals are exposed to light their yellow-brown cells continue to contain oil globules. Thus, in one experiment, animals were placed, some in filtered sea-water in the dark, others in filtered sea-water with extra nitrogen in the light. After fourteen days (August 21—September 5), though there was no fat in the algal cells of those kept in the dark, fat-globules were still present in the yellow-brown cells of the light-kept animals. It is therefore to be concluded that the fat-globules of the algal cells of *C. paradoxa* are reserve products of the photosynthetic activity of these cells.

It follows naturally from the foregoing experiments and conclusions that the fatty reserves of the yellow-brown cells are drawn on to supply material for the growth and metabolism of these cells. But there is evidence that the reserves of fat in the algal cells are also drawn upon and utilised by the animal tissues.

The general proof that the yellow-brown cells do pass on food substances to the animal tissues will be given later. That the reserve-fat of the algal cells is one of the substances transferred to the animal the following observations render probable :

In the first place, the tissues of animals whose yellow-brown cells are rich in reserve-fat also contain large numbers of globules of similar nature. These fat-globules, lying in cells of the animal tissue, have the same optical properties as, and give reactions similar to, those of the algal cells. In the second place, the appearance of the fat and its relation to the yellow-brown cells is, in freshly captured animals, most suggestive of secretion, recalling almost the appearance

of a cell of a mammary gland in its active stage. The large, clear, anterior end of the yellow-brown cell, only visible in fresh caught specimens which by the nature of their habitat have just been exposed to high light-intensity, is seen often to contain one large oily droplet. In some, one or more large droplets are situated in the deeper part of the clear anterior end, whilst in others a single large oil-drop lies close against its anterior margin, separated only from the animal tissue by the finest of membranes. Finally, other large globules may be seen lying just outside the colourless anterior borders of yellow-brown cells, and presenting every appearance of having been extruded from them (Pl. 27, figs. 8 and 9). It is not, of course, suggested that all the fat-globules, of which great numbers occur in the body of fresh-caught animals, are derived from the algal cells. Doubtless some fat comes from the digested solid food, diatoms, foraminifera, copepods, tetraspores, and the like on which *C. paradoxa* feeds so copiously. But it is claimed that the algal cells, when actively photosynthesising, regularly pass on the excess of their assimilate in the form of fat to the animal tissues.

These observations throw light on certain others which are recorded in the paper on "*Convoluta roscoffensis*" (Keeble and Gamble, 1907). Here, in some preparations, rows of fatty granules are to be seen passing from the green, algal cell to the neighbouring animal cells. It is highly probable that this fat represents the translocation-form of the ternary substance which *C. roscoffensis* obtains from its contained green cells. In this former paper, where proof was given that food-substances do actually pass from the green cells to the animal, it was suggested that the reserve-starch of the green cell travelled from that cell to the animal tissue in the form of sugar. But, having regard to the known fact that in plants starch is very readily converted into fat it may well be that the reserve-starch of the green chlamydomonadine cell of *C. roscoffensis* undergoes conversion into fat before passing out from the algal cell into the animal tissues.

(d) The Fate of the Yellow-brown Cells.—The yellow-brown cells persist throughout the normal life of *C. paradoxa*. Comparatively few in the very young, the number increases with the size of the animal. A casual observer, noting this large number of cells in the body of the adult, many of them of great size and many undergoing division, would be tempted to conclude that the yellow-brown cells were living parasitically upon the animal.

The evidence just given, together with that which follows, demonstrates that this way of looking at the relationship between *C. paradoxa* and its yellow-brown cells is erroneous. For, beside the tribute of fat which it exacts regularly from the algal cells, the animal has another and more drastic mode of exploiting them.

If animals are kept in darkness in sea-water filtered through a Pasteur-Chamberland filter, they exhibit, of course, starvation phenomena and become reduced greatly in size. This reduction in size is greater, and takes place much more rapidly in such dark-kept than in light-kept animals.

Thus animals of the same history were put in filtered sea-water on August 29th, 1907, and kept some in light and some in darkness. After nine days the light-kept animals were recognisably less reduced in size than the dark-kept. Measurements gave, in divisions of the Leitz ocular micrometer, oc. 2, obj. 3 (= L. 2. 3) $140 \times 60 = 8400$ in the case of a light-kept: $75 \times 44 = 3300$ in that of a dark-kept animal. That is the area of the former measured 142 sq. mm., the area of the latter 56 sq. mm., or $2\frac{1}{2}$ times less.

The experiment, which was repeated frequently in the course of this research, demonstrates that, in the light, supplies are forthcoming from yellow-brown cell to animal, and that, in the dark, such supplies are either altogether lacking or much smaller in amount. In other words, the experiment confirms the previous conclusion that the yellow-brown cells are the seat of photosynthetic activity, and that products of this activity pass from algal cell to animal tissue.

Animals kept for a long period in ordinary filtered sea-water, even though they are exposed to the light, become ultimately reduced almost to microscopic size. Thus, examples put in filtered water (July 24th, 1907) measured (L. 2. 3) $120 \times 83 = 9900$. After a month (August 23rd) a specimen measured $60 \times 30 = 1800$, i. e. $\frac{1}{5}$ of its original size. That such small animals as *C. paradoxa* are able to withstand starvation for such long periods points definitely to the conclusion that they are living at the expense of their algal cells. Microscopic examination of animals kept in light and darkness in filtered sea-water shows that, as the animal tissues waste, so the yellow-brown cells become more and more reduced both in size and numbers.

That this reduction of the algal cells is more rapid in "dark-filtered" than in "light-filtered" is seen in the results of the experiment already cited, in which, after nine days, the size of "light-filtered" animals was to that of "dark-filtered" as 2.5 : 1. At the time these measurements were taken, the records for the algal cells in the two cases of light-kept and dark-kept animals ran thus:—Light-filtered, number of yellow-brown cells double that in dark-filtered.

Size of yellow-brown cells:—Light-filtered, most 4 divisions (ocular micrometer, L. 2. 6) $= 14.8 \mu$; some 6 div. $= 22.2 \mu$ and more. Dark-filtered, most 2 div. $= 7.4 \mu$; some 3 div., some 5 div. $= 18.5 \mu$.

This rapid degeneration of algal cells in animals kept in filtered sea-water in the dark offers some means of obtaining information as to the nutritive relations which exist between yellow-brown cells and animal. For the purpose of this enquiry, animals of similar origin were placed in the dark, some in filtered sea-water, others in unfiltered sea-water with weed rich in food-material (diatoms, etc.) from the *Paradoxa* zone. After nineteen days the number of yellow-brown cells was greatly reduced in both. It is true that the reduction in numbers and in size had proceeded further in the dark-unfiltered than in the weed-fed animal, and that, whereas no normal yellow-brown cells were to

be observed among the former, among the latter some few intact normal yellow-brown cells persisted. Since there is no apparent reason why the algal cells of animals supplied with proper food should suffer curtailment of supplies merely because they are placed in the dark it is reasonable to conclude that, whatever may be the contribution toward the raw material of the food which the yellow-brown cells receive from the animal, they do not receive elaborated material from that source. The somewhat slower reduction of algal cells in the fed animals indicates that, in the presence of food derived from the outside, the yellow-brown cells are in some small measure spared.

Taking all the facts together: from the behaviour of the animals and algal cells in filtered sea-water (light), and in filtered sea-water (dark), and in ordinary sea-water with weed (dark), it must be concluded that, in darkness, the algal cell failing to photosynthesise material for itself and unable to obtain supplies of elaborated food material from the animal, falls a victim to the digestive activity of the surrounding animal tissues. In its weakened state, brought about by starvation, it can no longer offer resistance to this digestive activity, whereas in its normal state it buys off this fate at the price of the tribute in kind, in the form of fat, which it pays continuously to the animal.

Histological examination of the dark-kept animals supplies evidence that degeneration of the algal cells is not a mere decay within the animal body, but is the result of a process of digestion exerted on them by the animal tissues. The first sign of this digestive process consist in a reduction in size and a more spherical shape of the yellow-brown cells. The chloroplasts become smaller and rounder, though the nucleus and the clear anterior end of the algal cell may persist. Each reduced algal cell may now be seen lying in a distinct digestive vacuole containing a pink fluid. The pigment of the chloroplasts is dissolved, and diffusing out of the cell may colour the vacuolar fluid brown. At this stage the chloroplasts are greenish, but later they become colour-

less. Finally, nothing appears to be left of the algal cells, but heaps of few or many colourless, curiously persistent, granules. The time required for the algal cells to be reduced to such granular remnants varies considerably; in some experiments it took fourteen days, in others nineteen days or longer.

Experiments were instituted in order to ascertain whether the remnants of the yellow-brown cells left in the body after prolonged exposure to darkness were capable of regenerating into normal yellow-brown cells; but, as an understanding of the results obtained requires a knowledge of the appearance of the algal cells in their young stages, the results are deferred till after this appearance has been described (p. 460). It may, however, be mentioned here that, when a redevelopment of algal cells was induced in animals which had been kept previously for long periods in darkness, the animals which, during their sojourn in darkness, had become reduced to the size of larvæ, began at once to grow again. Thus, after fourteen days darkness three animals measured superficially in divisions of the ocular micrometer L. 2. 3:— $77 \times 53 = 1.18$ mm.; $71 \times 43 = .87$ mm. and $48 \times 28 = .37$ mm. Average = .8 mm. Whilst animals similarly treated but subsequently brought into the light and supplied with weed, under which conditions algal cells reappeared, measured, after four days exposure to the new conditions of light and food, $92 \times 55 = 1.46$ mm.; $74 \times 54 = 1.15$ mm.; $78 \times 40 = .9$ mm. and $50 \times 30 = .5$ mm. Average 1 mm.

Though, by reason of original differences in size, too much weight cannot be given to individual measurements, the phenomenon of apparent increase was so uniform as to admit of the conclusion that growth of the animal is bound up with the presence of living yellow-brown cells.

(e) The Origin of the Yellow-brown Cells.—A larval *Convoluta paradoxa*, as it hatches out from its egg-capsule, contains no yellow-brown cells nor any precursors thereof. *C. paradoxa* is in this respect precisely similar to

C. roscoffensis, which, as has been shown, is at birth altogether free from algal cells. That *C. paradoxa* hatches as an uninfected larva is easily shown; indeed, whereas in *C. roscoffensis* the difficulty is to prevent infection by the algal cells, in *C. paradoxa* the difficulty is to induce it.

If the egg-capsules of *C. paradoxa* are placed in ordinary sea-water, without any weed from the *Paradoxa* zone, the animals do not become infected. They feed on diatoms, etc., which may be present in the water, yet not only do they not increase in size, but rapidly become smaller, and fail altogether to show signs of development. Thus, a series of measurements of just-hatched animals gave length by breadth in divisions of ocular micrometer L. 2. 3:—3500, 3600, 4500, 5725, or in sq. mm.: 1.02, 1.04, 1.3, and 1.6; whereas a series of measurements of uninfected animals of various ages all put with water rich in diatoms and other food-material gave 1600, 1920, 2400, 2720, 3600, 4125, 4750, or in sq. mm.: .5, .56, .69, .78, 1.05, 1.2, and 1.4. That is, whereas the average size of the just-hatched animal is about 1.2 sq. mm., the average size of 7 animals supplied with food but not with the infecting organism is .88 sq. mm.; and this in spite of the fact that the food-material (diatoms, etc.) is taken up readily enough.

In order to induce the development of the yellow-brown cells in the larvæ it is not sufficient to cause them to hatch out in water from the *Paradoxa* zone. Weed from the zone must be provided, and the supplies of this weed replenished several times during the first few days after hatching. Even so, infection does not follow inevitably. In cases where, by the adoption of this procedure, infection ensued, it was found to occur very rapidly.

For example, in one instance, animals were caught on August 12th; on the 14th eggs were found on the small pieces of weed left with them. The young, hatched on the 16th, were put in a separate vessel with water and fresh weed from the *Paradoxa* zone. On the 17th the specimens examined showed no signs of yellow-brown cells. Another piece of

weed from the *Paradoxa* zone was added, and on the following day infection was observed. The results of this experiment are summarised in Table I (Infection Record), where it will be seen that, of 14 animals examined, 8 showed no sign of infection, and 6 showed infection in various stages: from the stage in which one yellow-brown algal cell was present in the animal to that in which large numbers of yellow-brown cells occurred. This was one of the most successful of the many infection-experiments which were carried on in 1906 and 1907. In others the proportion of infected to uninfected animals was smaller, and in some cases no infection was obtained. From this it is to be concluded that the changed conditions in the laboratory affect the infecting organism adversely or that its distribution in the weed is sporadic. Yet another possibility must not be lost sight of, namely, that under some circumstances, e. g. when the conditions, as, for example, those obtaining in the laboratory, are somewhat unfavourable, the yellow-brown cells taken up by the larvæ may suffer digestion just as was shown to be the case with the yellow-brown cells of the adult. As indicative of the probability that this fate may overtake the infecting algal cell, several records occur in the notes of infection experiments to the effect that a larva, exhibiting no normal yellow-brown cells, nevertheless contained digested remnants of such cells. The fact that in nature no *C. paradoxa* are to be met with which do not contain large numbers of yellow-brown cells is no argument against the probability of this view, since, under natural conditions, reinfection would doubtless occur. On the other hand, the conception that it is not a foregone conclusion that, once arrived in the body of *C. paradoxa*, the algal cell will grow and divide, contributes a little toward an understanding of the way in which the present relations between algal cell and animal may have originated. The animal, it may be conceived, plays with respect to the yellow-brown cells the part which that discriminating Providence, Natural Selection, plays with respect to living things in general. Not all the entering algal cells pass its test; nor,

indeed, all those belonging to the chosen race of yellow-brown algæ, but only those that can withstand digestion. How some possess this power, or acquire it, may likewise be imagined. When, as often happens, the body of *C. paradoxa* is gorged with solid food—in one case upwards of 70 diatoms were counted in a single animal—the activity directed toward the digestion of any single cell is likely to be reduced. Thus, it may be that the glut of solid or reserve food in the body may act temporarily as a protection to the yellow-brown cells. Profiting by this respite, the algal cells may react adaptively by change in osmotic power, or in permeability of plasmatic membrane, and so prepare to resist the action of the normal digestive juice of the animal. By what chemical mechanism the animal cells come to tolerate the presence of these foreign cells, or by what means the fatty reserves are discharged from the algal cells, it is impossible to say. An investigation of the proteases and other enzymes of *C. paradoxa*, particularly with a view to determine in what medium they act, might throw some light on the enigma of what maintains the balance between foreign algal cell and animal so equipoised that the former behaves like an integral part of the latter. To discuss such questions in the present state of our knowledge of invertebrate physiology, though tempting, is vain.

Apart, then, from all theoretical considerations, experimental proof is forthcoming that the eggs of *C. paradoxa* contain no yellow-brown cells; that larvæ, unless exposed to the weed of the *Paradoxa* zone, remain uninfected, and that infection may be induced by bringing the recently hatched animals into contact with this weed. Thus, except for the fact that it is much more uncertain in *C. paradoxa*, there is with respect to infection a complete agreement between *C. paradoxa* and *C. roscoffensis*. Both animals require to be reinfected in each succeeding generation. In neither is there any transmission of infecting organism from one generation to the next.

The greater certainty of infection of *C. roscoffensis*

under experimental conditions is, as has been shown (Keeble and Gamble, 1907), in part due to the regular and close relation which exists between the infecting organism in its free state and the egg-capsules of the animal and in part to the ubiquity of the infecting organism. In that species the free, four-flagellated, Chlamydomonadine alga which constitutes the infecting organism settles habitually on the gelatinous egg-capsules, and, undergoing division, gives rise in the empty capsules to spherical colonies. It was the observation of this fact which led to the isolation of the infecting organism of *C. roscoffensis*.

In *C. paradoxa* this habit on the part of the free infecting organism of frequenting the egg-capsules does not appear to obtain, or, if it obtains, is much more difficult of demonstration.

Repeated experiments were made in which the capsule-remnants, left after the larvæ had escaped, were put in filtered sea-water and kept under observation; but, though these capsules developed a fairly luxuriant flora of diatoms, brown flagellates and yellow-brown cells resembling in number of chloroplasts and in refractive inclusions the yellow-brown cells of the adult *C. paradoxa*, it was not found possible to induce the infection of larval animals by putting them in contact with these possible sources of infection. Too much weight must not, however, be attached to these negative results; for the experimental difficulties are greater in the case of *C. paradoxa* than in the case of *C. roscoffensis*, partly because the former animals are more difficult to obtain in any considerable numbers, and partly because of the difficulty of imitating in the laboratory the conditions under which *C. paradoxa* lives in the open.

For the same reasons all attempts to isolate the infecting organisms or to obtain it in its free stage have failed. The yellow-brown cells of the adult or young infected animal cannot be induced to grow when removed from the body of the animal. The same fact has been established with respect to the green algal cells of *C. roscoffensis*. They also fail

to develop when removed from the body. There can be little doubt that this failure on the part of the green cells of *C. roscoffensis* to maintain themselves when separated from the animal is due to the fact that they undergo whilst in the animal body partial nuclear degeneration. The same is probably true of the yellow-brown algal cells of *C. paradoxa*. It being impossible to isolate viable yellow-brown cells from the animal the only alternative is to seek among the weed for a corresponding form, to cultivate it, and apply the infection test. Though yellow-brown cells, resembling in the most striking way those of the animal, are to be met with occasionally, especially in the capsule flora (Plate 28, fig. 17), and though in one instance a brown cell with elongated anterior end very suggestive of a flagellated organism was observed on an egg-capsule (Plate 28, fig. 16), the isolation of the free alga which, when in the animal, gives rise to the yellow-brown cell, has not yet been accomplished.

The earliest stage yet observed shows the body of the animal to contain a single algal cell (Plate 28, figs. 10—15). The size of this cell varies considerably in the different cases in which it has been seen. In one example the cell was spherical, $7.4\ \mu$ in size, with greenish-yellow chloroplasts. In another it was of oval shape, measured $16.5\ \mu$, and exhibited, like the yellow-brown cells of fully infected animals, but in a more marked manner, a peripheral series of flattened yellow chloroplasts with a number of pale grey-yellow chloroplasts occupying the body of the cell. In yet other cases, in which more than one cell was present, the infecting alga exhibited a large, clear, transparent region, a group of flattened brown chloroplasts pointed at either end pressed against the wall of the cell, and a number (8—16) of pale yellow structures more like daughter-cells than mere chloroplasts. This appearance was strikingly exhibited in one case, where the animal contained five algal cells, of which the largest was $37\ \mu \times 37\ \mu$, the others $16\text{--}18\ \mu$. The large cell was surrounded by transparent thickish wall, against one side of which the elongated, dark brown chloro-

plasts were pressed, whilst in the centre a large number (thirteen counted) of irregular, oval, pale yellow-brown bodies occurred (Pl. 28, fig. 15 *a*, *b*, *c*). These yellow-brown structures contained each a central dark spot, and each when seen in profile showed the appearance of a vertical cleft extending through about half its depth. The wall by which these yellow-brown bodies were enclosed underwent change of shape due to the movement of the animal, whence it may be assumed that it consisted of animal vacuole-wall together with extremely delicate algal cell-wall. During examination the vacuole burst, and the contained irregularly oval, pale brown bodies were discharged into the body, and lay in groups of twos and fours. None of the other vacuoles of the body burst, and so there is a possibility that what was witnessed was the liberation of a group of daughter-cells from a mother-cell. On this view the peripheral chloroplasts are to be regarded, as already suggested, as the partially degenerate chloroplasts of this mother cell and the yellow-brown irregularly oval bodies as daughter cells, and not merely as chloroplasts. This interpretation of the phenomena finds support from facts connected with the development of yellow-brown cells in the body both of the larval and adult animal. Thus, in the former, besides the large cells described, just infected animals not infrequently contain minute yellow or yellow-green cells with only three peripherally placed chloroplasts, one oval and two somewhat elongated and pointed (Pl. 28, fig. 12). In the adult animal also, beside the large yellow-brown cells with numerous chloroplasts, there occur minute yellow cells, consisting now of a single, now of several chloroplasts.

Figures, Pl. 28, figs. 11, 12, 13, 18, represent these various stages in the development of the yellow-brown cells of larval and adult animals. The paleness of the colour—greenish-yellow, grey-yellow, yellow—both of the small cells of the adult, and of the irregular, oval structures contained in the large, developing cells of just-infected animals supports the view that the infecting organism has a colourless, saprophytic, free stage as well as a yellow-brown, holophytic, free

stage, and that infection may arise from the former stage. The more normal, yellow-brown appearance presented by other algal cells which occur in just-infected animals likewise suggest that the alga may also be ingested in its holophytic stage. Like variations in appearance are presented by the infecting organism of *C. roscoffensis*, in its earliest stage in the body of that animal; and in this case it has been demonstrated that these variations in the earliest recognisable condition of the infecting organism are due to the fact that the latter may be ingested at almost any stage of its life-history, viz. as a green resting-spore, as a colourless resting-spore, as a green or colourless, non-motile daughter-cell, or as a four-flagellated, green, typically chlamydomonadine cell: all of which stages have been observed in the life history of the infecting organism of *C. roscoffensis*. Once within the body of *C. paradoxa* the yellow-brown cell develops rapidly, the daughter-cells to which it gives rise are sown about the body, and undergo further growth and division. By this means, and also possibly by formation of spores, the yellow-brown cells of the body, now to be numbered by hundreds, impart, together with the orange glands, the characteristic colour to the animal.

To return to the question of the reappearance of normal yellow-brown cells in animals which, after long exposure to darkness in filtered sea-water, are brought into contact with weed of the *Paradoxa* zone (p. 454).

The appearance presented by the new crop of algal cells, the smallness of their numbers, their large size and their resemblance to those of just-infected, larval animals, their occurrence side by side with colourless heaps of undigested granular remnants of the destroyed earlier crop of yellow-brown cells, all suggest that reinfection has taken place. And the fact that, when reinfection takes place, growth of the animal is resumed indicates how intimate has become this relation between alga and animal.

(f) The Nature of the Infecting Alga.—Pending the discovery of the free stage in the life-history of the yellow-

brown alga, nothing of certain value can be said as to the systemic position of this organism. It is evidently distinct from the Zooxanthellæ of Radiolarians. For, whereas these yellow-brown cells possess two chloroplasts, the infecting alga of *C. paradoxa* possesses, in its fully-developed state, a large number (ten or more); and, whereas the reserves of Zooxanthellæ consist of starch or some undetermined substance, those of the infecting alga of *C. paradoxa* consist of fatty globules.

The pigments of the algal cells consist of a yellow-brown substance soluble in water and probably similar to Phycochrysin, a pigment described by Gaidukov as occurring together with a chlorophyll-like, green pigment in *Chromulina rosanoffii* a member of the Chrysomonadineæ.

The brilliant emerald-green reaction given by the yellow-brown cells when treated with strong sulphuric acid is stated by Zimmermann to be given also by Diatomin, the brown pigment of the Diatomaceæ. The fact that the pigments of the Chrysomonadineæ are held to be allied to those of the diatoms gives some slight indication that perhaps the yellow-brown alga of *C. paradoxa* may prove to be allied to the Chrysomonadineæ rather than to the Cryptomonadineæ, to which group the Zooxanthellæ of Radiolaria have been referred; but the evidence is too slender to admit of more than a conjecture.

(g) The Significance of the Relation between Animal and Yellow-brown Cell.—The facts set forth in the preceding pages have demonstrated the intimacy of the relation between *C. paradoxa* and its yellow-brown cells. So dependent is the animal on these cells that, apart from them, it is incapable of growth or development. Larvæ which escape infection, although they take up solid food, fail to develop, decrease steadily in size and die. In like manner the algal cell, once in the body of *C. paradoxa*, becomes an integral part of that body, and is no more capable of independent existence than is any somatic cell of the animal. The yellow-brown cell stands to the animal cells in the same rela-

tion as a chloroplast-containing cell of one of the higher plants stands to the colourless cells of that plant. The parallel is even closer than might seem from the foregoing for, as will be shown immediately, just as the chloroplast-containing cell of a plant receives unelaborated food-material from the colourless cells and, in return, provides them with organic food substances, so the yellow-brown cell receives from the cells of the animal the raw material from which it elaborates organic food substances, and in exchange passes on organic food substances to the animal tissues. As was pointed out in a previous paper (Keeble and Gamble, 1907), a precisely similar relation holds between green *Chlamydomonas* cell and *C. roscoffensis*. Viewed from the standpoints of the animals, *C. roscoffensis* and *C. paradoxa*, the relations between them and their respective algal cells admit of accurate definition. *C. roscoffensis* and *C. paradoxa* are obligate parasites. In the absence of their respective infecting organisms these species would become extinct.

But if the standpoint is shifted so that the relation is regarded from the point of view, so to speak, of the yellow-brown cell, the definition of this relationship will vary according as attention is directed to the individual yellow-brown cell or to the species of which that cell is a member. To the species, the relation is a meaningless episode; to the individual, ingested, yellow-brown cell, it is a great event. All that ingestion means to the species is that a certain, probably small, proportion of its members meet their fate in the body of *C. paradoxa*. It is only another hazard in the struggle for existence. The algal cells which are incorporated into the tissues of *C. paradoxa* never escape and so never impress the species with the consequences of that event; any modification which the algal cells may undergo as a result of their sojourn in the animal body can leave no mark on the species.

To the species *C. paradoxa*, infection or non-infection are matters of life or death; to the species "infecting organism,"

ingestion means only a somewhat increased death-rate. Hence, whilst to the infecting organism the effects of the relation are nil, these effects are permanently recorded in changed habit and modified development in the animal. Although to the species "infecting organism" it means so little, to the individual ingested algal cell incorporation in the tissue of the animal means much. The rapid growth and multiplication of the ingested algal cells indicate that the tissues of the animal offer a medium highly favourable to the development of those cells. So striking is this, that observers not infrequently infer from it that the alga is living parasitically in the animal body. Though this has been shown in the preceding pages not to be the case, the problem remains: to what peculiar conditions obtaining in the tissues of the animal is due this luxuriant vegetative development of the algal cell?

It was suggested, when this same problem was under consideration in the case of *C. roscoffensis*, that the luxuriant growth of the ingested algal cells was due to the favourable position which they occupy with respect to nitrogen. It is known that the amount of nitrogen present in sea-water in a form available to plants for synthetic purposes is extremely low; so low that it is highly probable that the amount of nitrogen is the factor which limits the development of marine plants and animals. Thus, according to Johnstone (1907), who bases his calculations on Raben's estimates, "the amount of nitrogen-compounds in Baltic and North Sea water may be taken as about .2 mgr. in a litre, or 2 parts in one million." Whence it follows that the infecting organism, living free in the sea, must be hard put to it, in common with its competitors, to obtain sufficient nitrogen. Once in the body of *C. paradoxa* the state of affairs, so far as nitrogen is concerned, is changed. Since this animal possesses no excretory system, its waste nitrogen, stored within the body, is at the disposal of the alga. As von Graff has suggested acutely (1891), the transverse bands of refractive substance, white by

reflected, black-brown by transmitted light, which occur in the body of *C. paradoxa* consist probably of urates (Pl. 26, figs. 4 and 5). The granular substance of which these bands are composed is always present, although the amount varies very considerably. Even in the just-hatched larva the bands are indicated as grey-black patches formed by groups of sparse granules. As the animals mature the amount of granular substance increases, though at the time of egg-laying it may disappear almost entirely. Now the hypothesis that the yellow-brown cells may utilise uric acid for their proteid-synthesis presents no difficulty. For instance, it has been established that holoplytic plants may utilise for this purpose organic nitrogen in many different forms, e.g. urea, uric acid, asparagin, leucin, tyrosin, guanin, kreatin, hippuric acid, etc., and, moreover, it has been shown that uric acid may replace nitrates in water-cultures of the higher green plants (Pfeffer, 1900).

The problem of the luxuriant development of the yellow-brown cells in the body of *C. paradoxa* may be thus stated in terms of the nitrogen-hypothesis:—*C. paradoxa* contains stores of waste organic nitrogen, presumably in the form of urates. Such substances are known to serve as sources of nitrogen to various plants. Is the yellow-brown cell able to utilise such substances?

In order to answer this question two modes of experimentations were adopted. The first method was as follows:—Batches of animals of similar sizes and origin were kept for some time in filtered sea-water with uric acid, and their condition compared with that of control animals. In one experiment of this kind of animals which had been kept in darkness with weed for nineteen days and which had undergone considerable reduction in size and in the number of their contained yellow-brown cells, some were put in filtered sea-water with uric acid, others were left with weed in the dark. After three days (September 9th—12th) the animals which had remained with weed in the dark were very colourless, and had lost most of their yellow-brown cells;

whilst those exposed to the light and supplied with uric acid were pale brown in colour, and were seen, on microscopic examination, to contain a very considerably larger number of intact yellow-brown cells.

Another experiment was started on August 28th with three similar lots of animals. These lots may be designated "filtered, light," "filtered, light, uric," and "filtered, dark." After twenty-one days (September 18th) the reductions in size of the animals and in the numbers of their yellow-brown cells were very great in the "filtered, dark," and least in the "filtered, light, uric." The measurements of animals taken as samples were—"filtered, dark" 55×20 divisions (oc. micrometer, L. 2. 3); "filtered, light, uric" 90×40 . That is the animals in the light with uric acid were more than three times as large as those in filtered water in the dark.

The experiment was continued. During the following weeks the "filtered, dark" and "filtered, light" animals dwindled, lost all their yellow-brown cells, became of microscopic size, and finally died. On October 21st specimens in "filtered, light, uric" were still alive, of recognisably brown colour, and possessed of many normal, yellow-brown cells. Finally, on October 28th a "filtered, light, uric" specimen was still alive, and, though reduced considerably in size, was rich in yellow-brown cells showing no sign of degeneration or of digestion.

When it is remembered that the animals were kept in limited supplies of sea-water only occasionally renewed, and that, during the time of the experiment, they were brought from Brittany to Reading, the effect of the uric acid in maintaining the yellow-brown cells and the animals alive for the space of two months will appear the more remarkable. From the experiment it is clear that the yellow-brown cells utilise uric acid as a source of nitrogen. It follows also that when the conditions admit of photosynthesis and when uric acid is supplied as a source of nitrogen the yellow-brown cells not only maintain their own existence, but also that of the animal.

That the algal cells may contribute not only fatty but also

proteid material to the animal, the results of the second mode of experimentation render highly probable.

Here, in lieu of determining the effect of uric acid on the life of algal cell and animal, its influence on egg-laying was investigated. The results of these experiments are summarised in Table II. In the first experiment of this series (started August 21st), five lots, each of twelve animals, were placed under the conditions indicated in columns 1—5 of the Table.

As the numbers in these columns show, but one clutch of eggs was laid in "filtered sea-water, light," one in "filtered sea-water, dark," none in "dark, weed" (column 5), four in "light, weed" (column 3), and six in "filtered, light, uric, and potassium nitrate" (column 2).

The next experiment (August 28th) shows that uric acid alone suffices to induce the production of eggs. For, whereas in "filtered sea-water, light" only eight clutches (column 1), and these containing but few eggs, were produced by fifty animals; seventeen clutches (column 2), with larger numbers of eggs, were laid by an equal number of animals kept in filtered sea-water in the light with uric acid.

The third experiment shows that nitrogen in the form of potassium nitrate is also efficacious in inducing egg-laying (Table II, 2b).

The total number of egg-clutches produced in these three experiments are:—in "filtered sea-water, light," 9; in "filtered sea-water, light, and added nitrogen" (uric acid, potassium nitrate, or both), 27.

These results would appear to prove that during the later stages of egg-development, when reserves are finding their way from animal to egg, a considerable portion of these reserves is obtained by the animal from the yellow-brown cells. The small number of egg-clutches laid in filtered water in the light indicates that the reserves of the animal tissues do not suffice to supply the eggs with material for their full development. It is noteworthy in this connection that, as before stated, the granular substance of the refrac-

tive bands undergoes marked reduction in amount, and frequently almost entirely disappears at the time of egg-laying. The stimulatory action of added nitrogen on egg-production may be, and probably is, due to the transmission from the algal cell to the animal, not only of the fatty assimilate, but also of products of proteid synthesis.

It is possible, however, that this increase in egg-production is an indirect rather than a direct consequence of added nitrogen. It is known from Boussingault's classical researches (1860) that nitrogen-starvation reduces enormously the photosynthetic activity of the green plant. So here it may be that the poor results in "filtered sea-water, light," and the good results of "filtered sea-water and added nitrogen, light" are due, the former to the curtailment of fat-synthesis by the algal cell in the nitrogen-starved animal; the latter to the active fat-synthesis by the algal cell well supplied with nitrogen. However this may be, the two sets of experiments just described serve to account for the luxuriant development of the yellow-brown cells within the body of *C. paradoxa*. In their free state these algæ, like all marine plants, run grave and frequent risks of nitrogen-starvation or, at all events, have their increase limited by the shortage of available nitrogen. Wherever there is any leakage of nitrogen in any form—and traces of combined nitrogen must be given off from all such animals as the Accelous Turbellaria—it is to be presumed that marine, motile plants will congregate. Congregating about *C. paradoxa* they will be ingested, and if, as happens in the case of the yellow-brown cell, they withstand digestion, they find rich stores of nitrogen—the waste nitrogen of the animal's metabolism—at their disposal. Thus they solve the problem of how to obtain sufficient nitrogen. To the individual infecting organism it is a solution; to the species it is none, for in effecting the solution to this nitrogen-problem the yellow-brown cell dooms itself to death without issue. It is to be noted that the association between algal cell and animal, though it has precisely the same significance in *C. roscoffensis* as in *C. paradoxa*, has gone a

step further in the former species. For, unlike *C. paradoxa* which continues all its life to feed voraciously, *C. roscoffensis* ceases very early to ingest solid food.

It is also noteworthy that the interpretation of the relation between animal and algal cell here offered throws light on certain facts concerning the distribution of algal cells in the bodies of various marine animals. The analyses of Natterer and Raben have demonstrated that the amount of combined nitrogen present in sea-water is less during the warm months, e. g. August, than during the cold months of the year, and that it is less in the warmer seas (Mediterranean) than in the colder seas (Baltic and North Sea) (Johnstone, 1907).

Now, as stated in the paper on *C. roscoffensis*, certain animals possess green or brown algal cells in one part of their range of distribution, but lack them in other stations. "Thus *Noctiluca* is colourless in the North Atlantic, and green in the Indian Ocean. British *Alcyonium* have no *Zoochlorellæ*, whereas the closely allied *A. ceylonicum* possesses them. It seems probable, indeed, that the maximum development of these associations occurs in the warmer seas." It would seem also probable that this parallelism between presence of algal cells and poverty of nitrogen is no coincidence, but that the former is causally connected with the latter.

The paleness of the colour of the infecting organism in its earliest stage of existence within the body of *C. paradoxa* suggests that this organism has in its free stage both holophytic and saprophytic phases. The similar behaviour of the infecting organism of *C. roscoffensis* has also been established. In its earliest stage within the body of the animal it may be altogether colourless. Such alternative phases, holophytic and saprophytic, pigmented or colourless, are known to occur in the life-histories of various of the lower organisms; for example, Diatoms (*Nitzschia*), certain Chlamydomonadineæ and Flagellates (*Euglena*). It is stated that the colourless phase may be induced by increasing the amount of soluble carbohydrate in the culture medium, or, in *Nitzschia*, according to Karsten, by augmenting the supply of organic

material (Czapek, 1905). But in the cases of the infecting cells of *C. paradoxa* and *C. roscoffensis* the development of assimilatory pigment appears to be associated with the increase in the amount of available nitrogen. And if this is the case it would seem probable that the colourless phase is brought about, not by excess, but by lack, of nitrogen. The suggestion is worth hazarding that the colourless saprophytic stages of such organisms as Diatoms, *Euglena*, etc., is a symptom of nitrogen-hunger. This hypothesis is at least as probable as that now prevalent. For, failing adequate supplies of nitrogen, no amount of carbohydrate-photosynthesis will avail; indeed, the more the carbohydrate-photosynthesis, involving, as it must do, the wearing out and reconstruction of the nitrogen-containing chlorophyll-machinery of pigment and plastid, the acuter will be the nitrogen-hunger; whilst, on the contrary, a shutting down of the photosynthetic process will economise nitrogen, and so postpone the evil day of nitrogen-starvation. Though the facts are not yet available for a confident statement, the hypothesis may be proposed that saprophytism generally may depend for its inception on nitrogen-hunger. It is tempting to push this provisional hypothesis to its limits, and to imagine that the great saprophytic group of the Fungi owes its origin to the changed mode of nutrition enforced upon it by lack of nitrogen. Lacking nitrogen, the photosynthetic activity of a green cell is greatly reduced or brought to a standstill; the chlorophyll machinery ceases to be worth its upkeep, and, wearing out, is too costly in nitrogen to be replaced. The organism will obtain directly from its environment as much carbon as is of use to it, together with as much nitrogen as it can get. It becomes a saprophyte.

Should this hypothesis be established, *C. paradoxa* and *C. roscoffensis* will rank high in interest among animals as suggesting the route along which far reaching evolution has travelled.

GENERAL CONCLUSIONS.

1. *C. paradoxa* occurs within a narrow belt of seaweed on the shore. It exhibits tidal migrations within this belt. The migratory movements are the resultant reactions to the various directive stimuli to which, in its changing environment, it is subject.

2. The egg laying and hatching are periodic. The periods synchronise with those of the neap-tides.

3. The eggs and just-hatched larvæ contain no yellow-brown cells. Preserved from infection, e.g., by hatching and maintaining in filtered sea-water, *C. paradoxa* remains free from yellow-brown cells (G. & K.).

4. By bringing uninfected larvæ in contact with seaweed from the *Paradoxa* zone infection is induced (G. & K.).

5. The infecting organism is an alga different from *Zooxanthella* of Radiolarians; its free stage is unknown. In the ingested state it is characterised by many chloroplasts, a colourless anterior end, and by the possession of fat globules in its colourless protoplasm.

6. Once introduced into the body of *C. paradoxa* the infecting organism multiplies rapidly.

7. The fat-globules of the algal cell are food-reserves. They arise as the result of the photosynthetic activity of the algal cells.

8. The reserve-fat of the algal cells is translocated from those cells to the animal tissues, and serves these tissues as food-material.

9. The ingested, yellow-brown, algal cell becomes, physiologically, an integral part of the animal, contributing towards its nutrition, and incapable of a separate existence.

10. The yellow-brown algal cells are indispensable to the animal. Uninfected animals fail to develop.

11. Nevertheless, starved animals digest their algal cells till no trace of these cells remains. Such disinfected animals

are capable of re-infection. With re-infection the growth of the animal is resumed.

12. The yellow-brown cells utilize in their constructive metabolism the waste products of the nitrogen-metabolism of the animal. The waste nitrogen of the animal is not excreted but is stored in the body, probably in the form of urates.

13. Animals deprived of solid food, but kept in the light in filtered sea-water to which uric acid is added, conserve their yellow-brown cells and maintain their lives longer than do animals not supplied with uric acid.

14. Similarly animals provided with uric acid lay many more eggs than are laid by animals kept under precisely similar conditions, but not supplied with uric acid.

15. The interpretation of the relation between yellow-brown cell and animal depends on the point of view :

From that of the animal, it is a case of obligate parasitism.

From that of the species "infecting organism," it is an insignificant episode, involving the loss of that, probably small, proportion of its members which are ingested.

From that of the individual ingested yellow-brown cell it is a solution of the nitrogen problem—a successful method of obtaining large supplies of nitrogen.

TABLE I.—Infection Record. Trégastel, 1907. Catch 12th; eggs laid 14th; hatched 16th (with weed of *Paradoxa* zone).

Date.	No. of infected animals.	No. of algal cells.	No. of uninfected animals.
17th	—	—	Several
18th	3	1 small algal cell. 1 large algal cell. 5 algal cells, 2 large, 3 small.	2
19th	—	—	2
21st	1	24 algal cells.	
22nd	2	Numerous.	2
30th	—	—	1
31st	—	—	1
Totals	6	—	8

TABLE II.—Egg-laying under various Nutritive Conditions. *Convoluta paradoxa*. Trégastel, 1907.

		LIGHT.			DARK.	
		1. Filtered sea-water.	2. Filtered sea-water + uric acid + KNO_3 .	3. Unfiltered sea- water + seaweed from Paradoxa zone.	4. Filtered sea-water.	5. Unfiltered sea- water + seaweed from Paradoxa zone.
August 21.—Of animals caught same day, 12 placed under each of the conditions: 1—5.						
Examined	Aug. 25	1 clutch of eggs	5 clutches	0	1	0
"	" 27	0 "	1 "	4	0	0
August 28.—Of animals caught same day, 50 placed under each of the conditions: 1 and 2 <i>a</i> .						
Examined	Aug. 31	0 "	7 "	Filtered sea-water + uric acid only = 2 <i>a</i>		
"	Sept. 4	4 "	8 "			
"	" 7	4 (few eggs in these clutches)	2 "			
August 29.—12 animals placed under each of the conditions: 1, 2 <i>b</i> .						
Examined	Sept. 2	0 clutch of eggs	4 "	Filtered sea-water + KNO_3 2 = 2 <i>b</i>		
Total egg-clutches laid under conditions 1 and 2 .		9	27			

LITERATURE REFERRED TO IN THE TEXT.

1. 1860. BOUSSINGAULT.—‘Agronomie,’ vol. i; see also Jost’s ‘Physiology’ (Eng. trans.), p. 133, 1907.
2. 1871. CIENKOWSKI.—“Ueber Schwarmerbildung bei Radiolarien,” ‘Schultzes Archiv f. Mikrosk. Anat.,’ vol. 7, 1871. (Cites Haeckel’s blue iodine reaction).
3. 1885. BRANDT.—“Fauna und Flora des Golfes Neapel,” ‘Radiolarien,’ 13, 1885. (Cites Müller’s brown iodide reaction.)
4. 1891. VON GRAFF.—‘Die Organisation der Turbellaria acoela,’ Leipzig, 1891.
5. 1892. KLEBS.—“Flagellaten studien,” ‘Jahrb. f. wiss. Zool.,’ vol. 55, 1892.
6. 1893. CRATO.—“Morph. u. mikrochem. Untersuch. über die Physoden,” ‘Bot. Ztg.,’ 1893; and “Über die Hansteen’schen Fucosankörner,” ‘Ber. d. D. bot. Ges.,’ 1893, 9.
7. 1896. KOCH.—“Untersuch. über die bisher für Öl oder Phloroglucin gehaltenen Inhaltskörper der Fucaceen,” ‘Diss. Rostoch,’ 1896. Cit. Oltmanns, ‘Morph. u. Biologie der Algen,’ vol. 2, p. 151, 1905.
8. 1897. MEYER.—“Untersuch. u. einige Flagellaten,” ‘Rev. Suisse de Zoologie,’ vol. 5, 1897.
9. 1900. HANSTEEN.—“Über das Fucosan als ersteres scheinbares produkt der Kohlensäure assim. bei den Fucaceen,” ‘Pringsh. Jahrb.,’ 1900, vol. 35, 611.
10. 1900. PFEFFER.—‘Physiology of Plants’ (Eng. trans.), vol. i, p. 405, where the literature is cited. 1900.
11. 1902. HUNGER.—“Über das assimilations produkt der Dictyotaceen,” ‘Pringsh. Jahrb.,’ 1902, vol. 38.
12. 1903. GAMBLE and KEEBLE.—“The Bionomics of *Convoluta roscoffensis*,” ‘Q. J. M. S.,’ vol. 57, 1903.
1904. KEEBLE and GAMBLE.—“The Colour Physiology of Higher Crustacea,” ‘Phil. Trans. Roy. Soc. London,’ Series B, vol. 196, 1904.
13. 1905. CZAPEK.—‘Biochemie der Pflanzen,’ vol. i, p. 481, Fischer, Jena, 1905.
14. 1905. OLTMANNs.—‘Morph. u. Biologie der Algen,’ Jena, 1905, vol. ii, p. 147.
15. 1907. JOHNSTONE.—“The Law of the Minimum in the Sea,” ‘Science Progress,’ vol. ii, No. 6, Oct., 1907.
16. 1907. KEEBLE and GAMBLE.—“The Origin and Nature of the Green Cells of *Convoluta roscoffensis*,” ‘Q. J. M. S.,’ vol. 51, Part 2, May, 1907.

EXPLANATION OF PLATES 26—28,

Illustrating Mr. Frederick Keeble's paper on "The Yellow-Brown Cells of *Convoluta paradoxa*."

PLATE 26.

FIG. 1.—Weed from *Paradoxa* zone with *C. paradoxa* ($\times 3$). Egg capsules laid on weed seen as orange dots. The large weed *Pycnophycus*; the small weeds *Ceramium* and *Rhodomela*.

FIG. 2.—Egg capsule ($\times 40$) with orange-pigmented eggs attached to weed of *Paradoxa* zone.

FIG. 3.—Egg ($\times 300$) showing the pigment bodies irregular and dumb-bell shaped; but no yellow-brown cell.

FIG. 4.—Young *C. paradoxa* ($\times 70$) two days after hatching. Body gorged with diatoms, etc., and containing one yellow-brown cell. The chief excretory band, already indicated, running transversely, a little distance behind the mouth.

FIG. 5.—*C. paradoxa* ($\times 50$) showing the yellow-brown cells and the concrement granules (black by transmitted light).

FIG. 6.—The yellow-brown cells ($\times 70$) as seen through the epidermal tissues in the living state. The orange glandular structures are also visible.

PLATE 27.

FIG. 7.—The yellow-brown cells (L. 2, $\frac{1}{10}$) as seen when the animal is examined soon after capture.

FIG. 8.—The cells as seen when the animal is examined immediately after capture. The clear anterior end of the yellow-brown cells is well marked, whereas in Fig. 7 it is not generally visible; and the cells, as well as the animal tissues, contain numbers of fat globules which are absent or very scarce in Fig. 7.

FIG. 9.—Yellow-brown cells showing fat undergoing excretion from their clear anterior ends.

PLATE 28.

FIG. 10.—*C. paradoxa* ($\times 20$). (*a*) Mature female with eggs; (*b*) mature male; (*c*) immature animal seen from dorsal, and (*d*) from ventral surface. The bands and masses of excretory substance (concrement granules) appear

white by reflected light. In the mature female the amount of excretory substance is considerably less than in the male and immature stages.

FIGS. 11, 12, 13.—Yellow-brown cells ($\times 330$) as seen in just infected animals. In Fig. 11 the infecting cell has many chloroplasts differentiated into elongated, "boat-shaped," and polygonal. In Fig. 13 only three chloroplasts occur, but the differentiation into the two forms is already indicated.

FIG. 14.—Young *C. paradoxa* ($\times 45$) 1—2 days after hatching; containing one large infecting cell and numerous colourless vacuoles.

FIG. 15.—*a*. The infecting cell of Fig. 14 enlarged (L. 2, $\frac{1}{10}$), showing peripheral, elongated, brown chloroplasts, and more central group of yellow, polygonal bodies, more suggestive of spores than of chloroplasts. *b*. The same cell undergoing changes of shape, due to the movements of the animal. *c*. The contents of (*b*) liberated in the body of the animal.

FIG. 16.—Egg capsules of *C. paradoxa* showing yellow-brown cells, one with pointed clear anterior end attached to the egg membrane.

FIG. 17.—Yellow-brown cells identical with those of the infecting alga occurring in the remnants of the egg capsule of *C. paradoxa*.

FIG. 18.—*a*, *b*, *c*, *d*. Yellow-brown cells showing nucleus. *e*. Minute yellow-brown cells showing stages of development from *e*, with single chloroplast to *e*₂ with several and *e*₃ with numerous chloroplasts (L. 8, $\frac{1}{10}$).



Keeble, Frederick. 1908. "Memoirs: The Yellow-Brown Cells of *Convoluta paradoxa*." *Quarterly journal of microscopical science* 52, 431–479.

View This Item Online: <https://www.biodiversitylibrary.org/item/52504>

Permalink: <https://www.biodiversitylibrary.org/partpdf/201073>

Holding Institution

Harvard University, Museum of Comparative Zoology, Ernst Mayr Library

Sponsored by

Harvard University, Museum of Comparative Zoology, Ernst Mayr Library

Copyright & Reuse

Copyright Status: Public domain. The BHL considers that this work is no longer under copyright protection.

This document was created from content at the **Biodiversity Heritage Library**, the world's largest open access digital library for biodiversity literature and archives. Visit BHL at <https://www.biodiversitylibrary.org>.