STUDIES ON THE PHYSIOLOGY OF CORALS

IV. THE STRUCTURE, DISTRIBUTION AND PHYSIOLOGY OF THE ZOOXANTHELLAE

BY

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WITH NINETEEN TEXT-FIGURES AND TWO PLATES

CONTENTS

1. INTRODUCTION.

This paper is concerned with the brown unicellular algae or zooxanthellae which are invariably present in vast numbers within the tissues of all true reef-building corals and also in many other reef organisms. Only the conditions within the Madreporaria are here described in detail. The two papers which follow will be concerned with accounts, respectively, of experiments set up to investigate the possibility that zooxanthellae are a source of food to the corals, and to study the gaseous exchange between corals and zooxanthellae.

Final conclusions as to the true significance of the relationship between the zooxanthellae and the corals will not be reached until all this work on the physiology of corals comes to be reviewed in the concluding, seventh paper of the series.

This and the two following papers represent the joint work in the field and in the laboratory at Low Isles of the authors stated. In all cases the papers have been written and illustrations prepared by the senior author, who is responsible, with the concurrence of his collaborators, for the conclusions arrived at.

2. LITERATURE.

Whilst the majority of those who have studied the histology of reef-building corals (for complete list see Buchner (1921)) have commented on the invariable presence within the tissues of zooxanthellae, few detailed descriptions of these have been given. Almost no work has been done on their physiology. Some work has been carried out on the physiology of zooxanthellae and of green zoochlorellae in other Coelenterata, and in Protozoa, Porifera and Turbellaria. An account of this work and its bearings on the results given in this and the two following papers will be provided in the final paper of this series.

Zooxanthellae, all workers are agreed, occur only in the endoderm of corals. Duerden (1902), as a result of his exhaustive examination of West Indian corals, states than zooxanthellae " are usually distributed throughout the polyp, but are more numerous in the exposed tissues (column wall, disk, tentacles) than in the endoderm of the mesenteries and skeletrophic tissues ; they even occur within the internal canals of the perforate genera *Madrepora* and *Porites*, but are never found free or detached within the polypal cavities except in larvae." Matthai (1914) notes their restriction to the endoderm, and their greatest abundance in all the exposed regions of the soft parts. He gives a good short account of the zooxanthellae, and provides better figures than earlier workers. He describes the zooxanthellae as being round, " the protoplasm staining pink (with eosin), the nucleus excentrically placed and granular in appearance. In addition there is, in most algae, a homogeneously dark-stained body—in all probability a pyrenoid—with a transparent ring round it."

Boschma (1924) gives probably the best account of the zooxanthellae. He found them present in all species of the thirty-eight genera of Indo-Pacific reef-building corals which he examined. Only in *Dendrophyllia* (which, as pointed out in the first paper of this series, is to be regarded as a deep-water coral which has extended its vertical distribution) were they absent. He describes the zooxanthellae as being yellow in colour and spherical in form, 7 to 10μ in diameter. He was unable to distinguish chromatophores, but states that the living cell always contains one, and occasionally two, refractile granules which stain a brownish violet with iodine. He interprets this reaction as showing the presence of an amyloid assimilation product somewhat different from that of higher plants which colours a deep blue after similar treatment. Boschma also describes a more excentrically placed granular nucleus which is a little smaller than the assimilation product, and can only be distinguished in living material after the addition of acetic acid. After fixation he obtained best results by staining with Heidenhain's iron-hæmatoxylin or with safranin and light green. Only the central portion of the assimilation product—the pyrenoid—takes the stain, being surrounded, as Matthai also noted, by a clear area. The protoplasm of the zooxanthellae, Boschma states, is frequently vacuolated.

Other work by Boschma and Vaughan dealing with the nature of the association between corals and zooxanthellae will be discussed in Paper V, and work by Vaughan, Gardiner and Verwey on the gaseous exchange between them in Paper VI of this series.

3. MATERIAL AND METHODS.

A great variety of Madreporaria as well as certain Alcyonaria and other coelenterates were examined in the course of this research. These animals were collected on Low Isles Reef or on Batt Reef, or were dredged in various localities between the Great Barrier and the mainland. The greater part of the work here recorded was carried out at the Laboratory on Low Isles. Material was fixed in Bouin, Flemming or Carnoy, and subsequently sectioned and stained at the Plymouth Laboratory. Sections were invariably cut 6μ thick. The combination of safranin in 70% alcohol and light green in 90% alcohol was found the most suitable stain for general purposes, though Heidenhain's iron-haematoxylin and Delafield's haematoxylin with erythrosin were also employed, and Mayer's muci-haematin for the detection of mucus. Thanks are due to Prof. E. J. Goddard, of the University of Queensland, for providing details of methods and the necessary reagents for the identification of cellulose. Material was macerated by Hertwig's method, which consists in placing the tissue in a mixture of 0·04% osmic acid and 0·2% acetic acid in sea-water for a few minutes, and then washing out repeatedly in a solution of 0·2% acetic acid in sea-water. Hydrogen ion concentration was determined colorimetrically by means of Clark and Lubs indicators. Mrs. Yonge carried out oxygen determinations, using the Winkler method, and also tested for phosphorus by the colorimetric method of Denigès, as developed by Atkins. She received previous instruction in both these methods from Mr. A. P. Orr. Mrs. Yonge has also given further important assistance by cutting sections. Acknowledgments are also due to Miss S. M. Marshall for carrying out experiments on the culture of the zooxanthellae, and to Mr. G. W. Otter for the photograph reproduced in Plate II, fig. 6.

4. OCCURRENCE OF ZOOXANTHELLAE IN REEF ORGANISMS.

Zooxanthellae were found in all species of all genera of true reef-building Madreporaria examined. Species of the following genera were examined : *Acrhelia*, *Seriatopora*, *Pocillopora*, *Stylophora*, *Euphyllia*, *Leptastrea*, *Cyphastrea*, *Echinopora*, *Galaxea*, *Favia*, *Favites*, *Goniastrea*, *Coeloria*, *Platygyra*, *Merulina*, *Hydnophora*, *Tridacophyllia*, *Caulastrea*, *Acanthastrea*, *Symphyllia*, *Lobophyllia*, *Trachyphyllia*, *Fungia*, *Herpetolitha*, *Döderleinia*, *Psammocora*, *Pavona*, *Coeloseris*, *Pachyseris*, *Astreopora*, *Turbinaria*, *Montipora*, *Acropora*, *Goniopora* and *Porites*. Zooxanthellae were invariably contained within the planulae of *Pocillopora* and *Porites*, and in others of unknown origin obtained in tow-nettings.

The only coral taken at or near the surface of reefs which never contained zooxanthellae was *Dendrophyllia*. Both *Dendrophyllia nigrescens*, which was not uncommon on the under-side of boulders around Low Isles, and *D. manni*, which is very abundant on the surface of the fringing reef at Kaneohe Bay in the Island of Oahu, in the Hawaiian Islands, contained no zooxanthellae. Planulae of the latter species were examined and also

found devoid of zooxanthellae. But, as emphasized in Paper I of this series, *Dendrophyllia* is not to be regarded as a true reef builder, but as a deep-water coral which has extended its vertical distribution.

Zooxanthellae similar in all respects to those of the Madreporaria were invariably found in great numbers in the Alcyonarian corals, *Tubipora* and *Helipora*, as well as in all other Alcyonacea, such as *Sarcophyton*, *Lobophytum*, *Sinularia*, *Xenia* and *Clavularia*. The gorgonids, *Isis* and *Melitodes*, also contained zooxanthellae, but in smaller numbers, and they were extremely abundant in the zooanthid, *Palythoa*, which was one of the commonest animals on the surface of Low Isles Reef. All actiniarians examined, notably the large *Stoichactis* and *Actinodendron*, contained great numbers of zooxanthellae. So far as they were examined, there appeared to be no difference in the type of zooxanthellae found in any of these Actinozoa.

The hydrozoan coral, *Millepora*, invariably contains great numbers of zooxanthellae, which are apparently distinct from those of the Actinozoa. The hydroid *Myrionema*, which was very abundant about the roots of the mangrove trees, contained so many zooxanthellae that it was invariably coloured brown by them. These, again, are unlike those of the Actinozoa, being a little smaller, less regular in outline, and differing in details of their internal structure. A full account of these is given by Dr. E. A. Fraser in Volume III of these reports.

The ubiquity of zooxanthellae in reef organisms is further emphasized by their presence in members of the Protozoa, Mollusca and Tunicata, as well as in the Coelenterata. They were found in the foraminiferan, *Polytrema*, they were always present in great numbers within the thickened mantle edges of the clams, *Tridacna* and *Hippopus* (a full account of which will be given in a later paper in this volume), while Dr. A. B. Hastings, when examining the collections of colonial tunicates, discovered the presence of zooxanthellae in members of the genera *Trididemnum*, *Didemnum* and *Diplosoma*. Her report appears in Volume IV.

The significance of the widespread abundance of zooxanthellae in reef organisms and the reasons why they are confined to certain groups of animals will be discussed in the final paper of this series, after the various reports have been published.

5. STRUCTURE AND LIFE-HISTORY OF ZOOXANTHELLAE FROM CORALS.

No differences in average size or in any detail of structure were observed between the zooxanthellae of different genera of Madreporaria. It is assumed, therefore, that the same species is present in them all (and also in other Actinozoa). Some account of the different zooxanthellae from *Millepora* will be given later.

The zooxanthellae of the Madreporaria (Plate I, figs. 1–3) are spherical, and vary in diameter from 6 to 14μ, the majority being between 7 and 10μ. In life they are a yellowish brown, and little internal structure can be distinguished.

The sharp outline of healthy zooxanthellae indicates the presence of a firm wall. The nature of this was first of all investigated. Scrapings were made of the coenosarc of *Galaxea fascicularis*, which contains vast numbers of zooxanthellae, and this material was fixed in 70% alcohol. The yellow-brown colouring matter was quickly dissolved

out, leaving the zooxanthellae colourless. The following tests for the presence of cellulose were then applied :

1. A dilute solution of iodine stained the cell-walls yellow.

2. After staining with strong iodine, the addition of 25% sulphuric acid caused the cell-walls to swell greatly and turn distinctly blue.

3. The addition of chlorzinc iodine caused a swelling of the cell-walls and the appearance of a greenish-blue colour.

4. Freshly prepared cuprammonia (Schweitzer's reagent) caused the cell-walls to disappear owing to the dissolution of the cellulose.

5. After the addition of calcium chloride iodine solution, the walls of the zooxanthellae turned a dull reddish pink.

All these five tests, therefore, gave positive results, and demonstrate without any doubt the important fact that the zooxanthellae are surrounded by a relatively stout wall of cellulose.

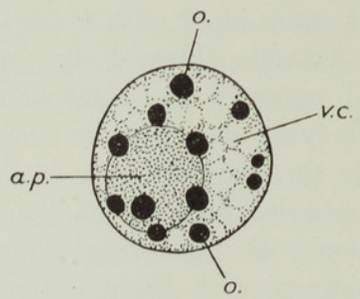
The nucleus (*n.*) is relatively large, and contains granular masses of chromatin with no indication of a nucleolus. It stains black with Heidenhain's iron haematoxylin (Plate I, figs. 1 and 2) and red with safranin (Plate I, fig. 3). It usually lies close to the assimilation product (*a.p.*) and pyrenoid (*p.*), and under high powers is revealed as concavo-convex, the concave side lying alongside the assimilation product and pyrenoid.

The assimilation product is the only structure visible in the living zooxanthellae. It consists of a large, refractile, spherical body with a diameter frequently almost half that of the entire cell. After fixation with Flemming, it stains a dull red with safranin (Plate I, fig. 3, *a.p.*). After treatment with dilute acetic acid followed by iodine it stains a dark brown, but, after careful focusing under the oil-immersion objective, an underlying reddish-violet colour can also be distinguished. This agrees with Boschma's statement already quoted, and indicates, as he suggests, the presence of some amyloid substance, though not of true starch. After fixation with Bouin (sometimes also after fixation with Flemming) and staining with safranin, a central pyrenoid of about half the diameter of the assimilation product is all that can be distinguished (Plate I, figs. 1 and 2, *p.*). It lies within a clear area, which consists either of material which fails to stain, or has been dissolved out by the fixing and preserving fluids, the latter alternative being the more probable. In the majority of cases only one pyrenoid and assimilation product are present in each zooxanthella, but in a number of instances two are present, as shown in Plate I, fig. 2, the nucleus lying between them. It is possible that this stage may precede division.

The cytoplasm, which stains green with light green or red with erythrosin, is very vacuolated (Plate I, figs. 1–3, *v.*). Material fixed with Flemming shows the presence also of droplets of osmicated oil (Plate I, fig. 3, *o.*) in the cytoplasm. Scrapings of the edge-zone tissue of *Lobophyllia* were made and fixed in 5% formalin, and later stained for several hours in a solution of Sudan III in a mixture of 9 parts of glacial acetic acid and 1 part of alcohol, after which they were washed in water and mounted in glycerine. On examination under the oil-immersion it was found that in a number of cases the stain had penetrated into zooxanthellae, and numerous relatively large, red droplets of oil could be clearly distinguished. The appearance of such a stained zooxanthella is shown in Text-fig. 1, where the great abundance of oil droplets (*o.*) is clearly indicated. Although the first product of photosynthetic activity would appear to be the carbohydrate

which accumulates around the pyrenoid and forms the assimilation product, the greater part of the reserve food within the zooxanthellae apparently takes the form of droplets of oil or fat.

The life-history of the zooxanthellae appears to be very simple. Under favourable conditions they increase rapidly within the tissues by a process of simple division. This was frequently seen in both fresh and macerated material (see Text-figs. 2 and 3) and in sections, but unfortunately the exact process of division has not been determined owing probably to the great speed with which this takes place. Certain sections gave evidence that the nucleus divides mitotically, the pyrenoid having already divided. Immediately after division the two daughter-cells are contained within the same tissue-cell, at first flattened at the opposing sides, but later rounding off (see Text-figs. 3 and 2 respectively), but whether they continue to remain there until that cell divides, one passing to each daughter-cell, or whether one of them is transferred to another tissue-cell, it is impossible to say.



Text-fig. 1.—Zooxanthella from endoderm of edge-zone of *Lobophyllia corymbosa*, fixed 5% formol and stained with Sudan III. × 2400. *a.p.*, assimilation product; *o.*, oil droplets; *v.c.*, vacuolated cytoplasm.

No evidence of the presence of spores was ever obtained. Miss S. M. Marshall failed to find free zooxanthellae in any of the very numerous water samples from the anchorage at Low Isles or from the regular boat stations. They are transferred directly from the parent to the offspring by way of the planulae, which invariably contain very great, though varying, numbers of them. Miss Marshall, who gives full details in a later paper in this volume, counted the numbers of zooxanthellae in planulae of *Porites*, which varied in length from 0·5 to 1 mm., and found that they varied from 1150 to 7400, while she estimates a population of zooxanthellae of not less than 25,000 in the larger planulae of *Pocillopora*.

At what stage in development the zooxanthellae pass from the parent to the young is unknown. Dr. T. A. Stephenson, who examined the gonads of species of *Favia* and *Lobophyllia* throughout the year, failed to find zooxanthellae in the ova. Although he was never successful in finding perfectly ripe eggs, it would seem probable that infection with zooxanthellae takes place after fertilization.

Miss Marshall also attempted to rear the zooxanthellae in Miquel's solution, in diluted Miquel, and in boiled coral tissue diluted and brought to a pH approximating to that found normally in the living tissues, but invariably without success.

All the evidence thus goes to show that the zooxanthellae of corals—unlike the *Chlamydomonas* present in *Convoluta roscoffensis* which is found free in the sea and, forms

spores (Keeble and Gamble (1907))—can live only within the tissues of the coral, and are transmitted direct from parent to offspring by way of the planulae.

The zooxanthellae in the hydrozoan coral, *Millepora*, are apparently different from those of the Madreporaria. This was not realized at Low Isles, owing to the fact that externally there is little difference between the different zooxanthellae, and they have about the same average size. As a result, the material fixed did not, unfortunately, include any fresh *Millepora*. Specimens which had been starved and fed in the starvation experiment to be described in Paper V were fixed in Flemming and Bouin, but in neither case with the success that attended the fixation of Madreporaria. The appearance of the zooxanthellae in the sections prepared from this material is certainly unlike that of those in Madreporarian corals. *Millepora* from various regions in the Pacific and the Atlantic was kindly supplied by Prof. J. Stanley Gardiner and by the British Museum, but all had been preserved in alcohol, and sections prepared from this material failed to show the structure of the zooxanthellae in the necessary detail.

These zooxanthellae are certainly more variable in size and shape than those from the Madreporaria, and there is a central nucleus, but usually no well-developed pyrenoid. According to Moseley (1881) and Mangan (1909) the zooxanthellae from *Millepora* may divide into four, and the appearance in certain of the experimental material does seem to confirm this. This never occurs in the zooxanthellae from the Madreporaria. Finally the cellulose wall, so well defined in the other zooxanthellae, is either absent or very thin in those from *Millepora*. Moseley and Mangan both state that it is absent, but we find it difficult to be absolutely confident about this.

No figures of these zooxanthellae are provided owing to the poor quality of the fixed material, and they are here discussed principally with a view to emphasizing the necessity for more detailed work on them, and the nature of their relation to *Millepora* and the other hydrozoan corals.

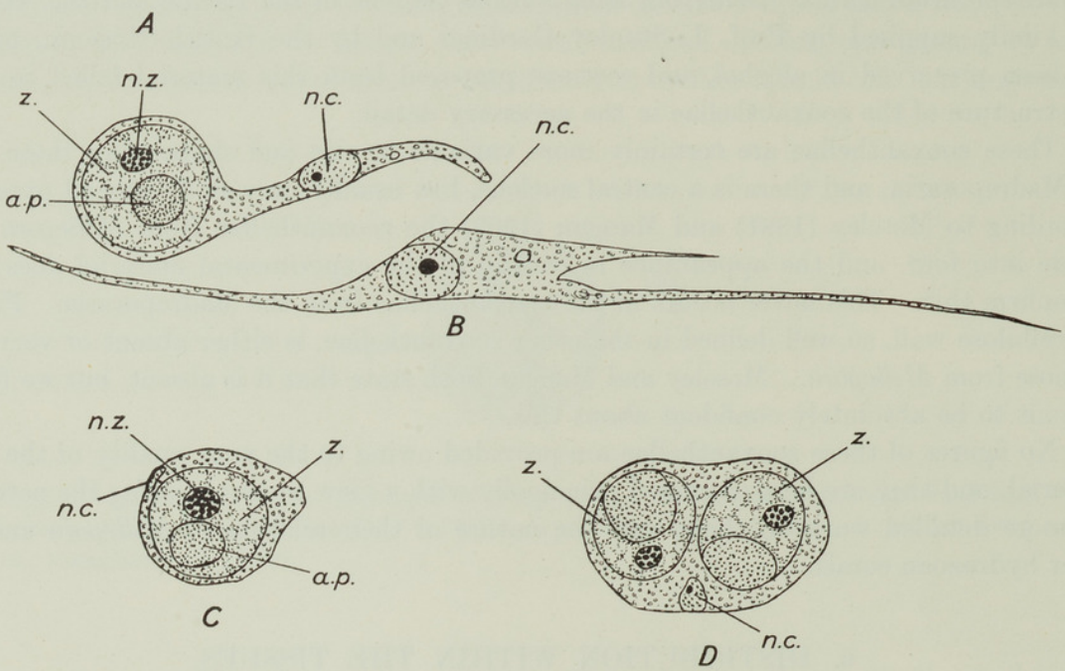
6. DISTRIBUTION WITHIN THE TISSUES.

Zooxanthellae occur only in the endoderm, and are never under any circumstance present in the ectoderm or in the mesogloea. They are most numerous in those regions, such as the disc, oral cone, tentacles and coenosarc, which are most exposed to light, though they may be present in all regions of the endoderm, even within the internal canals of the perforate corals.

The conditions typically found within the tentacles of *Pocillopora bulbosa* are shown in Plate I, fig. 4. No zooxanthellae are present in the ectoderm (*ec.*), which is ciliated, and contains mucus-glands (*m.g.*) and nematocysts (*nem.*). The endoderm (*en.*) is packed with zooxanthellae (*z.*), which occupy a great part of this area. Although in some instances there is evidence that they are contained within cells, the nuclei of which appear smaller and more darkly staining than those of the epithelial cells, the exact relationship between the zooxanthellae and the tissue-cells is impossible to determine satisfactorily in sections. The majority of the zooxanthellae in the portion figured are healthy, and they divide freely in this region. Degenerating zooxanthellae occur, but very infrequently, in this region, and Plate I, fig. 4, shows one of these (*z.d.*). All internal structure has been lost, the interior of the cell being occupied by an irregular mass which is blackened by the osmic acid. There are numerous fat-globules (*f.*) in the endoderm, but there is absolutely no

evidence that these come from the very rare degenerating zooxanthellae. Essentially similar conditions are revealed in sections of the disc, oral cone and coenosarc, deeper tissues varying only in the smaller numbers of zooxanthellae present.

The greatest obstacle to the completion of this research has been the interpretation of the histology of corals. In Paper III attention was drawn to the conclusions of Matthai (1923) that all tissues of Astraeid corals are syncytial. Sections of many species of corals lend support to this view, at any rate so far as the endoderm is concerned. Yet, when tissues of the " absorptive " zone of the mesenterial filaments were macerated, discrete cells (figured in Paper III) were obtained which contained carmine and zooxanthellae

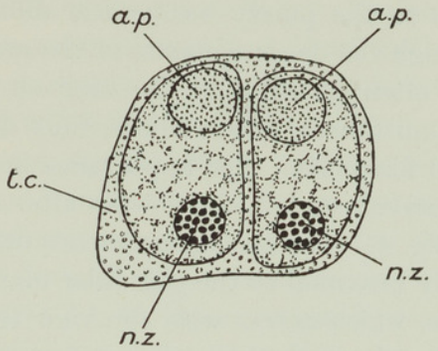


Text-fig. 2.—*Euphyllia glabrescens*, cells from endoderm of edge-zone obtained by maceration by Hertwig's method. × 1650. A, C, D, cells containing zooxanthellae ; B, cell without zooxanthellae ; *a.p.*, assimilation product ; *n.c.*, nucleus of tissue cell ; *n.z.*, nucleus of zooxanthella ; *z.*, zooxanthellae.

prior to their excretion into the coelenteron. This would appear to indicate the presence, at least, of discrete wandering cells, which certainly occur in the mesogloea.

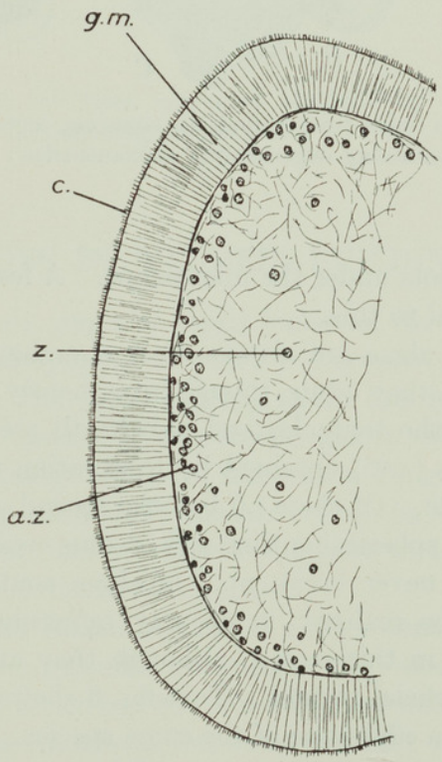
Material was macerated in the hope of determining the exact relationship between the zooxanthellae and the cells of the corals. Text-fig. 2 shows four cells (A–D) from the endoderm of the edge-zone of *Euphyllia glabrescens* after maceration. Of these cells, A, C and D contain zooxanthellae (*z.*), two of which—presumably the products of recent division—are present in D, and one each in A and C. The nucleus of the tissue-cell (*n.c.*) can be seen in all four cells. Text-fig. 3 shows a tissue-cell obtained by maceration from the endoderm of the edge-zone of *Symphyllia recta*, and this contains two zooxanthellae which have not yet rounded off after division. Endodermal tissues from many species and genera of Madreporarian corals were macerated, and in no case were zooxanthellae found not contained within tissue-cells. There was no evidence to show that the remaining

endoderm might *not* be syncytial, although, unfortunately, this was not definitely determined, because its importance was not realized until much later after sections had been cut.



TEXT-FIG. 3.—*Symphyllia recta*, containing two zooxanthellae, cell from edge-zone obtained by maceration. × 2480. *t.c.*, tissue-cell; other lettering as before.

Maceration, therefore, has confirmed the impression gained from the examination of sections, such as the one shown in Plate I, fig. 4 (but more clearly displayed in Text-figs. 10, 12, 17 and 18), that zooxanthellae are *invariably intracellular*. The next problem



TEXT FIG. 4.—*Lobophyllia corymbosa*, side view of mesenterial filament pressed out for examination under a coverslip. × 180. *a.z.*, "absorptive" zone; *c.*, cilia; *g.m.*, glandular margin; *z.*, zooxanthella.

which demands solution is whether they are *always* contained in wandering cells. These may occur anywhere in the tissues, one (*w.c.*) being shown in Plate I, fig. 4. In the course of this paper, evidence will gradually be accumulated pointing more and more definitely

to the conclusion that *zooxanthellae may always be contained in wandering cells*, and that they are never present in the general mass of the endoderm. Further work on living material can alone decide this question. The fact that they never occur in the ectoderm and mesogloea may be due to the purely mechanical difficulties of transporting such relatively large objects through the dense material of the mesogloea.

The zooxanthellae are often plentiful in the base of the mesenteries, but, except under certain abnormal circumstances, which will be fully discussed later, they are never abundant in the mesenterial filaments. The appearance in life of a filament from *Lobophyllia corymbosa*, when observed under low powers after being stretched out under a coverslip, is shown in Text-fig. 4. The zooxanthellae are most numerous in the " absorptive " zone (*a.z.*) immediately proximal to the glandular margin (*g.m.*). The latter region *never* contains zooxanthellae, which agrees with the view that it is of ectodermal origin. The " absorptive " zone is usually marked by a band of zooxanthellae of varying intensity of colour according to the number of algae present. Possible reasons for this variation will be discussed later. Some of these zooxanthellae are reduced in size and dark brown in

TEXT-FIG. 5.—*Symphyllia recta*, cells from endoderm covering septa obtained by maceration. × 1240. A, C, D, cells containing degenerating zooxanthellae ; B, cell containing healthy zooxanthella.

colour, appearing as dark spots under the microscope. A few zooxanthellae are scattered about in the region proximal to this.

Although degenerating algae are always most abundant in the " absorptive " zone of the mesenterial filaments, they occur, though infrequently, as already shown in Plate I, fig. 4, in the endoderm of the tentacles and also in all other regions of the endoderm. Text-fig. 5 shows four cells (A–D) obtained by maceration from the endoderm covering the septa in *Symphyllia recta*. Of these all but the second contain degenerating zooxanthellae which have lost their spherical outline and become condensed, dark coloured masses. It is significant that they never break up. B alone contains a normal zooxanthella. This text-figure, it must be realized, in no way represents the normal abundance of degenerating zooxanthellae in this region, although they are somewhat more numerous here than in the more superficial regions. Text-fig. 6 shows a degenerating zooxanthella in macerated tissue from the edge-zone of the same species.

It will be noted that the occasional presence of degenerating zooxanthellae throughout the endoderm and their much greater abundance in the " absorptive " zone of the mesenterial filaments, both point to the conclusion that, like the carmine and iron saccharate injected into the edge-zone of *Lobophyllia corymbosa* described in Paper III of this series, they are *excreted* into the coelenteron *viâ* the " absorptive " zone of the mesenterial filaments. Further, and much more positive, evidence in support of this view will be presented later.

Conditions in the planulae and early post-larval stages are essentially the same as in adult corals. Freely-swimming and recently settled planulae of *Pocillopora bulbosa* were fixed in Flemming and subsequently sectioned. Zooxanthellae were seen in great numbers in the endoderm, but never in the ectoderm or in the glandular margin of the mesenterial filaments. They were much more numerous than usual in the " absorptive " zone of the mesenterial filaments, but the great majority of them were perfectly healthy. There was a very great accumulation of fat in the tissues, but this clearly comes, not from the zooxanthellae, but from large, rounded vesicles whose contents blacken with osmic acid, and which have a diameter about double that of the zooxanthellae. They are extremely numerous in the endoderm and occupy most of the central lumen of the planulae. After the mesenterial filaments are formed, these vesicles can be seen, clearly in process of digestion, within the " absorptive " zone, but a general decrease in intensity and increasing degree of fragmentation throughout shows that they are also utilized *in situ*. It is reasonable to assume that these vesicles form a reserve of food supplied by the parent, which enables the planula to maintain itself during the free-swimming period and early settled stages.

TEXT-FIG. 6.—*Symphyllia recta*, cell from edge-zone obtained by maceration and containing a degenerating zooxanthella. × 2480.

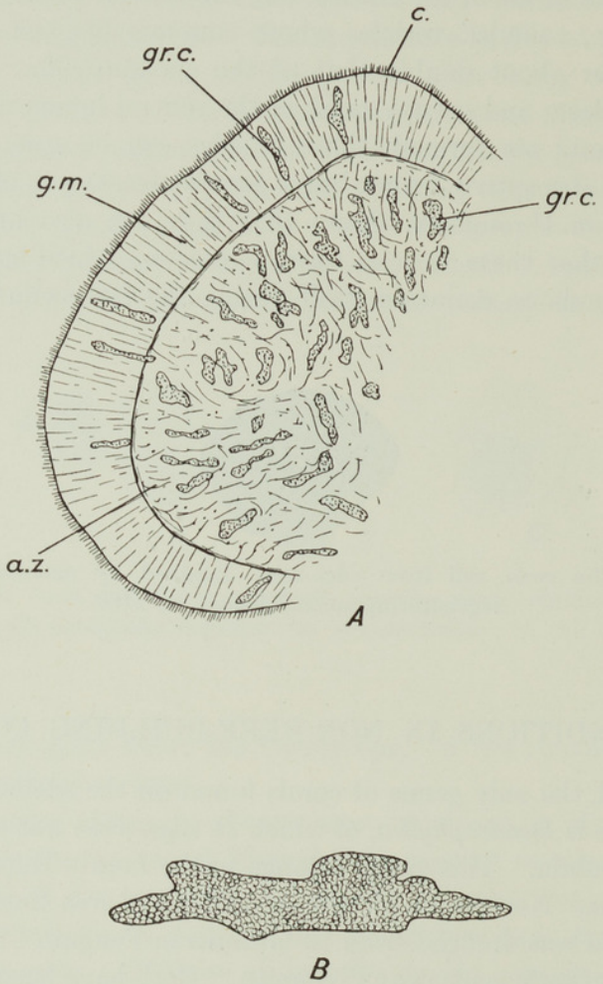
7. CONDITIONS IN NON-REEF-BUILDING CORALS.

As already stated, the only genus of corals found on the surface of reefs which never contains zooxanthellae is *Dendrophyllia*, of which *D. nigrescens* was examined at Low Isles and *D. manni* at Honolulu. This genus belongs to the family Eupsammiidae, which also includes *Balanophyllia*. No species of this second genus was found near the surface of reefs, but *B. bairdiana* was dredged from 16 fathoms in Penguin Channel, near Low Isles, while *B. regia* occurs in rock pools near Plymouth. Both have been examined and neither contains zooxanthellae. Conditions in these two genera of this very well-defined family are so similar that they can be discussed together.

In both *Dendrophyllia* and *Balanophyllia* the tissues contain great numbers of irregularly-shaped bodies containing granular corpuscles and often of a relatively large size and yellow or green in colour, which are not present in any other Madreporarian examined. They are most numerous in the endoderm, but do occur in the ectoderm and in the glandular margin of the mesenterial filaments. The appearance of a living mesenterial filament of *D. nigrescens* after being pressed out for examination under a coverslip is shown in Text-fig. 7A. The great abundance of these granular corpuscles (*gr.c.*), both in the "absorptive" zone and in the glandular margin, will be noted, and also their very irregular outline. Sections reveal their presence in especially large numbers in the deeper region of the endoderm, lying against, but never actually within, the mesogloea. They are abundant in all regions of the endoderm, but less numerous in the ectoderm.

Those lying within the " absorptive " zone of the mesenterial filaments show no sign of being broken down or " digested," although in fresh tissue numerous brown and red granules of moderate size can be distinguished in this region, and these very possibly do represent products of digestion.

Boschma (1924) observed these corpuscles in *D. micranthus* (=*nigrescens*), *D. coccinea*, and in a species of *Balanophyllia*. He suggests that they may be algae which have become



TEXT-FIG. 7.—*Dendrophyllia nigrescens*. A, side view of mesenterial filament pressed out for examination under a coverslip. × 180. B, cell containing granular corpuscles, obtained by maceration. × 1150. *a.z.*, " absorptive " zone ; *c.*, cilia ; *g.m.*, glandular margin ; *gr.c.*, cells containing granular corpuscles.

" highly reduced." He brings forward the finding of MacMunn (1902) that *Dendrophyllia* contains a chlorophylloid pigment, and his own observation that these corpuscles are found in the coelenteron mixed with the food remains—in the same way as zooxanthellae —as further evidence in support of his views.

These corpuscles strongly resemble the granular " albumen " gland-cells of Actiniaria (see Stephenson (1928)). Their colour is their only point of resemblance to zooxanthellae. They have no definite shape, those deeper in the tissues being usually more rounded than those more superficially placed, and none of the structure of the zooxanthellae. After maceration they are revealed as containing a mass of minute granules which blacken

with osmic acid, their appearance being shown in Text-fig. 7B. Unlike zooxanthellae they *never* occur within tissue cells. A nucleus was never distinguished in macerated material. After fixation with Flemming the granules are blackened with osmic acid, and it is thus very easy to detect the presence of the corpuscles in sections. Where the granules are not packed very closely, as in the great majority of cases they are, a nucleus can sometimes be distinguished after staining with safranin, as shown in Plate I, fig. 5. This nucleus is smaller and more darkly staining than that of the ordinary tissue-cells, but it is quite unlike that of the zooxanthellae, while there is no pyrenoid. Material fixed and decolorized in 70% alcohol was treated with chlorzinc iodine, with iodine and with calcium chloride iodine, but in no case was there any evidence of the presence within or around the corpuscles respectively of either starch or cellulose.

It is thus impossible on histological grounds to agree with Boschma's opinion that these corpuscles are of algal origin. Moreover further evidence to the contrary will be presented later in this paper when describing the results of experiments on the utilization of carbon dioxide and phosphorous by the zooxanthellae, and again in Paper VI of this series when dealing with the production of oxygen by the zooxanthellae.

There does, however, seem good reason for thinking that the corpuscles are wandering cells, and that their contents represent the accumulation of the products of excretion. It is notable that they occur in corals which lack the supplementary excretory system provided by the zooxanthellae, but which live in warmer water than the deep-water corals, and so have a more active metabolism. Their presence in the coelenteron after ejection from the " absorptive " zone and their green or yellow colour (possibly due to chlorophyll which has been taken in with the food and which cannot be digested) can both be explained on this assumption.

The true deep- or cold-water corals do not contain zooxanthellae, although Gardiner (1929) has shown the presence of similar bodies in *Gardineria antarctica* taken from over 200 fathoms. He can only account for their presence in a coral from this depth on the assumption that they have lost their chlorophyll and become parasitic on the coral polyp. The absence of zooxanthellae in *Heterocyathus*, *Heteropsammia* and *Flabellum* is stated by Gardiner (1930) in *Stephanotrochus*, *Cyathohelia*, *Odontocyathus* and *Stephenophyllia* by Boschma (1924), while observations of the senior author have confirmed their absence in *Caryophyllia* and *Lophohelia*.

Duerden (1902) states that the tissues of *Astrangia danae* contain no zooxanthellae, and that in *Astrangia solitaria* and *Phyllangia americana* they are " nearly or wholly absent." Boschma (1925) states that at Woods Hole colonies of *Astrangia danae* can be obtained both with and without zooxanthellae, and he describes the means whereby he infected those without zooxanthellae. His experiments and conclusions will be dealt with in the general discussion which concludes this paper. It is noteworthy that these Atlantic genera, *Astrangia* and *Phyllangia*, both occur in shallow water, but it appears from Duerden's descriptions that they normally live in dark or shady places which would account for the frequent lack of zooxanthellae.

8. PHYSIOLOGY OF ZOOXANTHELLAE.

The zooxanthellae possess chlorophyll which, in the presence of the radiant energy of sunlight, builds up the amyloid assimilation product. In the absence of light, as will

be shown in later sections of this paper, the zooxanthellae cannot live. It has already been shown that they are most numerous in the superficial regions of corals, where they can obtain most light. The process of photosynthesis involves the utilization of carbon dioxide and water and the production of oxygen. Paper VI of this series will be concerned with the description of a long series of experiments dealing with the conditions controlling the production of oxygen by the zooxanthellae, and the relation of this to the oxygen consumption, *i. e.* respiration, of the corals. Miss S. M. Marshall, in a separate paper, will give an account of work done on the gaseous exchange in coral planulae.

Although exact determinations of the carbon dioxide consumption of zooxanthellae were not made, estimations of the pH of the water from sealed jars in which corals containing zooxanthellae were kept, first in light and later in darkness, provide significant evidence of the utilization of carbon dioxide by the zooxanthellae in the light. Table I summarizes the results of a series of such experiments. Full details of the experimental conditions, which were identical with those for the oxygen experiments, will be given in Paper VI.

TABLE I.

Change in pH of sea-water in sealed glass jars of almost 3-litre capacity containing corals with and without zooxanthellae at the end of 9-hour periods, first in light and then in darkness. The same jars used for both series of experiments, being sunk in the sea in open and closed crates respectively.

Coral.	Volume.	Light.				Darkness.			
		Temperature (°C.).	pH.			Temperature (°C.)	pH.		
		On. Off.	Initial.	Final.	Difference.	On. Off.	Initial.	Final.	Difference.
Control	..	29·0—29·7	8·32	8·32	0	29·0—28·9	8·32	8·32	0
Porites	103 c.c.	,, ,,	8·32	8·32	0	,, ,,	8·32	8·26	−0·06
,,	130 ,,	,, ,,	8·32	8·35	+0·03	,, ,,	8·32	8·24	−0·08
Favia	157 ,,	,, ,,	8·32	8·19	−0·13	,, ,,	8·32	7·95	−0·37
,,	120 ,,	,, ,,	8·32	8·19	−0·13	,, ,,	8·32	8·0	−0·32
Galaxea	45 ,,	29·7—30·5	8·32	8·45	+0·13	30·0—29·5	8·32	8·27	−0·05
,,	50 ,,	,, ,,	8·32	8·42	+0·10	,, ,,	8·32	8·29	−0·03
Fungia	31 ,,	,, ,,	8·32	8·43	+0·11	,, ,,	8·32	8·18	−0·14
,,	32 ,,	,, ,,	8·32	8·45	+0·13	,, ,,	8·32	8·20	−0·12
Pocillopora	56 ,,	28·5—29·5	8·32	8·19	−0·13	27·9—28·7	8·32	7·80	−0·52
,,	46 ,,	,, ,,	8·32	8·20	−0·12	,, ,,	8·32	7·82	−0·50
		Average difference = −0·001				Average difference = −0·219			
Control	..	29·2—31·5	8·32	8·32	0	29·2—29·0	8·32	8·32	0
Dendrophyllia	40 c.c.	,, ,,	8·32	8·20	−0·12	,, ,,	8·32	8·21	−0·11
,,	30 ,,	,, ,,	8·32	8·22	−0·10	,, ,,	8·32	8·22	−0·10
,,	28 ,,	,, ,,	8·32	8·23	−0·09	,, ,,	8·32	8·23	−0·09
		Average difference = −0·103				Average difference = −0·10			

It will be seen from an examination of this table that for ten typical reef-building corals from five different genera, the average change in the pH of the water surrounding them after nine hours in light was a fall of only 0·001. Although carbon dioxide was constantly being produced by the corals, it was being utilized to such an extent by the zooxanthellae in their tissues that the pH of the small body of water surrounding the corals never fell very low in any case, and in a number of instances rose appreciably. The same corals under exactly the same conditions but in complete darkness caused an average fall in pH of 0·219 in the surrounding water. This was due to the accumulation of carbon dioxide in the confined volume of water, the zooxanthellae being unable to utilize it owing

to the absence of light and, indeed, actually increasing it slightly by the carbon dioxide produced by them during respiration.

The second part of the table deals with experiments on *Dendrophyllia* which contains no zooxanthellae and shows very different results. In this case there was a fall of pH in the water of the same extent in both light and darkness. There is no evidence, therefore, of the presence in the tissues of this coral of any plant possessing chlorophyll.

The carbohydrate formed as a result of photosynthesis is accumulated around the pyrenoid as the assimilation product. Much of it is, as we have seen, apparently stored in the form of oil-droplets in the cytoplasm of the zooxanthellae. Diatoms store their reserve food in the same manner. Evidence was produced in Paper II of this series indicating that the zooxanthellae probably possess an enzyme capable of converting starch and similar polysaccharides into glucose and also, possibly, a lipase which breaks down fats and oils into fatty acids and glycerol.

Much of the carbohydrate accumulated in the zooxanthellae must be converted into the protein needed for the repair of waste tissue, for growth and for division. The formation of protein from carbohydrate involves the addition of nitrogen, available in the form of nitrates or of the salts of ammonium, as well as, in certain cases, of phosphorus available only in the form of phosphates and of sulphur available in the form of sulphates. All of these substances, in addition to carbon dioxide, are continually being formed as a result of katabolic processes in the coral and have then to be excreted.

Work on the assimilation of these substances as an indication of protein synthesis by the zooxanthellae had to be confined to estimations of phosphorus.* The reagent for nitrate determination proved, unfortunately, useless. Unlike the formation of carbohydrates by photosynthesis, protein synthesis proceeds equally well in darkness and in light.

The results of an experiment on the changes in the phosphorus content of confined quantities of sea-water in which corals, with and without zooxanthellae, were kept, are shown in Table II.

TABLE II.

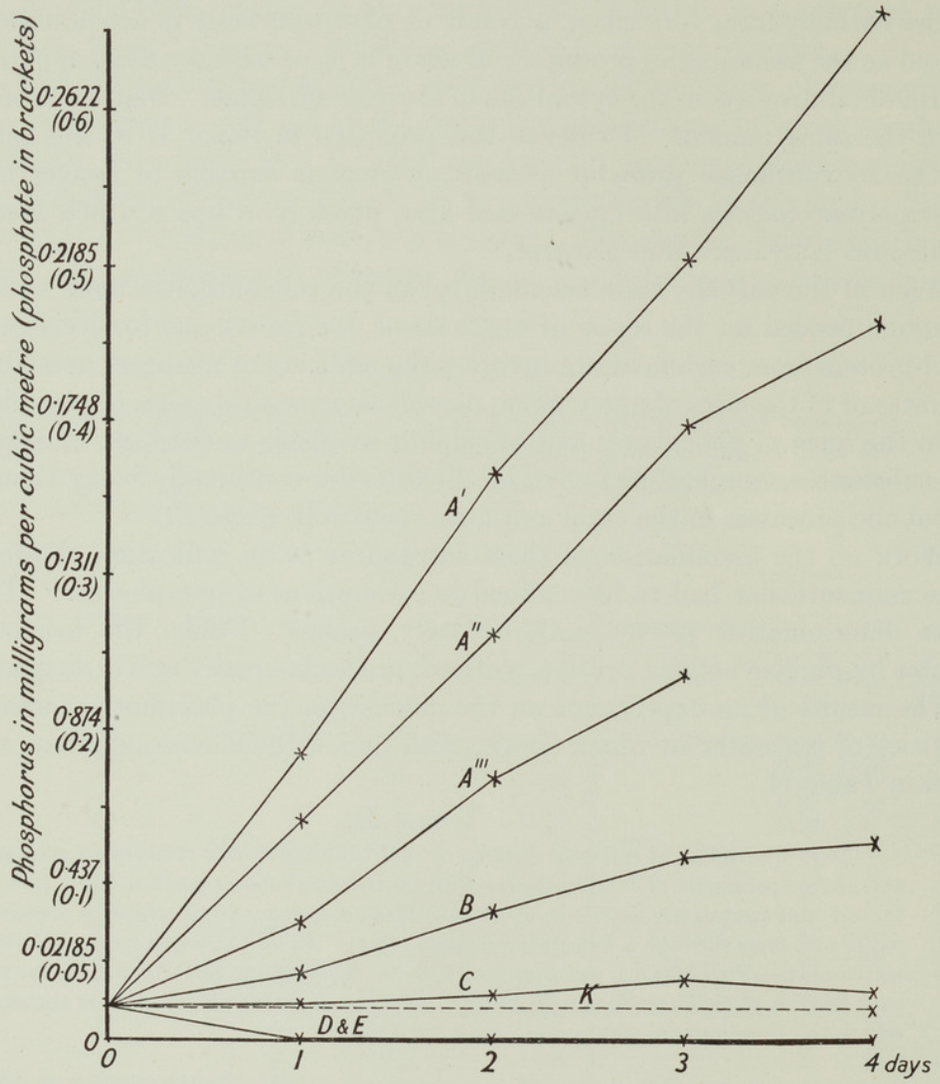
Four specimens of different genera of reef building corals containing zooxanthellae and three specimens of *Dendrophyllia* with no zooxanthellae placed in glass jars open to the air and containing 2500 c.c. of twice filtered sea-water (once through a coarse filter paper and once through a fine sintered silica filter). In addition one control jar with sea-water only. Two 100 c.c. samples and 100 c.c. for washing out 100 c.c. flasks removed each day for phosphorus determinations. All kept in a cool, shady place in the aquarium, where the temperature was round about 25° C.

Coral.	Phosphorus in mg. per cubic metre.					Total phosphorus in mg., allowing for removal of fluid daily.				
	Initial	1 day.	2 days.	3 days.	4 days.	Initial.	1 day.	2 days.	3 days.	4 days.
Favia	3·41	0	0	0	0	0·0085	0	0	0	0
Porites	3·41	0	0	0	0	0·0085	0	0	0	0
Psammocora	3·41	3·85	5·29	7·65	5·24	0·0850	0·0096	0·0128	0·0173	0·0135
Fungia	3·41	7·39	15·99	23·90	26·35	0·0085	0·0185	0·0375	0·0524	0·0564
Dendrophyllia (1)	3·41	32·6	68·08	100·07	143·55	0·0085	0·0815	0·1596	0·2203	0·2897
,, (2)	3·41	24·69	48·86	71·14	99·94	0·0085	0·0617	0·1149	0·1572	0·2032
,, (3)	3·41	13·11	31·94	47·72	..*	0·0085	0·0328	0·0742	0·1041	..*
Control	3·41	..	..	..	3·54	0·0085	..	..	..	0·0089

* Jar broken.

* As stated in Section 3, phosphorus was estimated as phosphate, and the figures for phosphorus—*i. e.* phosphorus *immediately available for assimilation by plants*, not total phosphorus—were obtained by multiplying the figures so obtained by 0·437.

The results in actual quantities of phosphorus and phosphate in the water are shown graphically in Text-fig. 8. It will be noted that in the case of two of the reef corals, *Favia* and *Porites*, the phosphorus content of the water fell to zero during the first day and remained there for the duration of the experiment. In other words, the zooxanthellae utilized not only the phosphorus which would normally have been excreted into the water



TEXT-FIG. 8.—Graph showing exchange of phosphorus between corals and surrounding sea-water. See Table II. A′, A″, A‴, *Dendrophyllia* (1), (2), (3); B, *Fungia*; C, *Psammocora*; D, *Favia*; E, *Porites*; K, control.

by the coral, but also all that was originally present—admittedly a very small amount—in the sea-water in the jars. In the case of *Psammocora* and *Fungia*, the phosphorus excreted by the coral exceeded the quantity used up by the zooxanthellae, only slightly so in the former (where the process was reversed after the third day), but by a considerable margin in the case of *Fungia*. Exactly the same relative results were obtained with these same corals in a second experiment.

The three specimens of *Dendrophyllia*—with no zooxanthellae—gave very interesting

results. Here, in all three cases, there was a great and very consistent increase in the amount of phosphorus excreted into the water during the course of the experiment. No zooxanthellae being present to remove it, this great increase—reaching a maximum of 3400% at the end of 4 days in the case of *Dendrophyllia* (1)—gives an indication of the very great amount of phosphorus which would be excreted into the water by *all reef-building corals* were it not for the presence within them of zooxanthellae. The volume of this particular piece of *Dendrophyllia* was only 40 c.c. This experiment also demonstrates very clearly the important source of nutrient salts which the zooxanthellae are in a position continually to tap.

In continuation of the above experiment, a colony of *Favia* was placed in the water in which *Dendrophyllia* (2) had been for four days. This contained phosphorus to the extent of 99·94 mg. per cubic metre. At the end of one day the water was again tested for phosphorus and with *negative* results all had been utilized by the zooxanthellae in addition to the amount produced during that period by the coral.

The results of this experiment led to the setting up of a further one, summarized in Table III, and the results of which are shown graphically in Text-fig. 9. In this experiment three typical reef-building corals were placed in jars containing filtered sea-water, to which had been added 50 mgrm. of phosphate (in the form of sodium phosphate) per litre.

TABLE III.

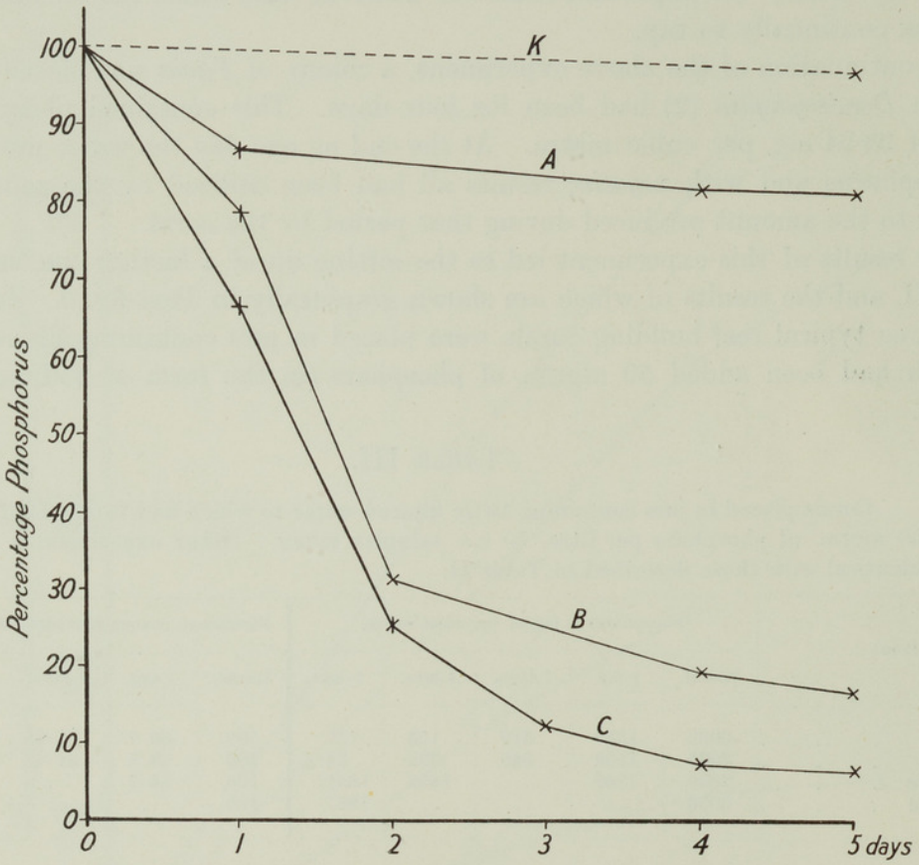
Corals placed in jars containing twice filtered water to which had been added about 50 mgrm. of phosphate per litre, 10 c.c. samples taken. Other experimental details identical with those described in Table II.

Coral.	Phosphorus in mgrm. per cubic metre.					Percentage change in concentration of phosphorus.				
	Initial.	1 day.	2 days.	4 days.	5 days.	Initial.	1 day.	2 days.	4 days	5 days.
Favia	2036	1359	519	153	137	100	66·7	25·5	7·5	6·7
Porites	2036	1599	640	398	34·4	100	78·5	31·4	19·5	1·68
Psammocora . . .	2036	1765	..	1656	1651	100	86·7	..	81·3	81·05
Control . . .	2036	..	..	..	1967	100	..	..	..	96·6

Here again the utilization by the zooxanthellae of the phosphate contained in the sea-water in addition to that produced by the corals in which they live is strikingly demonstrated, especially in the case of *Favia* and *Porites*. In *Psammocora* the tissues are very much thinner and the algal content as a result lower than in the other two corals. The small drop in the phosphorus content in the control jar is to be attributed to the development within it of phytoplankton during the course of the experiment. As the graph in Text-fig. 9 shows particularly well, there is a big initial utilization of phosphorus, but this falls off with the decrease in the phosphorus content, particularly after the third day. The point particularly to be borne in mind is that *the zooxanthellae are capable of utilizing much more phosphorus than is normally available for them.* One qualifying statement must be made: the corals during the course of the experiment were starved, and therefore presumably excreting less phosphate than usual. The results of other work on the changes in the phosphorus content in the water surrounding corals will be described later in this paper and in Paper V.

As already stated, no work could be done on nitrates or ammonia. Certain results

obtained by Pütter (1911), who worked on the actinian *Aiptasia* which contains zooxanthellae, are, however, of interest in this connection. He found that if no ammonia was present in the sea-water in which *Aiptasia* were placed, a small quantity was excreted into the water by the anemone ; that if the water contained a total of between 0·113 and 0·266 mgrm. of ammonia then no appreciable change took place during the period of the experiment ; while if the ammonia content exceeded this amount, reaching 0·57, 1·56 or 1·60 mgrm., or on certain occasions just attained 0·266 mgrm., there was



Text-fig. 9.—Graph showing percentage change in phosphorus content of sea-water in jars containing corals. See Table III. A, *Psammocora* ; B, *Porites* ; C, *Favia* ; K, control.

a reduction of about one half in the course of the experiment. He concluded, and with good reason, that the zooxanthellae present within the tissues of *Aiptasia* were responsible for the removal of ammonia from the surrounding water if it exceeded a certain concentration. The conditions closely parallel those already described for the change in the phosphorus content of the water surrounding *Psammocora* ; when the concentration is high, phosphorus is removed from the water ; when it is low phosphorus is added. The algal content in *Aiptasia* is lower than that in the great majority of reef corals, approximating more to the condition found in *Psammocora* than in *Favia* or *Porites*.

9. EFFECT IN NATURE OF ADVERSE CONDITIONS.

There is often no better method of determining the factors which govern the distribution and abundance of any form of life than the study of the effects upon this of adverse

or abnormal conditions. In the case of the zooxanthellae, the most important factor is clearly food supply, and the controlling agencies may be divided into two : (1) The intensity of the light which controls carbohydrate synthesis, and (2) the supply of nitrogenous material, phosphates and sulphates, which controls protein synthesis. Since the latter, and also a sufficient supply of carbon dioxide for carbohydrate synthesis, come largely from the coral, their abundance depends upon the metabolic state of the coral. If the coral is starved the zooxanthellae will be starved to some degree also. In addition to the food supply there are the physical factors other than light, the most important of which is probably temperature.

It proved possible to determine in some degree the effect *in nature* of varying intensities of light and of high temperature on the zooxanthellae. Under experimental conditions the effect of all three factors—light, starvation (*i. e.* lowering of the metabolism) of the corals and temperature—were investigated. This section of the paper deals with observations of the effect of these factors in nature, the next section with experimental data.

(*a*) Darkness.

On or near the surface of reefs, corals may occasionally be found, of which portions have grown round the underside of boulders or have become overgrown by other corals, in either case being almost or completely cut off from the light. The appearance of such a coral, a species of *Favia* in this instance, is shown in Plate II, fig. 6. The portion growing under normal conditions of illumination has the usual deep brown colour but the remainder, which lives in almost complete darkness, is pure white, although the tissues are perfectly healthy. An examination of the tissues of this coral revealed that the brown region contained the usual high content of zooxanthellae in the endoderm of the superficial regions, the mesenterial filaments also containing a certain number of them, all apparently in good condition. In the white region, on the other hand, zooxanthellae were very sparsely distributed. In the coenosarc (see Text-fig. 16) they were scattered here and there instead of occurring in solid masses, but those present were all healthy. No more than six in all were seen in any complete mesenterial filament, though here again they were to all appearance quite healthy. Further references to the conditions in this coral are given in the section dealing with experimental data on corals kept in darkness.

In this connection certain observations by Duerden (1902) on West Indian corals are of interest. He states (p. 437) that, " the polyps on the under, unexposed surface of colonies living in shady places are nearly always devoid of colour, although the individuals on the exposed area of the same colony are deeply pigmented. A remarkable instance of this occurs on the piles supporting the broad wharves at Port Royal. Numerous clumps of the corals *Oculina* and *Cladocora* grow attached to the piles ; the outer exposed colonies are of the usual brown colour, while those living on the inner pillars, which are cut off from the strong sunlight, are perfectly white, the corallum alone showing through the transparent tissues. It is manifest that a chlorophyll-bearing alga could not flourish under conditions where it is more or less deprived of light ; but except for this absence of coloration the coral polyps appear normal. Colonies of *Agaricia*, which usually are densely coloured, are found to be quite pale when living in the shady places often selected by these forms. The presence of zooxanthellae does not seem to be at all essential to the

life of coral polyps, seeing that colourless individuals in the shade flourish apparently as well as those in fully exposed places."

Corals dredged around Low Isles from depths of 7 and 9 fathoms invariably showed in the reduced number of their zooxanthellae the effect of diminished light. Division of the zooxanthellae and so their increase within the tissues will be slower owing to the much longer period needed for the accumulation of the necessary reserves of carbohydrate, fat and protein (the two last depending on the supplies of the first) needed for division.

In a *Galaxea* dredged from 7 fathoms the coenosarc was much paler than that of a typical specimen taken from the surface of the reef, and this was due to reduced numbers of zooxanthellae. There were also a few zooxanthellae scattered in ones and twos through the mesenteries, and all appeared in good health. In a species of *Favia* dredged from 9 fathoms, the same general difference between it and a typical specimen from the surface of the reef was noted. In *Tubipora* dredged from 9 fathoms, although the tentacles contained many zooxanthellae, these were by no means so abundant as in specimens taken from the surface of Batt Reef. So great was the difference that the tentacles of the specimen from deep water appeared white in comparison with the deep brownish green of those from the surface of the reefs. In all cases the algal content of corals from 7 or 9 fathoms was not more than half, probably considerably less, than that of corals from the surface of reefs.

(*b*) High Temperatures.

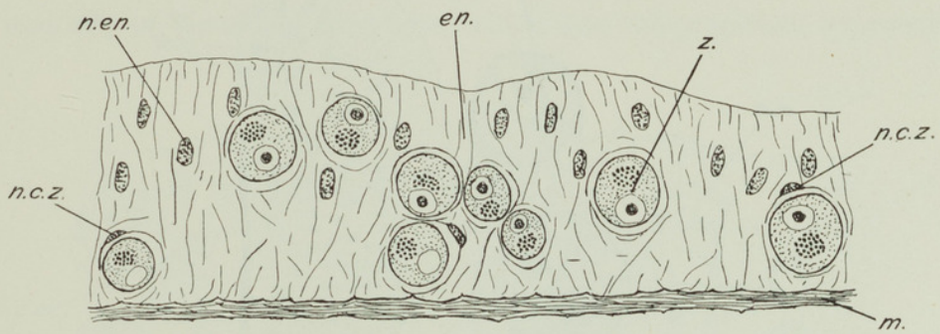
During the full moon spring tides in February, 1929, which happened to coincide with dead calm weather, the temperature at low tide during the day in the pools on the reef flat at Low Isles rose to very high figures, the highest recorded for the year. On February 22nd the senior author was impressed by the temperature of the water in the pools, which was literally hot to the touch, and a maximum thermometer reading of 35·1° C. was obtained. There was good reason for thinking that the temperature two days previously had been still higher, but unfortunately no thermometer readings were taken on that day. In the surface waters of the anchorage, Mr. F. W. Moorhouse recorded the highest temperature for the year, 33° C., on February 12th, and only 30·3° C. on the 22nd. The former date, however, was during neap tides, when the reef flat was not exposed during the day, when alone it could have been possible for the water in the pools to attain very high temperatures. Mr. A. P. Orr (see Vol. II) recorded maximum temperatures of 37·8° C. and 37·1° C. in sandy and coral pools respectively.

Very many corals exposed to the air and in some cases in the shallow pools were killed at this period. This is to be attributed to the exceptionally high temperature. Exposure to the air or to the intense light could not alone account for so much destruction. During the winter months very little destruction was observed, although the corals were exposed for much longer periods during the day (owing to the fact that during spring tides in the winter the lowest tides were during the day and the highest during the night, whereas the reverse was the case in the summer). Moreover, the light was as intense and the period of exposure as great, and often greater, during the other spring tides during the summer, but, as these never happened to coincide with such hot, calm weather, the temperature never rose to the same abnormal height.

When walking over the exposed reef flat during the next spring tides on March 21st,

great numbers of whitened skeletons of corals killed by the great heat a month previously, were seen. In addition there were a number of other corals, principally species of *Favia* and *Goniastrea*, which were equally white, but which, on closer examination, were found to be alive and apparently perfectly healthy, but with colourless, transparent tissues. They resembled in every way corals which had been living in the absence of light, and whose tissues consequently were almost or entirely without zooxanthellae. Either the great heat or the exposure to air or light of the previous month had presumably been responsible for this. Experiments, which will be described in the next section of this paper, indicate clearly that temperature was the cause, while, as already stated, exposure to light and air was more prolonged at other periods of the year.

Not less than twenty of these corals with colourless, transparent tissues were seen in a small area, and five of them, three species of *Favia* and two of *Goniastrea*,



TEXT-FIG. 10.—*Goniastrea* sp., section through endoderm of coenosarc of specimen exposed to great heat on reef flat and fixed in Bouin 4 weeks later. Stained safranin and light green. × 1250. *en.*, endoderm ; *m.*, mesogloea ; *n.c.z.*, nucleus of cell containing zooxanthella ; *n.en.*, nucleus of endoderm cell ; *z.*, zooxanthella.

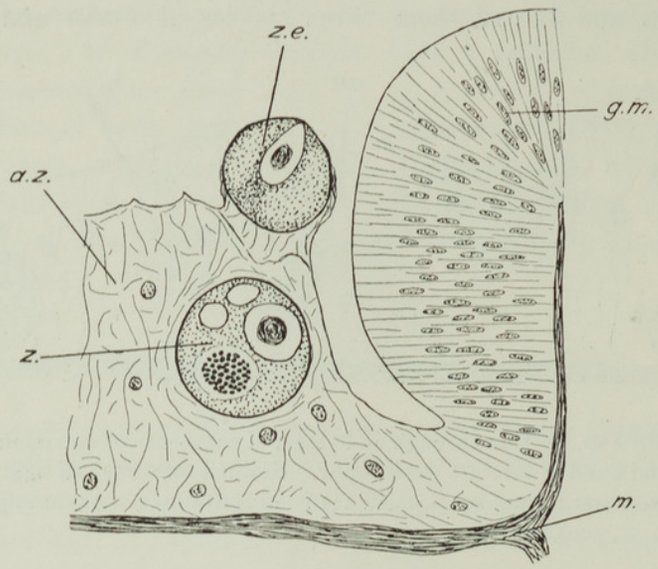
were marked with numbered stainless steel bars, and samples taken which were fixed in Bouin's fluid.

On 11th April, during the following new moon spring tides, these colonies were again examined—after a period, that is, of three weeks. All five were found to be perfectly healthy, the chipping off of the samples having done them no apparent damage. They were all distinctly brown in colour, although much paler than average colonies of the same species. Samples were again taken and fixed.

Owing to our absence in the Torres Strait for five weeks, a third examination could not be made until a further seven weeks and four days had elapsed, on 3rd June, during the new moon spring tides. All five colonies had by this time resumed their normal deep brown colour, *i. e.* the zooxanthellae had apparently multiplied until they had regained their normal abundance within the tissues. For the third and last time samples were taken and fixed.

Subsequent sectioning of two of the series, one *Favia* and one *Goniastrea*, revealed the condition within the tissues at each of the three periods. The first samples (taken four weeks after the corals had been exposed to very high temperatures on the reef flat) showed that the endoderm of the coenosarc, tentacles, disc and other superficial regions which normally are packed with zooxanthellae, contained very few, and these were scattered very irregularly. Text-fig. 10 shows a portion of the endoderm of the coenosarc

of the *Goniastrea*, the algal content being about the *maximum* seen. It will be noted that some of the algae are clearly contained within cells, the nuclei (*n.c.z.*) of which appear smaller and more darkly-staining than those in the tissues. Many similar regions were almost devoid of zooxanthellae. All those present were healthy, and there were many signs of recent division, although none is shown in the figure. There was an unusual accumulation in the tissues of refractile granules, and this may be correlated with the paucity of zooxanthellae. Zooxanthellae were relatively more numerous in the endoderm of the mesenteries, and were more plentiful than usual in the "absorptive" zone of the filaments. Many instances of algae being ejected were seen. An example of this is shown in Text-fig. 11, which shows one zooxanthella (*z.e.*) in process of ejection—*in exactly the*



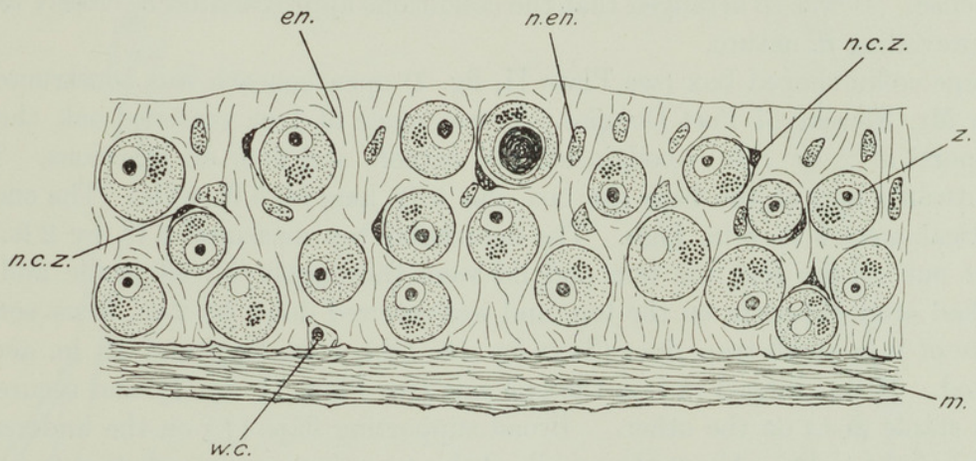
TEXT-FIG. 11.—*Goniastrea* sp., transverse section through portion of mesenterial filament from same colony as shown in text-fig. 10, fixed in Bouin 4 weeks after exposure to great heat. Stained safranin and light green. × 1666. *a.z.*, "absorptive" zone; *g.m.*, glandular margin; *z.e.*, zooxanthella in process of ejection. Other lettering as before.

same region where injected carmine was excreted, as described and figured in Paper III—and another (*z.*) a little deeper within the "absorptive" zone. The one being ejected shows some evidence of degeneration, the other appears perfectly healthy. Many zooxanthellae were found lying free in the coelenteron or on the surface of the glandular margins of the filaments, and the great majority of these as well as those in process of ejection were apparently healthy. There is thus no certain evidence—and this will be confirmed later in this paper—that the zooxanthellae were themselves adversely affected by the high temperatures to which the corals were exposed.

The second series of samples, taken when the corals were becoming brown again and seven weeks after they had been exposed to great heat, showed in sections very many more zooxanthellae in the endoderm of the superficial regions. All these algae appeared healthy and there were very many indications of recent division. In spite of this fact, however, no actual division stages were seen and the manner of division of the nucleus and pyrenoid could not be determined. It is clear that division takes place very rapidly.

Zooxanthellae were present in fair numbers in the mesenteries, but were exceptionally rare in the mesenterial filaments.

In the third series of samples, taken from the brown colonies, fourteen and a half weeks after exposure to great heat, zooxanthellae were seen in sections packed closely in the endoderm of all superficial regions, as in all healthy reef-building corals. A typical region from the endoderm of the coenosarc of the *Goniastrea* sectioned is shown in Text-fig. 12, and further evidence of the enclosure of zooxanthellae within tissue-cells is provided. A comparison between this and Text-fig. 10 will show clearly the difference between the *average* population of zooxanthellae in the endoderm of the coenosarc after the coral has recovered from the effects of its exposure to great heat, and the *maximum* population within the same region of the same coral four weeks after this exposure. In the " absorptive " zone of the mesenterial filaments, zooxanthellae were much more numerous than in the second sample, and perhaps even a little more numerous than in the first sample. The great majority of them, including some which were being ejected, appeared healthy.



TEXT-FIG. 12.—*Goniastrea* sp., section through endoderm of coenosarc of same colony as shown in two previous Text-figs., but fixed 14½ weeks after exposure to great heat. Stained safranin and light green. × 1250. *w.c.*, wandering cell. Other lettering as before.

There is thus evidence that, *under natural conditions*, corals may not only be killed by high temperatures, but that they may themselves survive although their contained zooxanthellae have been almost completely ejected. The question arises, are the algae directly affected, or are the corals so injured that the zooxanthellae are no longer able to live within them and so are ejected ? Although the former alternative appears at first sight the more obvious explanation, the apparently healthy stage of the zooxanthellae as revealed by sections and, in particular, the results of experiments to be described later in this and the following papers, indicate that the zooxanthellae are probably themselves uninjured, but that they are ejected as a result of the low metabolic state to which the corals are reduced owing to the highly unfavourable conditions to which they have been exposed.

Whatever the cause, the zooxanthellae are quickly carried *viâ* the mesenteries to the " absorptive " zone of the filaments, where, alone, they are ejected into the coelenteron. The mode of transport will be considered in the section dealing with experimental data. This process of ejection was still proceeding four weeks after the exposure of the corals to great heat, although probably then almost completed, for at that time the zooxanthellae in the endoderm of the superficial regions were already showing clear signs of division

and increase. No algae were being ejected at the end of seven weeks, while the zooxanthellae, provided with ample food, had greatly increased in the superficial regions, so that the normal brown colour had been half regained. At the end of fourteen and a half weeks the normal population of zooxanthellae had been regained, and the normal process of ejection of unhealthy or superfluous ones was again in progress.

10. EXPERIMENTAL DATA ON THE EFFECT OF ADVERSE CONDITIONS.

(*a*) Darkness.

In addition to the experiment in the aquarium in which corals were starved and fed in both light and darkness, to be described in Paper V of this series, an experiment was set up on the reef flat to test the effect of darkness upon corals and their contained zooxanthellae. It was so arranged that the conditions approximated as closely as possible to those prevailing in nature.

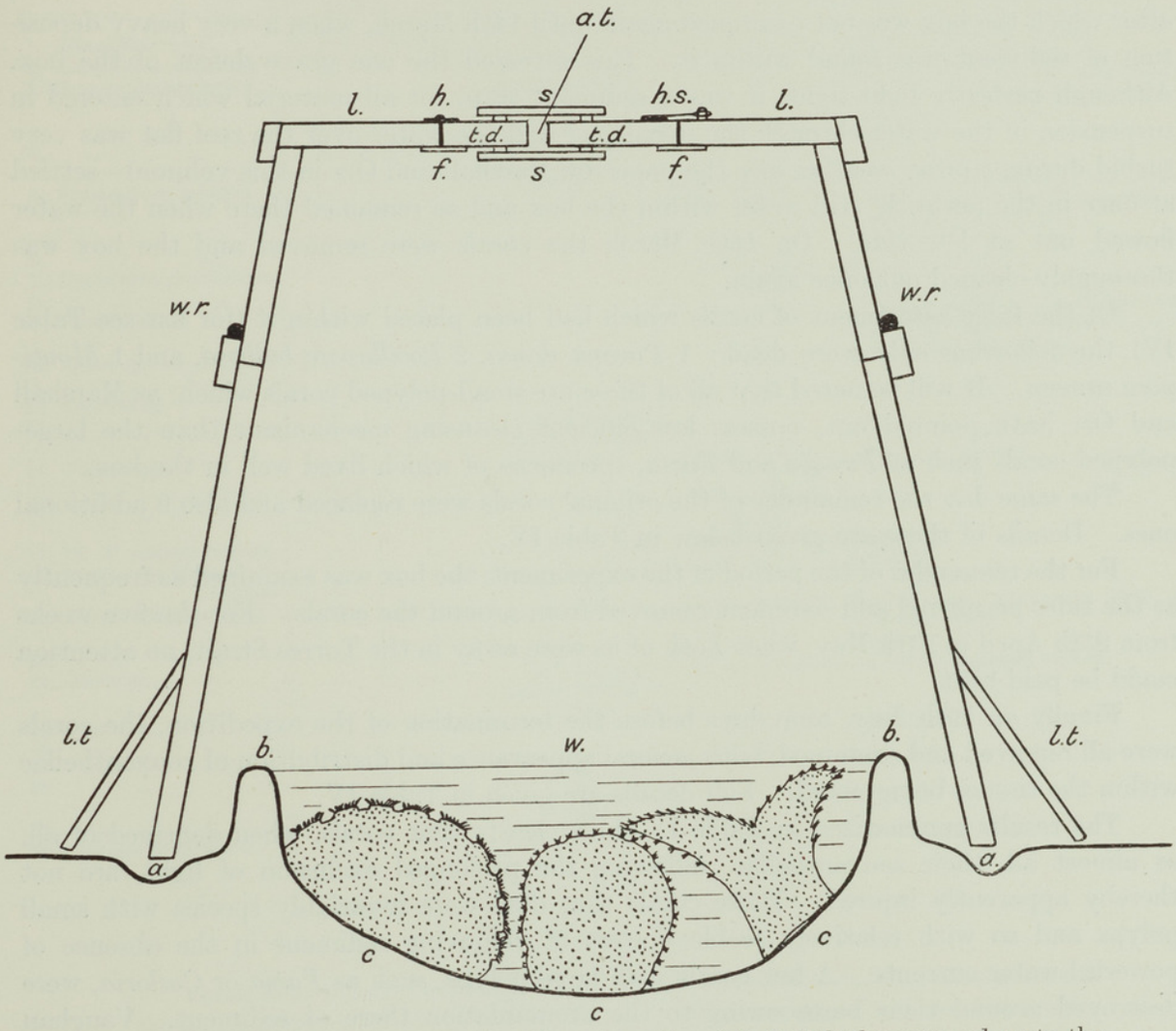
A large coffin-shaped box (see Plate II, fig. 7) open beneath was constructed to our design by Mr. Nielsen, of Port Douglas. It was made of 1-in. seasoned oak throughout, with supports of 2 by 1 in. wood inside the corners and over all junctions. The sides sloped outwards and at the base the box was 5 ft. long by 3 ft. wide. The ends, which were vertical, were 3 ft. 6 in. high. The detachable top measured 5 ft. by 2 ft. Before being put out on the reef, the box and lid were thoroughly tarred inside and outside. The lid had cross-supports on the top, and was screwed down by 2-in. brass screws. In the centre of it a small-trap door (Text-fig. 13, *t.d.*), 1 ft. long and 10 in. across, was constructed. It was hinged (*h.*) on the one side (see Plate II, fig. 7), and secured with a hasp and staple (*h.s.*) on the other. Broad supporting flaps (*f.*) on the underside made this entirely light-tight. About the middle of this trap-door was an aperture (*a.t.*), through which water could pass in and out, but through which light could not pass owing to the presence, above and below, of broad strips of tarred wood (*s.*).

This box, without the lid, was securely cemented down at low tide on the night of 27th December, 1928, in about the centre of the reef flat and near the western corner of Low Isles reef. It was further secured by stays of thick galvanized iron wire rope (see Plate II, fig. 7), which passed along the sides off the box (*w.r.*), and were fastened at either end to iron spikes driven into the coral of the reef flat and later cemented over. Four openings of moderate size (*a.*) were left in the cement which secured the box to the reef, one each in the middle of the sides and the ends. The entrance of light through these openings was effectively prevented by constructing traps of downwardly projecting flaps of wood (*l.t.*), with exactly fitting side pieces the whole being tarred black.

Originally the bottom was merely cleared of living coral and excavated to a depth of about 1 foot below the level of the base of the box. It then consisted of dead coral rock with many small apertures between adjacent blocks. It was soon found, however, that great quantities of sand and fine sediment worked their way up through these openings and speedily buried the corals which had been placed within. Consequently, on 4th February, 1929, all corals were removed from the box, and the bottom was excavated still deeper and then covered with a thick layer of cement. At the same time buttresses of cement (*b.*) were built up around the inner side of the openings, thereby raising the level

of the water, and causing the permanent retention, even over the lowest tides, of a layer of water about 1 foot deep. Text-fig. 13 gives a diagrammatic cross-section of the box through the middle of the sides after it was thus reconstructed.

All fine cracks between the planks forming the box were effectively blocked with putty and the lid was screwed on, and cracks left between this and the sides also puttied.



TEXT-FIG. 13.—Diagrammatic cross-section through centre of light-tight box secured on to the surface of the reef flat. × ⅛. *a.*, light-tight aperture at base ; *a.t.*, light-tight aperture in trap-door ; *b.*, buttresses of cement ; *c.*, corals ; *f.*, flaps of wood beneath trap-door ; *h.*, hinge ; *h.s.*, hasp and staple ; *l.*, lid ; *l.t.*, downwardly projecting flaps of wood over apertures at base ; *s.*, strips of wood above and below aperture in trap-door ; *t.d.*, trap-door ; *w.*, level of water within box at low water spring tides ; *w.r.*, wire rope stays.

After these unfortunate, for unavoidable, delays, the box was ready for use. It was absolutely light-tight. As the tide rose, water entered by the four openings (*a.*) around the base and gradually filled the box, air escaping through the light-tight aperture in the trap-door (*a.t.*), through which water flowed when the box was full.

On 17th February the base of the box was covered with an assortment of corals. At low tide on the 21st the temperature of the water within was found to be 29·8° C., and

outside 34·2° C., and on the day following 30·0° C. and 35·1° C. respectively, showing that the thick wooden sides effectively insulated the corals within from the great heat of that period.

On the 25th, following a big storm, examination of the contents of the box through the trap-door revealed the presence of a great deal of sediment within the box, and a species of *Acropora* was found dead, probably as a result of this. This sediment was removed, after which the box was not examined again until 14th March, when a very heavy deposition of sediment was found within it. This revealed the one grave defect of the box. Although perfectly light-tight, it was a sediment trap, for all material which entered in suspension in the water through the openings—and the water over the reef flat was very turbid during stormy weather (see the paper by Marshall and Orr in this volume)—settled at once in the perfectly still water within the box and so remained there when the water flowed out at low tide. On 15th March the corals were removed and the box was thoroughly cleaned out once again.

Of the large assortment of corals which had been placed within it (for list see Table IV), the following only were dead : 1 *Pavona danai*, 2 *Pocillopora bulbosa*, and 1 *Montipora ramosa*. It will be noted that all of these are small-polyped corals which, as Marshall and Orr have pointed out, possess less efficient cleansing mechanisms than the large-polyped corals such as *Fungia* and *Favia*, specimens of which lived well in the box.

The same day the remainder of the original corals were replaced and also 9 additional ones. Details of these are given below in Table IV.

For the remainder of the period of the experiment, the box was examined as frequently as the tides permitted and sediment removed from around the corals. For the five weeks from 25th April to 27th May, when both of us were away in the Torres Strait, no attention could be paid to it.

Finally on 19th July, nine days before the termination of the expedition, the corals were all removed and examined, both general appearance and distribution of zooxanthellae within the tissues being noted. Full details are given in Table IV.

The results summarized in Table IV show clearly that corals, when deprived of all, or almost all, their zooxanthellae, following the prolonged exclusion of light, are not thereby apparently injured. Those corals that died were invariably species with small polyps and so with relatively feeble powers of removing sediment in the absence of powerful water currents. A few corals with large polyps, such as *Favia* or *Coeloria*, were destroyed around their bases owing to the accumulation there of sediment. Vaughan (1914) carried out a similar series of experiments with Atlantic corals at Tortugas. A variety of common corals were placed in a light-proof live-car and examined after 14, 28 and 43 days. The great majority of them survived—he does not discuss the possible effect of sediment on those that died—but their tissues became pale or colourless.

The results, therefore, of this experiment and that of Vaughan agree with the observations made by Duerden and ourselves, that colourless corals may be found in nature in dark places. Taken together these experiments and observations indicate without any doubt that *individual* reef-building corals at any rate can flourish without contained zooxanthellae.

An experiment, summarized in Table V and shown graphically in Text-fig. 14, was carried out to determine the change in the phosphorus content of water in which a series of these corals, all of which had been in darkness for 152 days, had been placed.

TABLE IV.

Effect on corals of prolonged exposure to complete darkness in light-tight box on the reef flat.

Coral.	Date in box.	Period in box.	Condition at end of period in box.		
			General state.	Colour.	Presence of zooxanthellae.
1. *Symphyllia recta*	17 Feb.	152 days	Perfect	Very pale green	Almost completely absent.
2. *Lobophyllia corymbosa*	,,	,,	,,	Pale yellow	None found by teazing.
3. *Galaxea fascicularis*	,,	,,	Coenosarc gone	White	Moderate number in "absorptive zone" of mesenterial filaments, all dead.
4. ,, ,,	,,	,,	Ditto, ¼ killed by sediment	,,	Ditto.
5. *Psammocora gonagra*	,,	,,	Perfect	Very pale brown	More numerous than in other corals, though only small percentage of normal concentration.
6. ,, ,,	,,	,,	,,	,,	Ditto.
7. *Cyphastrea chalcidicum*	,,	,,	,,	Almost white	A very few only.
8. ,, ,,	,,	,,	,,	,,	,, ,,
9. *Fungia danai*	,,	,,	,,	Pale yellow	Moderate number, more numerous than in majority of other corals.
10. *Favia* sp.	,,	,,	,,	White	None found by teazing.
11. ,, ,,	,,	,,	,,	,,	,, ,,
12. *Coeloria* sp.	,,	,,	,,	,,	A very few only.
13. ,, ,,	,,	,,	Killed round base by sediment	,,	,, ,,
14. *Porites* sp.	,,	,,	Perfect	Light yellow	Moderate number.
15. ,, ,,	,,	,,	,,	,, ,,	,, ,,
16. ,, ,,	,,	,,	,,	Pale yellowish brown	,, ,,
17, 18. *Montipora ramosa*	,,	,,	Both dead, killed by sediment.		
19, 20, 21. *Pocillopora bulbosa*	,,	,,	All three dead, killed by sediment.		
22. *Psammocora gonagra*	15 Mar.	126 days	Perfect	Very pale brown	Rather more numerous than in other corals.
23. ,, ,,	,,	,,	,,	,, ,,	Ditto.
24. *Favia* sp.	,,	,,	Killed round base by sediment	Very pale yellow	Almost completely absent.
25. ,, ,,	,,	,,	Perfect	,, ,,	,, ,,
26. ,, ,,	,,	,,	,,	,, ,,	,, ,,
27. ,, ,,	,,	,,	,,	White	,, ,,
28. ,, ,,	,,	,,	⅓ killed by sediment	,,	,, ,,
29. ,, ,,	,,	,,	Dead	..	..
30. *Galaxea fascicularis*	,,	,,	Coenosarc gone	White	A very few only.

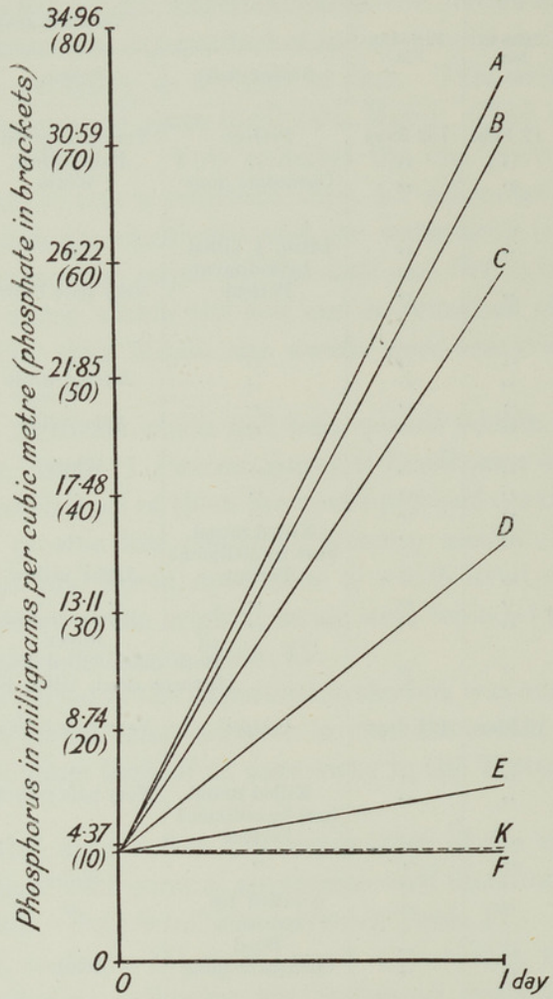
TABLE V.

Corals after 152 days in the light-tight box on the reef flat placed in glass jars, each containing 2500 c.c. of twice filtered sea-water. One control jar with sea-water only. Phosphorus content of the water estimated before the experiment and after 24 hours.

No.	Coral.	Phosphorus in mgrm. per cubic metre.	
		Initial.	After 24 hours.
2	*Lobophyllia*	4·20	30·79
5	*Psammocora*	4·20	33·12
7	*Cyphastrea*	4·20	6·68
9	*Fungia*	4·20	25·84
10	*Favia*	4·20	15·82
14	*Porites*	4·20	4·25
	Control	4·20	4·35

In no case it will be noted, did the phosphorus content in the water fall, though with *Porites* the increase was negligible. In all other cases the increase was very great, ranging from 59% to 688% (see Table VI), and thus exactly what would be expected as a result of phosphorus excretion from an animal undergoing normal metabolic processes. The

differences between the results obtained here and those from normal reef corals, and also *Dendrophyllia*, are shown in Table VI, which compares the results of experiments summarized in Tables II and V.



TEXT-FIG. 14.—Graph showing exchange of phosphorus between corals kept in darkness for 152 days and surrounding sea-water. See Table V. A, *Psammocora*; B, *Lobophyllia*; C, *Fungia*; D, *Favia*; E, *Cyphastrea*; F, *Porites*; K, control.

TABLE VI.

Percentage changes at the end of 24 hours in the concentration of phosphorus in the water surrounding corals kept in jars of 2500 c.c. capacity. Data taken from Tables II and V. Figures in columns 1 and 2 not directly comparable as different corals used, while the initial concentration of phosphorus in the water was a little higher in the second case (4·2 instead of 3·41).

Coral.	Percentage change in phosphorus content in water after 24 hours.		Difference.
	Normal corals. (Table II).	Coral in darkness for 152 days (Table V).	
Favia	−100	+276	376
Fungia	+117	+515	398
Psammocora	+ 13	+688	675
Porites	−100	+ 1	101
Lobophyllia	..	+633	..
Cyphastrea	..	+ 59	..
Dendrophyllia (1)	+856	..	..
,, (2)	+624	..	..
,, (3)	+284	..	..

Although the results of the two sets of experiments are not directly comparable owing to the different sizes of the corals employed and the small difference between the initial concentrations of phosphorus in the water (though the corals were approximately the same size and the lower initial phosphate concentration in column 2 tends to make the differences smaller than they actually are), yet the differences are far too great not to be significant. The increase in phosphorus content in the second column is, in four instances, of the same order of magnitude as the increase in phosphorus content of water in which the three specimens of *Dendrophyllia* were placed. And the latter, of course, contain no zooxanthellae. Moreover, it is far greater than the small increase in phosphorus content in the case of normal examples of *Fungia* and *Psammocora* recorded in column 1, while with *Favia* a drop to zero in phosphorus content *within* 24 hours becomes, after a *Favia* has lost its zooxanthellae, an increase of 276% ! It is difficult to understand the absence of change in phosphorus content in column 2 in the case of *Porites*, but it may be that this particular coral was in poor condition and its metabolic activity correspondingly reduced. It is significant in any case that the phosphorus content did not fall.

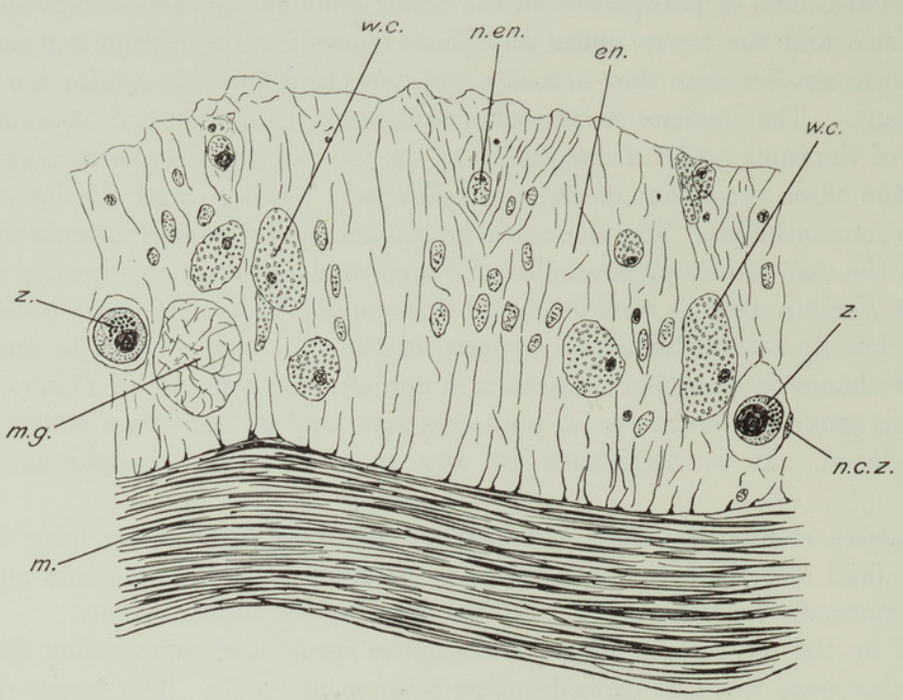
The above results, therefore, demonstrate yet more forcibly the large quantities of phosphate (and also there is every reason to presume, nitrates, ammonia and sulphate), which are normally removed direct from the coral by the zooxanthellae.

Work on the oxygen exchange between the corals and surrounding water in both light and darkness, was also carried out on these same corals. The results obtained will be described and discussed in Paper VI, together with all other work of this nature. They, also, indicate clearly the important part which the zooxanthellae play in the relations between the coral and the surrounding water.

Portions of certain of the corals used in this experiment, namely *Lobophyllia* (2), *Galaxea* (3), *Psammocora* (5), and *Favia* (10), all of which had been exposed to darkness for 152 days, were preserved in Bouin and later sectioned. Very few zooxanthellae were found in the sections. The typical conditions in the endoderm from the disc of *Favia* are shown in Text-fig. 15. As usual, the histology is not easy to interpret. Two zooxanthellae (*z.*), both of them reduced in size, alone appear in the portion figured. Both are clearly contained within tissue cells, the nucleus of one being shown. The cytoplasm of the epithelial cells is very vacuolated, and consequently stains very faintly with light green. Cell boundaries are as difficult as ever to determine, though the nuclei (*n.*) are conspicuous. The interesting fact is that there are numerous cells (*w.c.*) with granular somewhat refractile contents which stain readily with light green, and with small, rounded nuclei which stain darkly with haematoxylin or safranin, and are quite distinct from the larger, less darkly-staining nuclei of the epithelial cells. They occur, though much less frequently, in the ectoderm. These cells resemble closely the wandering " gland " cells which are so conspicuous in *Dendrophyllia* and *Balanophyllia*, though it is unfortunately impossible to say whether their contents blacken with osmic acid because no material was fixed with Flemming.

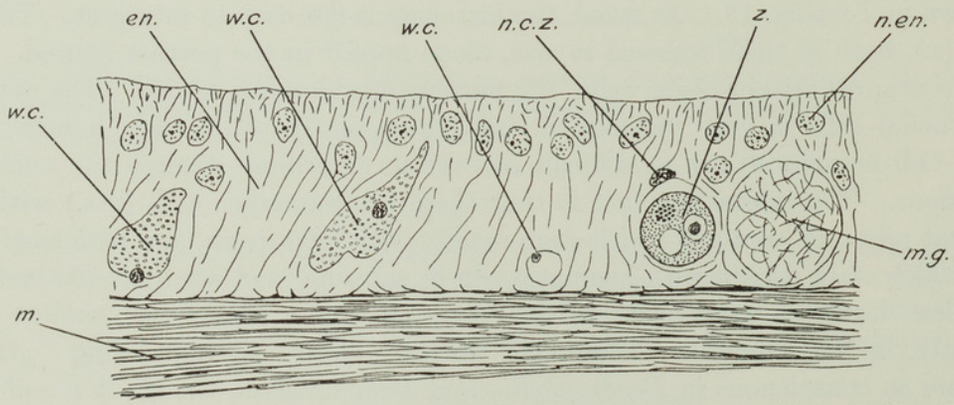
The presence of these cells in the tissues of corals from which zooxanthellae have been removed—they occur in all four genera—affords additional evidence in favour of the view previously put forward, that zooxanthellae are normally contained, not within the epithelial cells, but in wandering cells. These, after discharging their zooxanthellae by way of the " absorptive " zone of the mesenterial filaments, would appear to have resumed

their original function of excretion, which the presence of the zooxanthellae would render to a large degree superfluous.



TEXT-FIG. 15.—*Favia* sp., section through endoderm of specimen (No. 10, Table IV), kept in darkness for 152 days. Fixed Bouin, stained safranin and light green. × 833. *m.g.*, mucus-gland ; *w.c.*, wandering cell with granular contents. Other lettering as before.

To test these conclusions further, sections were cut of a portion of the whitened *Favia* previously discussed and shown in Plate II, fig. 6. Text-fig. 16 shows a typical strip of the endoderm of the coenosarc. There are very few zooxanthellae, though all

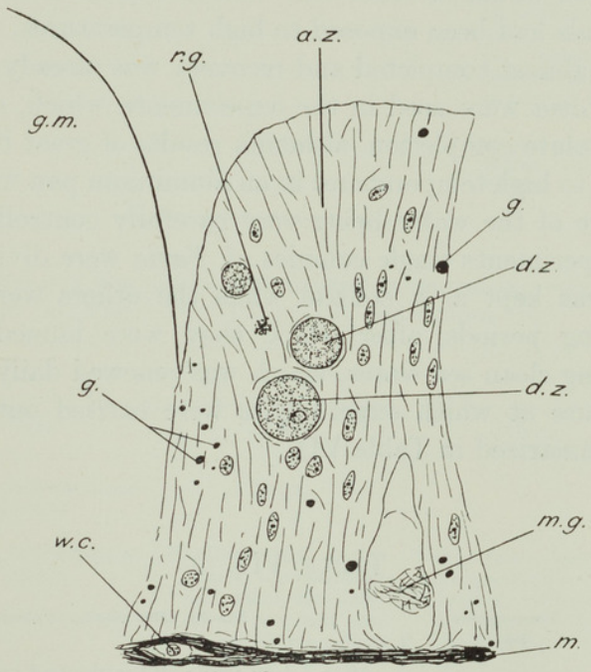


TEXT-FIG. 16.—*Favia* sp., section through endoderm of light area of colony shown in Plate II, fig. 6. Fixed Bouin, stained safranin and light green. × 1250. Lettering as before.

present are healthy. There are occasional mucus-glands (*m.g.*) and, notably, many wandering cells (*w.c.*) with the same granular contents and small, rounded, darkly-staining nuclei as those in the corals kept in the dark. Since this coral lived under normal conditions, the presence in it of these wandering cells indicates that their occurrence in the

experimental corals is not an abnormality due to the unusual conditions to which these corals had been exposed.

In the experimental corals, the great bulk of the zooxanthellae having been previously expelled, comparatively few were found in the mesenterial filaments. But, as shown in Text-fig. 17, which represents a portion of the " absorptive " zone of *Lobophyllia*, they are ejected in the usual manner. All three zooxanthellae shown are degenerating, as revealed by the absence of a clearly-marked nucleus or pyrenoid, but the cellulose wall remains intact, preserving the original spherical shape. In addition to the zooxanthellae, there are an unusually large number of granules (*g.*), some of them refractile (*r.g.*), and their



TEXT-FIG. 17.—*Lobophyllia corymbosa*, transverse section through portion of mesenterial filament of colony (No. 2) kept in darkness for 152 days. Fixed Bouin, stained safranin and light green. × 833. *d.z.*, degenerating zooxanthellae; *g.*, granules; *r.g.*, refractile granules. Other lettering as before.

presence may be the result of the absence of zooxanthellae, and so of the most potent agent for the removal of excretory products from within the tissues.

The ectoderm in all the corals sectioned, and especially in *Favia*, is characterized by an abnormal abundance of mucus-glands. This may be the result of the stimulus supplied by the abnormally heavy fall of sediment within the box.

It will be seen that information obtained from the study of sections confirms that previously acquired from examination of fresh and teazed material, and from experiments on the phosphate exchange between these corals and water in which they were kept. Prolonged exposure to darkness, while it inevitably destroys the zooxanthellae within their tissues, does *not* adversely affect the individual corals. This enforced return to primitive conditions, *i. e.* without zooxanthellae in the tissues, reveals the presence in the endoderm of large numbers of cells with granular contents, apparently wandering cells,

in which zooxanthellae may normally be contained, and which have now reverted to their original, excretory function.

(*b*) High Temperatures.

The fortunate discovery of the effect in nature of high temperatures on the algal content of corals, led to experiments being carried out to determine the exact conditions to which corals should be exposed if they are to expel their zooxanthellae without themselves being destroyed. It was also hoped that in this way the early stages of the process of expulsion would be observed. Observations in the field had commenced one month after the corals had been exposed to high temperatures, when expulsion of the zooxanthellae had been almost completed and recovery was already in progress.

Small colonies of *Favia* were used in the experiments, which, owing to lack of time, were never taken to absolute completion, although results of great interest were obtained. The corals were exposed to high temperatures in an aluminium pan with a capacity of some 2 litres, the temperature of the water being very carefully controlled during the experiment. In the later experiments single colonies of *Favia* were divided into a number of pieces, one of which was kept as a control while the others were exposed to certain temperatures for varying periods, after which they were immediately transferred to large glass jars containing clean sea-water, which was renewed daily.

The first temperature at which experiments were carried out was 40° C., and the results obtained are summarized in Table VII.

Table VII.

Coral.	Time.	Temperature.	Results, after placing in clean sea-water.
Favia 1	5 min.	40° C.	After 3 days corals all healthy and no ejection of zooxanthellae.
,, 2	10 ,,	,,	
,, 3	15 ,,	,,	
,, 4	30 ,,	,,	
,, 5A	1 hour	40° C.	After 1 day all showing signs of maceration, 5A least, coenosarc paler than usual, but no ejection of zooxanthellae. After 2 days all dead.
,, 5B	2 hours	,,	
,, 5C	3 ,,	,,	
,, 5D	4 ,,	,,	
,, 6A	1 hour	40° C.	After 17 hours A–D all slightly paler than K, especially on coenosarc between polyps. After 2 days all dead.
,, 6B	1½ hours	,,	
,, 6C	2 ,,	,,	
,, 6D	2½ ,,	,,	
,, 6K	Control	,,	
,, 7A	½ hour	40° C.	After 20 hours A unchanged in colour, B paler than K, and contents of coelentera of two polyps consisted of vast numbers of zooxanthellae with nematocysts and mucus, C similar but macerating. After 36 hours little change, but all except K macerating. After 60 hours all dead.
,, 7B	¾ ,,	,,	
,, 7C	1 ,,	,,	
,, 7K	Control		

Although some success was obtained with corals heated at 40° C. for half or three-quarters of an hour, when zooxanthellae were ejected, the results indicated that the temperature was too high, and that better results would be obtained by exposing the corals to somewhat lower temperatures for longer periods. Accordingly experiments were carried out at 36° C. (about the maximum temperature recorded in the pools of the reef flat), the results of which are shown in Table VIII.

TABLE VIII.

Favia 8 divided into five pieces, one retained as control (K), others placed in water at 36° C. for 2 hours (A), 2½ hours (B), 3 hours (C), and 4 hours (D).

Coral.	Condition after—			
	3 days.	4 days.	5 days.	7 days.
8A	Portion macerated; this removed; remainder healthy. No paling	Four polyps extruding mucus and great numbers of dead zooxanthellae. Paling.	Further maceration, but 10 healthy polyps; zooxanthellae extruded continually in minimum of mucus. Very pale. Many zooxanthellae in "absorptive" zone of mesenterial filaments apparently all dead	Dead.
8B, C, D	Dead	..	..	..
8K	Normal	Normal	Normal, mesenterial filaments with few zooxanthellae	Normal.

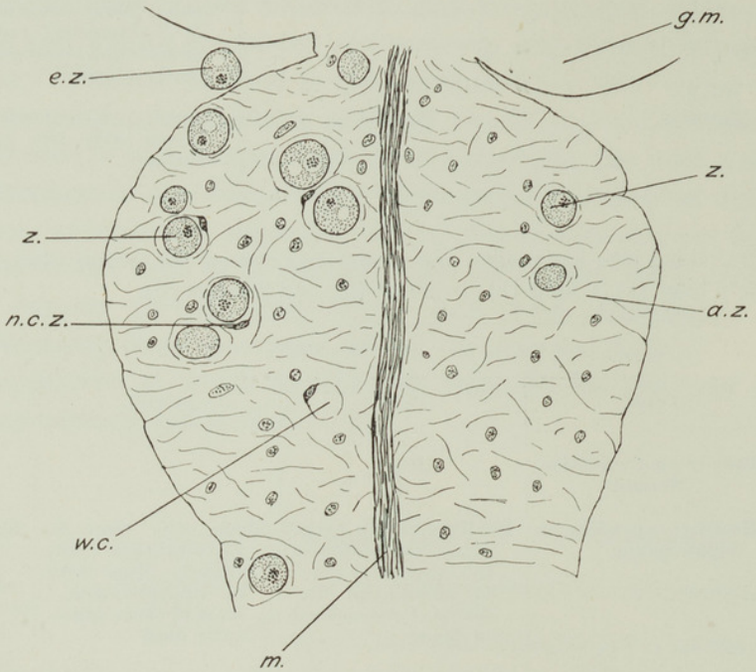
Favia 9 divided into five pieces, one retained as control (K), others placed in water at 36° C. for ½ hour (A), 1 hour (B), 1½ hours (C), and 2 hours (D).

Coral.	Condition after—				
	6 days.	7 days.	11 days.	23 days.	27 days.
9A	Normal	Pale, in poor condition	Dead	..	..
9B	,,	Normal	,,	..	..
9C	Dead	..	..	..	..
9D	Normal	Healthy, expanding at night, paling	Healthy, still paler. Many zooxanthellae in "absorptive zone" of mesenterial filaments. Portion fixed in Bouin	Much paler than K. Zooxanthellae extruded; dense layer in "absorptive zone," most of them apparently dead	Still perfectly healthy, expanding. Less extrusion of zooxanthellae; no longer dense layer in mesenterial filaments. Portion fixed Bouin.
9K	,,	Healthy, expanding	Healthy; few zooxanthellae in mesenterial filaments, most of them healthy	Normal; usual number of zooxanthellae in mesenterial filaments	Normal, expanding; few zooxanthellae in mesentrial filaments. Fixed Bouin.

An examination of the above table shows that *Favia* 8A and *Favia* 9D both gave satisfactory results. In both cases the corals had been exposed for two hours to a temperature of 36° C. The second experiment was particularly successful in that the coral remained healthy throughout. In both cases the tissues became gradually paler, while at the same time zooxanthellae began to appear in ever-increasing numbers within the "absorptive" zone of the mesenterial filaments. The greater number of these appeared to be dead. Later they were ejected into the coelenteron and then, mixed with mucus, poured out of the mouth. In the case of *Favia* 9D this process had practically ceased 27 days after the experiment began while the coral remained in perfect condition. *Favia* 8A did not survive the experiment. Lack of time prohibited the carrying out of further experiments, but there seems little doubt that, if sufficient experiments were conducted, the exact conditions necessary for completely ridding corals of their zooxanthellae could eventually be accurately determined.

As noted in Table VIII, portions of *Favia* 9D were fixed in Bouin 11 and 27 days after the beginning of the experiment, and also a portion of *Favia* 9K. This material has been sectioned, and the sections entirely confirm observations made on the living material.

Sections of *Favia* 9D 11 days after exposure to 36° C. revealed the presence of large numbers of zooxanthellae still in the endoderm of the superficial regions. The majority of these, judging by their staining reactions, appeared healthy. Sections of *Favia* 9K showed that zooxanthellae were distinctly more numerous, the conditions being, of course, normal. In *Favia* 9D 27 days after the experiment began zooxanthellae were distinctly less numerous than in the previous sample.

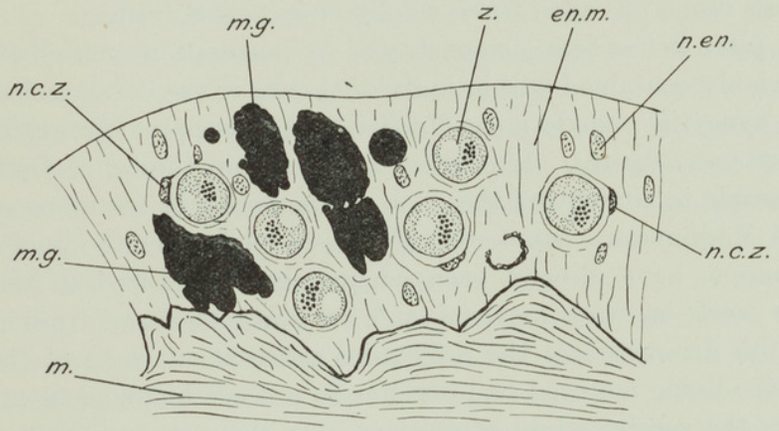


TEXT-FIG. 18.—*Favia* sp., transverse section through portion of mesenterial filament of colony 9D (see Table VIII) fixed in Bouin 11 days after exposure to a temperature of 36° C. for 2 hours. Stained Delafield's haematoxylin and erythrosin. × 833. *e.z.*, zooxanthella ejected at distal end of " absorptive " zone. Other lettering as before.

In agreement with the observations on the fresh material, the sections showed that zooxanthellae were present in abnormally large numbers in the " absorptive " zone of the mesenterial filaments 11 days after the experiment began. Typical conditions are shown in Text-fig. 18, where no less than 12 zooxanthellae appear in a section 6μ thick. Many of these very clearly enclosed within wandering cells, and one such (*w.c.*) also appears without such contents. Zooxanthellae were also numerous in this region in the sample taken after 27 days, but never so plentiful as in the first sample. In *Favia* 9K the usual number were found, perhaps a little more numerous than in material fixed immediately after removal from the sea, owing to the effects of poor nutrition, but many fewer than in either sample of *Favia* 9D.

The conditions in the first sample of *Favia* 9D are clearly intermediate between those in normal material and in the bleached corals taken from the reef flat one month after

they had been exposed to high temperatures. The process of removal of the zooxanthellae takes several weeks—which is not surprising when it is realized that each has apparently to be carried in a wandering cell through the tissues to the " absorptive " zone of the mesenterial filaments. They have to be taken by way of the mesenteries, and Text-fig. 19 shows typical conditions in a mesentery from the first sample of *Favia* 9D. As usual, cell boundaries in the endoderm cannot be made out, although there are numerous characteristic nuclei. Six zooxanthellae appear in the portion figured—an unusually large number for such an area of this region of the endoderm. Each is contained within a tissue cell, the nuclei of three of which are shown, and each appears smaller and stains more darkly than the other nuclei. Further evidence is thus provided that the zooxanthellae are contained in wandering cells which, when conditions are unfavourable, convey them to the mesenterial filaments for ejection. In the section, also, are a number of mucus-glands whose contents stain a uniform dark red with safranin. Such glands are always numerous



TEXT-FIG. 19.—*Favia* sp., transverse section through endoderm of mesentery of same sample of colony 9D as shown in preceding text-figure. Fixed Bouin, stained safranin and light green. × 1250. *en.m.*, endoderm of mesentery. Other lettering as before.

in this region, the surface of which, as observations on the living tissue demonstrate, is always ciliated, though the cilia can never be seen in sections.

The results of this experimental study of the effects of high temperature on corals and their contained zooxanthellae thus confirm the observations, previously recorded, on corals from the reef flat. Corals are killed if exposed to high temperatures for long periods, but they can survive moderately high temperatures if only exposed to them for short periods. But in this latter case the zooxanthellae may be expelled, in part or entirely. The question which remains undecided is whether the zooxanthellae are directly affected by heat or whether the lowered metabolic state of the coral is responsible, by starvation of the zooxanthellae (reduced supplies of carbon dioxide, nitrates, ammonia, phosphates, etc.) for their expulsion. In the case of *Favia* 8A, all the extruded algae certainly appeared to be dead, but in this case the coral itself failed to survive. In *Favia* 9D their appearance in the living material suggested that the zooxanthellae were dead, but sections failed to confirm this, assuming that the presence of an easily stained nucleus and pyrenoid is an indication that the zooxanthellae are alive. The best criterion of the death of the

zooxanthellae is undoubtedly a crumpling of the cellulose wall, but this is apparently so stout that it does not break down until some time after the plant is dead. The balance of evidence goes to show that the zooxanthellae are not themselves directly affected by high temperature, but by the lowering of the metabolism of the coral as a result of its exposure to high temperatures. It will be shown in Papers V and VI respectively that a similar expulsion of zooxanthellae follows a lowering of the metabolism of the corals as a result of starvation and of deprivation of oxygen.

11. DISCUSSION.

The investigations recorded in this paper represent an advance in many respects on previous knowledge concerning zooxanthellae and the nature of their relationship with the corals in which they live. As so frequently happens in scientific research, the problems raised are not less numerous than those wholly or in part solved.

In the first place it has been demonstrated by maceration methods that the zooxanthellae are invariably contained within individual cells of the coral, and are not scattered freely through a syncytial endoderm, as Matthai (1923) and previous authors have implied in their statements and figures. Indeed from the examination of sections alone it would have been impossible to controvert this view. The necessity for more accurate work on the histology of Madreporaria employing the most modern methods of histological and cytological technique have already been adequately emphasized in the course of this paper. Further work on fresh material and on macerations of fresh material is also indicated, and this demonstrates the difficulties inherent in work of this nature where observations of the living material had perforce to precede histological examination.

Nevertheless the careful study of sections does show the presence of zooxanthellae enclosed in cells in many instances, as shown in a number of the figures. There is also considerable evidence that the zooxanthellae are frequently, if not invariably, contained within wandering cells with rather smaller, more darkly staining nuclei than those of the body of the endoderm. Attention was drawn in Paper III of the series to the important work of Runnström on the histophysiology of the hydroid, *Clava squamata*, and to the account he gives of the wandering cells with granular contents which make their way through the syncytial endoderm, and also to the " granular vacuoles " which Matthai (1923) records in the tissues of Astraeid corals. The cells containing granular corpuscles (Plate I, fig. 5) which are so conspicuous in the tissues of the Eupsammiid corals, *Dendrophyllia* and *Balanophyllia*, neither of which ever contain zooxanthellae, are, as the evidence recorded in this paper clearly shows, probably also wandering cells of a similar nature. There is certainly no satisfactory evidence that they are of algal origin. Finally, there is the striking presence in corals which have been deprived of their zooxanthellae by exposure to darkness, either in nature or experimentally, of great numbers of wandering cells with granular contents.

There is thus very strong presumptive evidence that the zooxanthellae normally occupy the interior of wandering cells whose original function, which they still retain in the Eupsammiid corals, was the collection and removal of excretion from the tissues and probably also the distribution of food. The former function, which is of great importance in animals which have no other excretory mechanism, will be largely unnecessary when

zooxanthellae are present because these automatically remove all the principal excretory products, namely, carbon dioxide, phosphorus (as demonstrated experimentally in both cases in this paper), nitrogen in various forms, and sulphur, produced in the tissues as a result of katabolic processes. It may be that certain of the zooxanthellae are passed into the general body of the endoderm, which may be syncytial, but definite evidence on this point is lacking, and there is the important observation from macerated material which shows that *all* zooxanthellae are contained within distinct cells. This means that either the zooxanthellae are *always* in wandering cells, or else that the endoderm is *not* a syncytium.

A fact of the utmost importance which this research has brought out is that the *zooxanthellae are invariably rejected in the same manner and in the same region of the body.* They are carried in wandering cells by way of the endoderm of the mesenteries to the mesenterial filaments—there is no evidence for the view suggested in Paper III that material may be passed from cell to cell in the synctium. In the filaments they are ejected always in exactly the same place as was the injected carmine and iron saccharate whose fate was discussed in Paper III, namely at the extreme distal edge of the " absorptive " zone, next to the glandular margin. Macerated cells C and D in Text-fig. 1 on Paper III (see p. 88 of this volume) had already indicated, by the presence within them of both injected carmine and zooxanthellae, that this was the case.

In that paper it was demonstrated clearly that this " absorptive " zone is also the *only excretory region* in the body of the Madreporarian corals, and that it is indeed the only region in the body where interchange between the interior of the tissues and the exterior takes place. Boschma (1924, 1925, 1926) has based his theory that the zooxanthellae serve as a source of nutriment to the corals essentially on the presence in the " absorptive " zone of numerous degenerating zooxanthellae. Their presence in abundance in this region has been confirmed in this paper, but it has also been shown that similar degenerating zooxanthellae occur in smaller numbers elsewhere in the endoderm, and that under certain conditions—long exposure to darkness or exposure for short periods to high temperatures—the zooxanthellae are carried in great numbers to this zone and there ejected from the tissues. In short, there is just as much evidence that the degenerating zooxanthellae so abundant in the " absorptive " zone are, like the injected carmine and iron saccharate, in process of *excretion* into the coelenteron from the tissues, as that they are being digested there. It has, of course, already been demonstrated in Paper III that Boschma is perfectly correct in his view that this region is also concerned with digestion and absorption. The possibility that zooxanthellae are a source of nutriment to the corals will be discussed in detail in Paper V, which describes the results of an elaborate experiment set up to test the truth of this theory.

A last point in favour of the view that zooxanthellae are always contained within wandering cells is the absence of these algae in the mesogloea and ectoderm. This may not unreasonably be attributed to the mechanical difficulties of transporting such relatively large objects through the dense material of the mesogloea. Wandering cells are present in the ectoderm and also traverse the mesogloea as shown in Paper III, Plate I, fig. 5.

Another important fact is that individual reef-building corals can and do live well without zooxanthellae. This fact has already been established by Duerden (1902) in his work on West Indian corals. The ejection from the tissues of zooxanthellae starved to death by the subjection of the corals in which they lived to long periods of darkness in

the light-tight box was to have been expected, and confirmed, in more detail, the earlier experiments of Vaughan (1914). But the similar ejection of zooxanthellae from the tissues of corals exposed to high temperatures (35° C. and above) on the reef flat is a new and important observation which proved capable of experimental verification. Further evidence will be presented in Papers V and VI, showing that a similar ejection of algae follows starvation of corals or their exposure to low oxygen tensions. In other words, when the metabolism of corals is reduced in any way, a proportion of the zooxanthellae—the excess which can no longer be fed owing to the lowered production of carbon dioxide and the products of protein breakdown—is removed from the tissues of the coral. But the individual coral colony is not apparently any the worse for the absence of zooxanthellae. In *Astrangia danae* at Wood's Hole, as Boschma (1925) has shown, some individual colonies contain zooxanthellae and others do not. He was able to infect the former with zooxanthellae by feeding them with the minced tissues of injected colonies mixed with crab meat (in the absence of the latter the food was refused). Many of the zooxanthellae in the food were taken in at the " absorptive " zone, which soon became packed with them. Later a number found their way into other regions of the endoderm and began to increase by division. Others remained in the " absorptive " zone, where some of them degenerated as a result, in Boschma's opinion, of their digestion by the coral.

Although the presence of zooxanthellae within the tissues is certainly *not* essential to the life of individual colonies of reef-building corals—to the corals as a marine community which forms reefs conditions may well be different, but this will be discussed in the final paper of this series—to the zooxanthellae life in a madreporarian coral or other anthozoan is apparently essential. Miss S. M. Marshall failed to find zooxanthellae in any of the very numerous water samples from the anchorage at Low Isles and from the regular boat station which she centrifuged for nannoplankton, she also failed to culture them in any medium outside the body of the corals, while there is no evidence whatever of any flagellated spores or free-living stage such as is found in the *Chlamydomonas* which lives in *Convoluta roscoffensis*. The zooxanthellae have a thick cellulose wall, absent according to Keeble and Gamble (1907) in *Chlamydomonas*, and are thus well isolated from the tissues of the coral in which they live. From the animal they obtain the carbon dioxide, nitrogen, phosphorus and sulphur necessary for the synthesis of carbohydrates and proteins, and they are also well exposed to light within their superficial tissues, where the majority of them live. It has already been shown that they are most numerous in corals exposed to the greatest light intensity. The zooxanthellae appear, therefore, to be totally dependent on the coral or other anthozoan in which they live. But they are in no way injurious to them, living as they do entirely on the waste products of the animals, and which they remove automatically as soon as these are formed. It was shown in the course of this paper that they will also remove the same necessary substances from the sea-water around the corals. In this way the zooxanthellae constitute excretory organs of exceptional efficiency. The significance of this in the life of the corals will be fully discussed in the final paper of this series.

Another point of great importance which will be more fully discussed later is the ideal conditions for such an association between animals and plants which presents itself in the case of Madreporaria and allied Coelenterata. These animals, as shown in Paper II, are specialized for the digestion of protein, and must obtain a large part of their carbohydrates, a certain minimum of which will be essential for their metabolic processes, by

the breaking down of the proteins which they digest with such ease and rapidity. In this process unusually large quantities of nitrogenous material, phosphorus and sulphur must be produced, all of which will be available for the zooxanthellae.

Evidence as to the origin and nature of the relationship between corals and zooxanthellae will be materially increased by the results of experiments recorded in Papers V and VI. Final conclusions on these important matters must, therefore, be left to the final paper in this series, where the whole question of the significance of the remarkable prevalence of zooxanthellae in reef organisms belonging to a variety of widely different phyla will be fully discussed.

12. SUMMARY.

1. Species of thirty-five genera of reef-building Madreporaria were examined and all found to contain great numbers of zooxanthellae. These were also found in all planulae examined.

2. *Dendrophyllia* alone amongst Madreporarian corals which live near the surface of reefs possesses no zooxanthellae.

3. Zooxanthellae of the same type were equally numerous in the alcyonarian corals, *Tubipora* and *Heliopora*, and in all other Alcyonaria examined. They were also abundant in the zooanthid, *Palythoa*, in all Actiniaria examined and, in smaller numbers, in the gorgonids *Isis* and *Melitodes*.

4. Zooxanthellae of apparently a somewhat different type are abundant in the hydrozoan coral, *Millepora*, and in the hydroid, *Myrionema*.

5. Zooxanthellae also occur in the foraminiferan, *Polytrema*, in the mantle edge of the clams, *Tridacna* and *Hippopus*, and in a number of compound Ascidians.

6. This paper is concerned especially with the zooxanthellae of Madreporaria. These are yellowish-brown and spherical, varying in diameter from 6 to 14μ. They are bounded by a stout cellulose wall and contain a granular nucleus, and one, occasionally two, pyrenoids around which an amyloid assimilation product accumulates. The cytoplasm is vacuolated and contains numerous oil-droplets.

7. The zooxanthellae increase rapidly by division into two, but there is no evidence of the formation of spores. They are passed from the parent to the offspring by way of the planulae, but the exact stage at which these are infected is unknown.

8. Zooxanthellae were never found in centrifuged water samples, nor could they be cultured outside the body of the corals. There is thus no evidence that they can live apart from the corals.

9. Zooxanthellae are present only in the endoderm, being most numerous in the superficial regions. Although it is impossible to determine this from sections, macerated material shows clearly that the zooxanthellae are invariably contained within tissue-cells.

10. There is much evidence in favour of the view that zooxanthellae may *always* be contained within the wandering cells which certainly convey them from place to place in the tissues, and that they are never present in the general tissues of the endoderm.

11. The absence of zooxanthellae in the ectoderm and the mesogloea may be due to the mechanical difficulties of transporting such relatively large objects through the dense material of the mesogloea.

12. Zooxanthellae never occur in the glandular margin of the mesenterial filaments,

but, especially under certain conditions, they may be very numerous in the " absorptive " zone. Degenerating zooxanthellae are most abundant in this latter region, although they may be found anywhere in the endoderm.

13. The especial abundance of degenerating zooxanthellae in the " absorptive " zone provides the first evidence that they are excreted here, in the same way as the carmine injected into the edge-zone of corals, as described in Paper III.

14. Conditions are essentially the same in planulae and early post-larval stages.

15. In the Eupsammiid corals, *Dendrophyllia* and *Balanophyllia*, zooxanthellae are never present, but there are numerous yellow or green, irregularly-shaped bodies containing granular corpuscles. These occur in the ectoderm and the glandular margin of the mesenterial filaments, as well as in the endoderm. There is no evidence that they are algal in origin, as they have been considered to be, but abundant evidence that they are wandering cells containing granular masses of excrement.

16. The true deep- or cold-water corals contain no zooxanthellae, although the presence of apparently analogous bodies has been demonstrated by Gardiner (1929) in *Gardineria antarctica* from over 200 fathoms.

17. The pyrenoid of the zooxanthellae contains chlorophyll which, in the presence of light, forms the amyloid assimilation product, utilizing carbon dioxide and water and producing oxygen. Experiments with corals in sealed jars showed that the pH of the water falls appreciably after nine hours in darkness, but that it remains approximately constant during a similar period in light. This is due to the utilization of carbon dioxide by the zooxanthellae. In *Dendrophyllia* there is a similar drop in pH in both light and darkness.

18. The carbohydrate so produced is partially converted into oil and stored in that form.

19. Protein synthesis was followed by estimations of the phosphorus exchange between corals in glass jars and the sea-water surrounding them.

20. *Dendrophyllia* excretes large quantities of phosphorus, but reef corals containing zooxanthellae do not. On the contrary they frequently remove phosphorus from the water, even when this has been greatly increased by the addition of phosphate. The zooxanthellae are thus capable of utilizing for protein metabolism much more phosphorus than is normally produced by the katabolic processes of the corals in which they live.

21. These results are in agreement with those obtained by Pütter on the ammonia excretion in the actinian *Aiptasia*, which contains zooxanthellae.

22. The number of zooxanthellae in any coral depends, amongst other things, upon the intensity of light to which the coral is exposed. Reef-building corals from deep water (7 or 9 fathoms) and those nearer the surface which have grown on the underside of boulders, have both many less zooxanthellae than corals living, fully exposed to light, near the surface of reefs.

23. Corals exposed in nature to high temperatures, *e. g.* 35° C., may survive, but become colourless owing to the ejection of the great majority of their zooxanthellae. In the course of time, about three months in the case of colonies observed, the normal population of zooxanthellae is regained. Sections reveal that the zooxanthellae are ejected by way of the " absorptive " zone of the mesenterial filaments.

24. An experiment carried out in a large light-tight box cemented down on the reef flat showed that corals can survive exposure to complete darkness for 152 days, but that

practically all their zooxanthellae are killed and ejected, invariably by way of the " absorptive " zone of the mesenterial filaments. There was satisfactory evidence that the few corals that failed to survive were killed by the abnormally heavy fall of sediment in the box.

25. Reef-building corals which had been denuded of zooxanthellae in this way excreted large quantities of phosphorus, of about the same order of magnitude as *Dendrophyllia*.

26. In place of the zooxanthellae there appeared in the endoderm great numbers of wandering cells with granular contents. These were also found in sections of corals which had grown under boulders in darkness. It is suggested that these cells normally contain zooxanthellae, but that in their absence they had resumed their original function of excretion, as they normally do in *Dendrophyllia* and *Balanophyllia*.

27. Experiments carried out to determine the effect of exposing corals to high temperatures showed that a *Favia* kept in water at 36° C. for 2 hours ejected a large number of its zooxanthellae by way of the " absorptive " zone of the mesenterial filaments. They were then discharged from the mouth in mucus strings. The process appears to reach its maximum about 11 days after exposure to high temperature, and to be largely completed at the end of 27 days.

28. The zooxanthellae ejected in both the experimental corals and those observed in nature appeared often to be healthy. The conclusion is reached that the zooxanthellae are not themselves directly affected by high temperatures, but that the metabolism of the corals is lowered and the zooxanthellae, which can no longer obtain the necessary supplies of carbon dioxide, nitrogen, phosphorus, etc., are largely expelled.

29. Individual reef-building coral colonies live perfectly well without contained zooxanthellae.

30. The zooxanthellae, by automatically removing from the tissues the waste products of coral metabolism, constitute excretory organs of exceptional efficiency.

31. Attention is finally drawn to the ideal conditions for this association between animals and plants which prevail in Madreporaria and allied Coelenterata. Owing to their very limited powers of digesting carbohydrates (see Paper II), these animals probably obtain a large proportion of the carbohydrates necessary for metabolism by the breaking down of protein, which they digest with great rapidity, and the consequent liberation of nitrogen, phosphorus, sulphur, etc., in a form immediately available to the zooxanthellae.

REFERENCES.

BOSCHMA, H. 1924. On the Food of Madreporaria. Proc. Acad. Sci. Amst. XXVII, pp. 13–23, text-figs. 1, 2.

—— 1925. On the Feeding Reactions and Digestion in the Coral Polyp *Astrangia danae*, with Notes on its Symbiosis with Zooxanthellae. Biol. Bull. Wood's Hole, XLIX, pp. 407–439, text-fig. 1.

—— 1926. On the Food of Reef-Corals. Proc. Acad. Sci. Amst. XXIX, pp. 993–997.

BUCHNER, P. 1921. Tier und Pflanze in Intracellularer Symbiose. Berlin, 462 pp. pls. i, ii, text-figs. 1–103.

DUERDEN, J. E. 1902. West Indian Madreporarian Polyps. Mem. Nat. Acad. Sci. Washington, VIII, pp. 399–597, pls. i–xxv, text-figs. 1–18.

GARDINER, J. S. 1929. Coelenterata. Part IV : Madreporaria. (*b*) Turbinolidae and Eupsammiidae. British Antarctic (" Terra Nova ") Expedition, Zoology, V, pp. 121–130, pl. i.

—— 1930. Studies in Coral Reefs. Bull. Mus. Comp. Zoöl. Harvard, LXXI, pp. 1–16.

KEEBLE, F., and GAMBLE, F. W. 1907. The Origin and Nature of the Green Cells of *Convoluta roscoffensis* Quart. J. Micr. Sci. LI, pp. 167–219, pls. xiii, xiv.

MANGAN, J. 1909. The Entry of Zooxanthellae into the Ovum of *Millepora* and Some Particulars Concerning the Medusae. Quart. J. Micr. Sci. LIII, pp. 697–710, pl. xviii.

MATTHAI, G. 1914. A Revision of the Recent Colonial Astraeidae possessing Distinct Corallites. Trans. Linn. Soc. Lond. Zool. XVII, pp. 1–140, pls. i–xxxviii.

—— 1923. Histology of the Soft Parts of Astraeid Corals. Quart. J. Micr. Sci. LXVII, pp. 101–122, pls. vii, viii.

MOSELEY, H. N. 1881. On the Hydrocorallinae. "Challenger" Reports, Zoology, II, pp. 11–101, pls. i–xiv.

PÜTTER, A. 1911. Der Stoffwechsel der Aktinien. Z. allg. Physiol. XII, pp. 297–322.

RUNNSTRÖM, J. 1929. Zur Kenntnis der Histophysiologie von *Clava squamata* (O. F. Müller). Acta Zool. X, pp. 1–58, pls. i–iv, text-figs. 1–27.

STEPHENSON, T. A. 1928. British Sea Anemones, Vol. I. Ray Society, London, 148 pp., pls. i–xiv, text-figs. 1–41.

VAUGHAN, T. W. 1914. Reef Corals of the Bahamas and of Southern Florida. Yearb. Carnegie Instn., No. 13, pp. 222–226.

DESCRIPTION OF PLATE I.

Lettering employed: *a.p.*, assimilation product; *c.*, cilia; *c.a.*, clear area around pyrenoid; *c.w.*, cellulose wall; *ec.*, ectoderm; *en.*, endoderm; *f.*, fat-globule; *m.*, mesogloea; *m.g.*, mucus-gland; *n.*, nucleus of zooxanthella; *n.c.z.*, nucleus of cell containing zooxanthella; *n.gr.c.*, nucleus of cell containing granular corpuscles; *nem.*, nematocyst; *o.*, oil-droplet; *p.*, pyrenoid; *v.*, vacuole; *w.c.*, wandering cell; *z.*, zooxanthella; *z.d.*, degenerating zooxanthella.

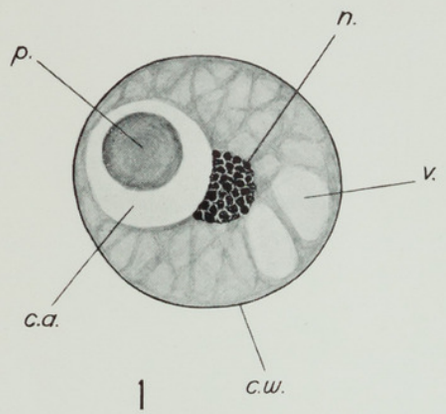
FIG. 1.—Zooxanthella from *Galaxea fascicularis*. Fixed in Bouin's fluid, stained iron haematoxylin, safranin and light green. × 3125.

FIG. 2.—Zooxanthella from *Galaxea fascicularis*, possessing two pyrenoids. Fixed in Bouin's fluid, stained iron haematoxylin, safranin and light green. × 3125.

FIG. 3.—Zooxanthella from *Pocillopora bulbosa*. Fixed in Flemming's strong fluid, stained safranin and light green. × 3125.

FIG. 4.—*Pocillopora bulbosa*. Transverse section through portion of a tentacle. Fixed Flemming's strong fluid, stained safranin and light green. × 625.

FIG. 5.—*Dendrophyllia nigrescens*. Cell containing granular corpuscles. Fixed in Flemming's strong fluid, stained safranin and light green. × 3125.



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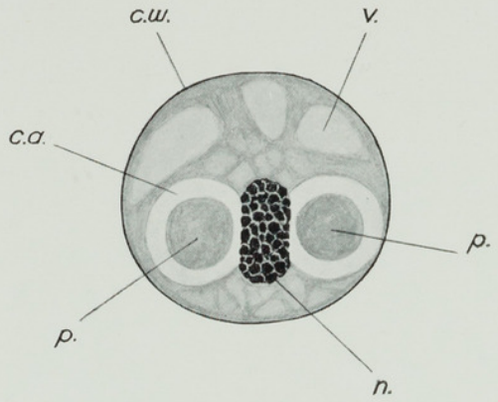

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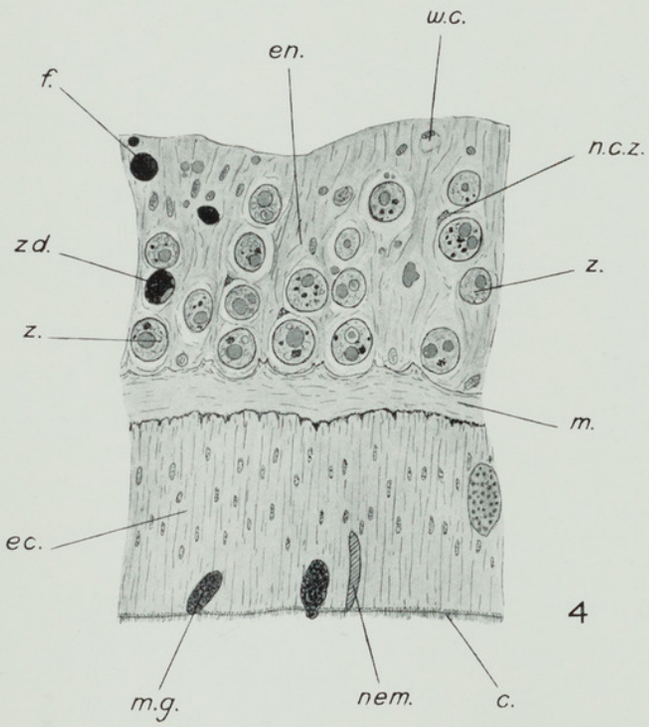

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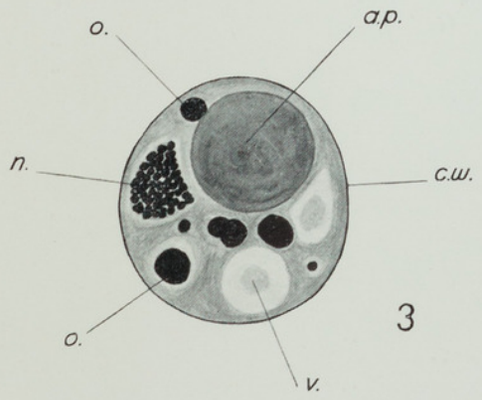

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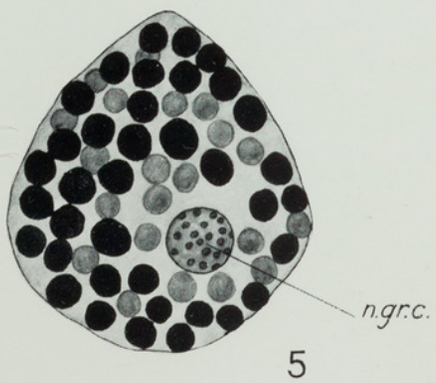

5

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DESCRIPTION OF PLATE II.

FIG. 6.—*Favia* sp. ; from underside of boulder, whitened portion on underside has grown in darkness. × ¾.

FIG. 7.—Light-tight box on reef flat, showing trap-door open, light-tight aperture at side and wire stays.

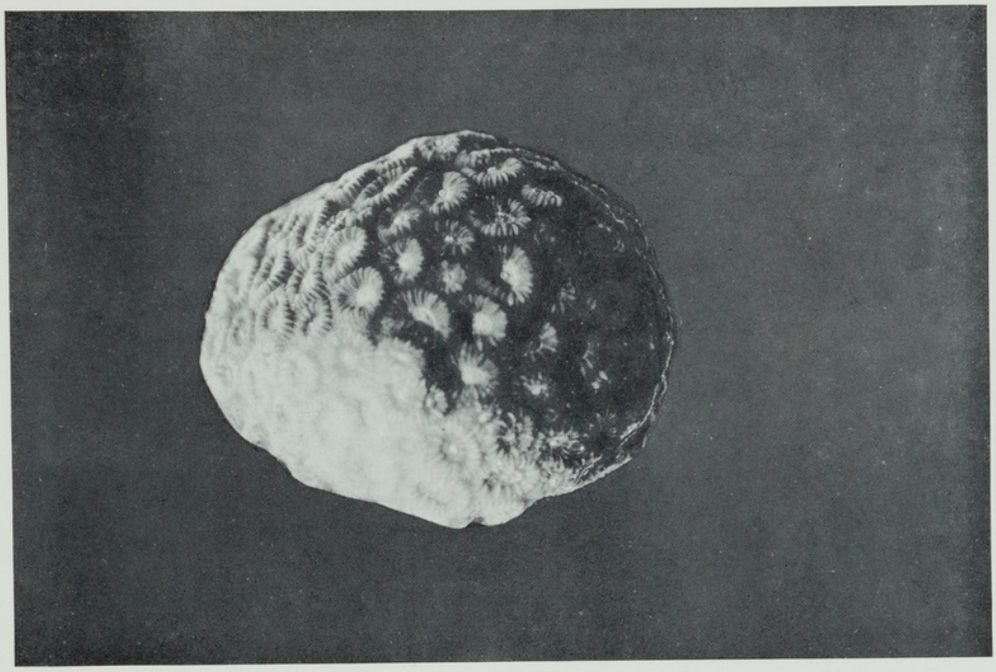

Photo G. W. Otter. FIG. 6.

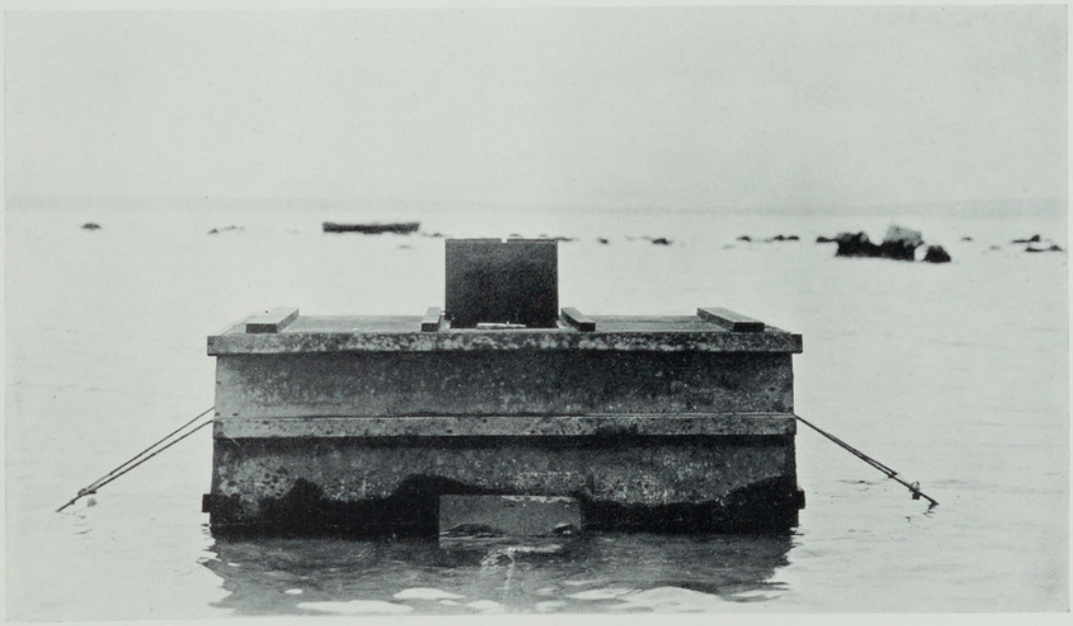

Photo M. J. Yonge.] FIG. 7. [*Adlard & Son, Ltd., Impr.*