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The anemone Anthopleura xanthogrammica varies in color from bright green to muddy brown. However, individuals found in caves are white. White specimens of Anthopleura xanthogrammica collected from dark caves along the Oregon coast were subjected to various experiments to determine if these cave dwelling animals carried any cells of the algal symbiont, and to ascertain whether adults could become infected by feeding, injecting and placing in the surrounding water algal cells which had been extracted from exposed anemones. Fresh smears and prepared sections showed that the white anemones were completely devoid of any algal cells and that adults of these apparently were not susceptible to infection by cells removed from naturally infected forms.

Algal cells extracted from exposed forms of A. xanthogrammica were of two colors: green and brown. Two-dimensional paper and thin layer chromatography with spectrophotometric analysis

proved that the green cells contained a complement of pigments characteristic of and unique to green algae while the brown cells contained a complement of pigments characteristic of and unique to dinoflagellates.

The Algal Symbionts of the Solitary Green Sea Anemone *Anthopleura xanthogrammica*

by

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THE ALGAL SYMBIONTS OF THE SOLITARY GREEN SEA ANEMONE ANTHOPLEURA XANTHOGRAMMICA

INTRODUCTION

Symbiotic relationships between unicellular algae and invertebrates have been known since 1871 when Cienkowski noted that green cells in certain radiolarians were not those of the host, but of what he described as parasitic algae. Since then there have been countless records of this kind of symbiotic relationship found in nearly every invertebrate phylum. Coelenterates show a very large number of examples of this association. The algae symbiotic with coelenterates fall into two general categories: zooxanthellae or the golden-brown algae and zoochlorellae the green algae. The zoochlorellae are found in fresh water hydrozoans, commonly Chlorohydra and Hydra (Goetsch, 1924). The zooxanthellae are restricted to numerous marine forms. Although there are many different marine coelenterates harboring symbiotic algae, only two forms of zooxanthellae have been isolated. Culture of the symbionts from various coelenterate hosts by McLaughlin and Zahl (1957, 1959, 1962) and Freudenthal (1962) has produced a typical dinoflagellate, Symbiodinium microadriaticum (Freudenthal, 1962), which seems to be the most common form found in a wide variety of coelenterates from many places. A form resembling a primitive dinoflagellate

was found in the Pacific Coast anemone Anthopleura xanthogrammica (McLaughlin and Zahl, 1959). The life cycles of these have been worked out, but only in vitro (McLaughlin and Zahl, 1957, 1959, 1962 and Freudenthal, 1962). They have only speculated as to how the in vitro life cycles are allied to the life cycles of the host animals.

The mechanism of transmission of the algal symbiont from parent to offspring is known in a few animals, but remains unknown for the majority. There are several ways in which transmission may be accomplished. The most common means would be by ingestion as in some protozoans (Buchner, 1953), some sponges (Limberger, 1918) and some turbellarian worms (Keeble, 1910). In some coelenterates there are algal cells contained within the eggs before fertilization as well as in asexual budding processes. This is exemplified by Chlorohydra viridissima with a zoochlorella (Muscatine, 1961). However not all the offspring of C. viridissima contain the symbiont (Droop, 1963). Algal cells within the cytoplasm of protozoans are shared between the two daughter cells at division (McLaughlin and Zahl, 1966).

Specimens of Anthopleura xanthogrammica found living in nearly completely darkened caves are white in color and seemingly devoid of any algal symbiont. These seemed to be suitable for experimentation in establishing or at least reviving the algal flora

within the animal. If these specimens of A. xanthogrammica contained within their tissues any algal cells either from the parent originally or from other sources,they should, upon exposure to sunlight become colored in the same manner as those living in the light. If, in A. xanthogrammica the means of transmission is by ingestion, these white forms should become infected with the symbiont by placing algal cells so that they are ingested with or as food, or taken in with the respiratory currents. A more artifical means would be injecting some of these cells into the tissues. The exposed and therefore colored forms of A. xanthogrammica are either brown or green, with various intermediate combinations of the two (see Figure 5). In their chapter, Endozoic Algae in the book SYMBIOSIS, S. M. Henry ed., McLaughlin and Zahl (1966) caution the reader with regard to this phenomenon.

> However the authors' work (McLaughlin and Zahl, 1959) on the Pacific anemone Anthopleura xanthogrammica indicates that caution must be exercised in the matter of speciation. The symbionts of this anemone are deep green in color, and as such would appear to be zoochlorellae. Yet, isolated and observed in axenic culture, these cells appear to be primitive dinoflagellates, probably of the Desmomonadales.

Spoehr, H., et al. (1943) stated that:

> A small unicellular brown-colored alga was found in the tissues of a common Pacific coast sea anemone, Bunodactis xanthogrammica. The pigments were found to be identical with those of the dinoflagellate Peridinium. Although the alga from Bunodactis lacks some of the morphological characteristics of dinoflagellates, the identity of pigment

> makeup suggests that it is probably a member of some closely related algal group.

The generic name of this anemone has since been changed from Bunodactis to Anthopleura. The "deep green" symbiont of McLaughlin and Zahl (1966) and the "brown colored alga" of Spoehr et al. (1943) seem to indicate some difference of opinion as to the actual color of these cells. McLaughlin and Zahl (1966) base their statements on Spoehr's findings and agree with Spoehr on the probable taxonomic position of the symbiont. If this is indeed one and the same alga, there is some factor causing a difference in the relative amounts of the various pigments. The anemones examined by McLaughlin and Zahl were probably the green form and those Spoehr used were brown. Frazer (1931) stated that a marine hydroid may contain zoochlorellae because of its green color. McLaughlin and Zahl (1966) mentioned in passing that Anthopleura elegantissima contained zoochlorellae as well as zooxanthellae. Dr. McLaughlin (in littera, 1968) wrote as follows concerning the validity of this statement:

> ...on page 258 of the chapter on Endozoic Algae, we mention that the animal contains a green symbiont. This normally would classify the symbiont as a zoochlorella whereas it appears to belong to a class of primitive dinoflagellates, probably the Desmomonadales cf. p. 260.

With these two exceptions there is no mention of any marine coelenterate containing a zoochlorella as a symbiont. Dr. McLaughlin (in littera, 1968) did speculate that Anthopleura could

contain a non-dinoflagellate symbiont, but he has only been able to isolate the dinoflagellate-like organism.

In order to clear up this speculation with regard to the color of the symbiont as related to the actual pigment makeup, a thorough and accurate analysis of the pigments from individuals of each extreme of this color variation might provide some evidence as to why the brown pigments in some individuals seem to mask the green chlorophylls, or vice versa. If it were a factor caused by environmental differences, then a group of individuals of the two color types kept in a common tank for a reasonable length of time should gradually acquire approximately the same color. Fogg (1965) had shown that cultures of Botryococcus braunii accumulated carotenoid pigments which masked the chlorophylls when the nitrate in the medium was exhausted.

Two-dimensional paper and thin-layer chromatography with spectrophotometric analysis was carried out on the algal pigments from the variously colored anemones. Experiments were conducted to determine the susceptability of adult anemones to infection by the algae.

METHODS AND MATERIALS

Collection Data

Anemones were collected from three localities along the Oregon coast: Yaquina Head near Newport, the south jetty of Yaquina Bay and Squaw Island near Coos Bay. The most effective collecting tool proved to be a short-bladed dull knife which, if inserted under the edge of the pedal disc and quickly moved around the entire edge while the fingers of the free hand were slipped under at the same time, removed the animal very easily with little if any tearing of the pedal disc. However if the anemones were subject to wave shock, if removal took too long, or if they were disturbed in any other way it was difficult to obtain an intact specimen.

Transportation of the specimens was carried out in two ways depending upon the length of time required from the collecting area to the holding tank. When it was a matter of less than an hour the anemones were carried in a bucket partially filled with sea water. But when the time in transit exceeded an hour they were packed in wet seaweed or burlap. If the water in the holding tank was not circulating, it was necessary to change it several times after the anemones were placed in it, because they secreted copious amounts of mucus for several hours, or until they became attached. The holding tanks were kept constantly illuminated with two 40 watt

"Gro-Lux" fluorescent tubes set 60 cm above the water surface to insure retention of the algal symbiont within the anemones. Anthopleura will become pale in color if they are not lighted sufficiently.

Fixation and Staining

All specimens were fixed by immersion of the entire animal into standard A.F.A. (alcohol-formalin-acetic acid) solution and by injecting quantities into the gastro-vascular cavity at the same time. They were left in the fixative for a period of not less than 14 days before any further preparation was carried out. Tissue samples for sectioning were taken from the oral disc including the tentacles, the midpoint of the column and near the basal disc. These wedge-shaped samples were cut on three planes: radially, longitudinally and tangentially. Imbedding was in paraffin. They were sectioned at 10μ stained with Bismarck brown and fast green and mounted in picolyte. Tissue samples were taken from anemones of the following color phases:

1. Green.
2. Brown.
3. Naturally-occurring white.
4. Artificially whitened forms.
5. Green which had been held in the dark then exposed to

sunlight.

6. Brown which had been held in the dark then exposed to sunlight.
7. Naturally white which had been exposed to sunlight alone.
8. Naturally white exposed to sunlight in sea water inoculated with cells removed from green forms.
9. Naturally white exposed to sunlight in sea water inoculated with cells removed from brown forms.
10. Naturally white injected with mixed green and brown cells then exposed to sunlight.

Attempted Algal Infection of Adult Anemones

Naturally occurring white forms collected from a cave in Squaw Island (Coos Bay) were first thoroughly washed in filtered sea water and then placed into individual one-gallon aquaria. Two were placed into filtered sea water only, two others into filtered seawater which had been inoculated with algal cells extracted from green anemones and two into filtered sea water innoculated with algal cells from brown forms. The covered tanks were kept at $50^{o}C \pm 5^{o}$. and situated so that they received full daily sunlight. The water was changed every three days for three weeks with fresh inoculant being added each time. After this, the water was changed every week and no more inoculant was added. After a total of 75 days, the

anemones were removed and fixed in A.F.A. Two of the naturally white anemones were fed daily for three weeks with bits of squid which had been injected with algal cells of both colors. Two received injections of mixed algal cells into the tissue of the column every three days for 12 days. These were kept in the full daily sunlight for 75 days then fixed in A.F.A. Six anemones, three each of the green and brown color were placed in blackened two-gallon aquaria and kept at room temperature for 60 days, the water being changed every week. At the end of this time two of the green and two of the brown forms were placed in individual one-gallon aquaria, covered with Plexiglas and kept in full daily sunlight for 45 days with weekly water change, then removed and fixed in A.F.A. The remaining two anemones were placed directly into A.F.A. One group of 12 each of the green and brown forms were kept in a common tank at the same level of illumination for one year.

Extraction Procedures

In order to separate the algal cells from the animal tissue, the anemone was first washed in sea water and all particles of rock and vegetation were picked off the outer surface. Then the anemone was split the length of the column and the gastro-vascular cavity was washed out with a stiff stream of fresh water. A medium-sized (2"-3" in diameter) anemone plus 100 ml filtered or artificial

sea water were placed in a Waring Blendor and blended at medium speed for six to eight minutes in a chilled blender jar. After allowing the foam to dissipate, the fluid was poured through glass wool. Because quite a bit of the algal material remained trapped in the glass wool, it was shaken in a container with 500 ml filtered or artificial sea water and this fluid again passed through glass wool. The filtrate now containing mostly algal cells and fine animal debris was centrifuged at medium speed, the supernatant poured off and discarded and the remaining algal cells washed and centrifuged 10 to 12 times or until they appeared to be free of animal tissue.

Pigment Extraction and Analysis

Initially two-dimensional paper chromatography (Jeffrey, 1961) was used to analyze the pigments extracted from the brown and green forms. Subsequently thin layer chromatography and spectrophotometric analysis were employed. Approximately 0.5 - 1.0 ml wet pack of algal cells was placed in a small amount of distilled water for 15 minutes, centrifuged down and the supernatant discarded. The cells were placed in 50 ml 90% acetone, blended at high speed in an ice-packed small volume Waring Blendor for 15 minutes and then put into the freezer for 12 hours. The extract was filtered through a coarse sintered glass filter into a separating funnel, mixed with an equal volume of diethyl ether and washed with no less

than five volumes of 10% NaCl. The ether layer was collected in a petri dish and evaporated to complete dryness by blowing a stream of clean cool air over the solution. To prevent any contamination with water the pigments were again dissolved in a small amount of diethyl ether to which a single drop of acetone had been added, transferred to another clean petri dish and again evaporated to dryness. If the pigments were not used immediately, the dish containing them was tightly wrapped in parafilm and then in several layers of heavy paper and stored in the freezer. If they were used immediately, the pigment residue was again dissolved in a small amount of diethyl ether, allowed to become concentrated through evaporation and spotted onto the paper. Whatman 3MM chromatography paper was used with the point of origin at the lower left corner of the nine inch square sheets. The paper was formed into cylinders, clipped with a paper clip and placed into a jar previously equilibrated with the solvent mixture. Four percent n-propanol in petroleum ether (USP) was used for the first dimension and 30% chloroform in petroleum ether (USP) was used for the second dimension. The chromatograms were run for about 45 minutes in each solvent and allowed to dry for several minutes after each run. The entire process from the beginning of extraction to the final drying of the chromatograms was done in the dark to prevent any pigment breakdown. Identification of the pigments was done by comparing the color and placement of the

pigment spots against known standards (Jeffrey, 1961) and by their absorption spectra under ultra-violet light.

Thin Layer Chromatography

The Kensco TLC apparatus was used. The slurry was prepared by mixing Silica Gel G (Merck) with distilled water until a mixture the consistency of thick cream was reached. Kensco K-4231 Mylar film or glass plates eight by two inches were spread with a 250μ thick layer of the slurry. The coated strips were dried at room temperature then placed in a desicating cabinet which served the dual purpose of activating them as well as providing dust free storage. Algal pigments were prepared in the same way as given for the two-dimensional paper method. These were spotted about one-half inch from the bottom in the center of the activated plates which were then placed in a previously equilibrated jar which contained a mixture of pet. ether (rgt) 58%, ethyl acetate (rgt) 30%, and diethylamine (rgt) 12 % (Riley and Wilson, 1965), at a depth of about one-fourth inch. The chromatograms were developed for about 45 minutes in the dark at room temperature, then removed and allowed to dry. The pigment spots were scraped off and each dissolved in two ml 95% ethanol and centrifuged at moderate high speed for five minutes. The now clear colored supernatant was pipetted off and placed in small vials in a light-proof box. Analysis was

carried out using a Beckman DB-G Grating Spectrophotometer with a Beckman ten-inch recorder providing the graphic records.

RESULTS

Fresh smears and prepared sections of naturally white anemones which had been subjected to 75 days of exposure to sunlight showed no trace of algal cells in either the experimental or the control animals. Artificially whitened green and brown specimens after 60 days in the dark still showed a few scattered shrunken algal cells in the gastrodermis. After exposure to sunlight, those forms which had been initially brown became brown again within 21 days and the green recovered their color within 30 days. Smears and sections from both of these recovered forms showed a complement of algal cells equal in size and number to those in the naturally occurring colored anemones. Upon injection of algal cells, a greenish spot appeared at the site of the injection, but this disappeared within one day and some of the cells were recovered in mucus extruded from the mouth. The group of 12 each of the brown and green forms, after one year in a common tank at the same level of illumination showed no apparent difference from their original coloration.

Smears of algal cells extracted from brown anemones showed a large number of individual, walled cells some spherical and some oblong about .010 - .015 mm in diameter (see Figures 6, 7 and 8). There were many dividing cells in two and occasionally four-cell groups enclosed in what appeared to be a cyst-like wall. Mixed with

these were occasional green cells which appeared to be identical to those in the green anemones. Smears made of algal cells extracted from green anemones showed mostly single or dividing cells in twos rarely in fours within a thin cyst-like wall. The individual cells about .006 - .008 mm in diameter had a single grass-green cup-shaped chloroplast enclosed within a cellulose wall. Mixed in with these were occasional larger golden-brown cells with a much vacuolated diffuse chloroplast enclosed in a relatively thicker cell wall (see Figures 6, 7 and 8). These cells appeared to be identical to those extracted from the brown anemones. The actual volume of wet pack extracted cells was greater from brown anemones of the same size than from the green.

Paper Chromatography of Cell Extract from Green Anemones

There follows a description of the spots resulting from paper chromatography as compared with Jeffrey (1961) (see Figures 1 and 2).

1. A yellow spot which moved all the way with both solvent fronts and fluoresced white under ultra-violet light indicated the presence of β-carotene. This was in the same position on the chromatogram as was the given β-carotene spot.
2. A yellow spot which was in the same relative position as

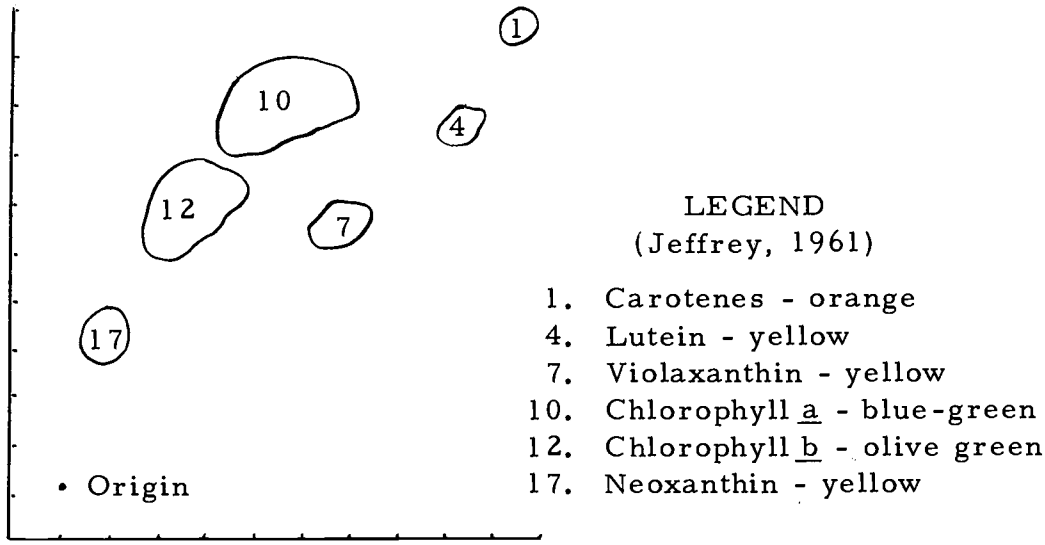

Figure 1. Chromatogram of Dunaliella tertiolecta (Jeffrey, 1961). A green flagellate.

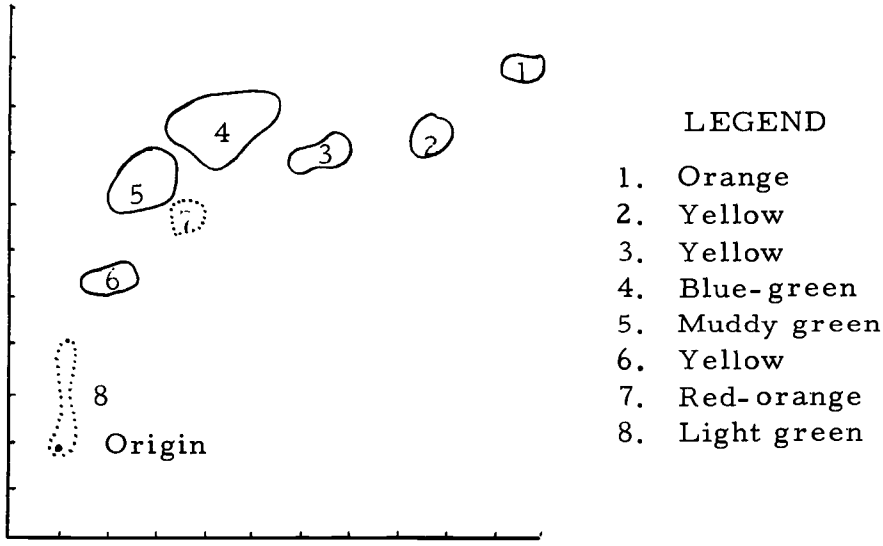

Figure 2. Chromatogram of cells extracted from green Anthopleura xanthogrammica.

the given lutein spot.

3. A yellow spot in the same relative position as the given violaxanthin spot.
4. A blue-green spot which fluoresced orange under ultraviolet light and corresponded in position to that of chlorophyll *a*.
5. A muddy green spot which fluoresced orange under ultraviolet light and was in the same relative position as that of chlorophyll *b*. Jeffrey (1961) described the color as "olive green".
6. A yellow spot just above the origin in the first dimension, but largely unmoved by the second solvent and in the same relative position as that of neoxanthin.
7. A very faint light green spot which remained at the origin in the same position as chlorophyll *c*.
8. A reddish-orange spot corresponding to peridinin. This was only a trace.

Paper Chromatography of Cell Extract from Brown Anemone

There follows a description of the spots resulting from the paper chromatography as compared with Jeffrey (1961) (see Figures 3 and 4).

1. A yellow spot which moved all the way with both solvent

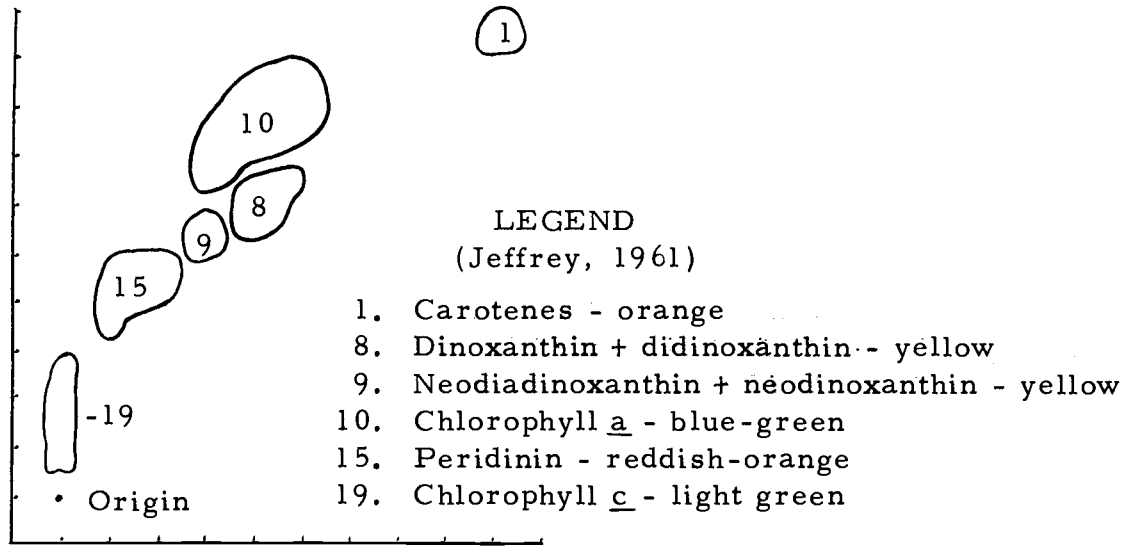

Figure 3. Chromatogram of Gymnodinium sp. (Jeffrey, 1961). A typical dinoflagellate.

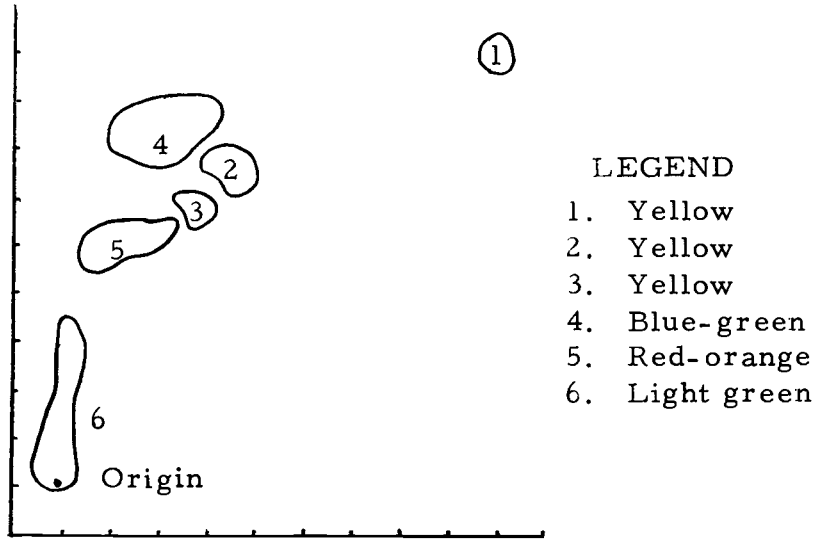

Figure 4. Chromatogram of brown cells from Anthopleura xanthogrammica.

fronts and fluoresced white under ultra-violet light indicated the presence of β-carotene. This was in the same position on the chromatogram as was the given β-carotene spot.

2. A yellow spot which corresponded in position to that of dinoxanthin + didinoxanthin.
3. A yellow spot which was in the same relative position as neodiadinoxanthin + neodinoxanthin.
4. A blue-green spot which fluoresced red-orange under ultra-violet light and corresponded in position to that of chlorophyll a.
5. An intense reddish-orange spot in the same relative position as that of peridinin.
6. A light green spot which remained at the origin and fluoresced red-orange under ultra-violet light indicated chlorophyll c.

Thin Layer Chromatography and Spectrophotometric Analysis of Algal Pigments

Using a Beckman DB-G Spectrophotometer with a Beckman 10 inch recorder. Readings are absorption peaks.

Pigments from Green Cell Extract

1. A yellow spot above the origin had two peaks at 468 nm and

438 nm. Identical with that of neoxanthin.

2. A yellow spot which had peak absorption at 472 nm and 442 nm. Identical with that of violaxanthin.
3. A yellow spot with peak absorption at 476 nm and 447 nm. Identical with that of lutein.
4. A green spot with peak absorption at 650 nm and 476 nm. Almost identical with that of chlorophyll b which has two peaks of 650 nm and 475 nm.
5. A green spot with peak absorption at 665 nm and 433 nm. Almost identical with that of chlorophyll a which has peak absorption at 665 nm and 432.5 nm.
6. An orange-yellow spot which had two broad peaks at 480 nm and 450 nm. Similar to that of the carotenes which range from 476 nm and 446 nm for α-carotene and 482 nm and 452 nm for β-carotene. This particular chromatography method does not separate the carotenes so this probably is a mixture of the two.

This analysis, according to Strain (1951), is characteristic of green algae (Division Chlorophyta).

Pigments from Brown Cell Extract

1. A green spot which remained at the origin with peak absorbtion at 632 nm, 584 nm and 446 nm. This was similar to

that for chlorophyll c with peak absorption at 624 nm, 585 nm, and 450 nm.

2. A reddish-orange spot with two broad peaks at 476 nm and 454 nm. Similar to that of peridinin given as about 475 nm.
3. A yellow spot with peaks at 478 nm and 448 nm. Identical with that of diadinoxanthin.
4. A green spot with peaks at 665 nm and 433 nm. Almost identical to that of chlorophyll a given as 665 nm and 432.5 nm.
5. A yellow-orange spot with two broad peaks at 478 nm and 448 nm. Similar to that given for carotenes--mixed ϵ - and β-carotene 440 nm, 470 nm, and 452 nm, 482 nm respectively.

This analysis, according to Strain (1951), is characteristic of dinoflagellates.

DISCUSSION

There has been little work done on the transmission of the algal symbiont from parent to offspring or from individual to individual among the colenterates. Muscatine (1961) working with Chlorohydra viridissima has done the majority of this work. The extensive work of McLaughlin and Zahl (1959) and Freudenthal (1962) on culturing of symbiotic algae leads to much speculation with regard to the in vivo life cycle of these algae: They found that the zooxanthella from the Pacific Coast anemone Anthopleura xanthogrammica did produce a motile flagellated form which resembled members of the Desmomonadales, a primitive dinoflagellate. This motile form could very well serve as a means to transmit the infection. The fact that there is this motile form would make the reader more inclined to believe that the method of infection is not as it is in C. viridissima where the symbiont cells are included within the egg, but rather by some other means involving this motile form. It has been suggested (McLaughlin and Zahl, 1966) that a lowering of calcium in the environment stimulates the formation of these motile forms, such as would be the case if the host were to die or eject its symbiont into the sea water.

The actual mechanism of transmission in A. xanthogrammica is still unknown. However, my experiments suggest that if the anemone did get a complement of the algal cells from the parent

that these were somehow lost or ejected completely when the animal took up its cave-dwelling existence. The fact that there was no proliferation of algal cells within these whitened forms upon exposure to sunlight is fairly good evidence that they were not there to begin with. The artificially whitened ones kept temporarily in absolute darkness regained their original algal complement within a short time after restoration to the light. This indicates that the anemone is able, at least for a short period of time, to retain some cells, and that these cells are able to begin growing again when exposed to sunlight. That the naturally white forms of A. xanthogrammica did not pick up any algal cells within their tissues after having been exposed to them in their surrounding water, ingesting them in their food and having some actually injected into their tissues offers evidence that the adult forms of this animal are not susceptible to infection by this stage of the algae. This would indicate that perhaps the initial infection takes place early in the life history of the anemone or in another stage of the algae. This agrees with Droop (1963) who stated:

> ...Coelenterates, on the other hand are generally held not to ingest algal cells, so that infection would be uncertain, even if their symbionts were numerous in the phytoplankton.

According to McLaughlin and Zahl (1966), and McLaughlin (in littera, 1968), the color of the Pacific Coast anemone, Anthopleura xanthogrammica has little or no bearing on the taxonomy of the algal symbiont contained within this animal. Spoehr (1943) analyzed the

pigments of an algal symbiont from this anemone and found them to be identical to those of a common dinoflagellate Peridinium. McLaughlin and Zahl (1959), isolated in axenic culture a motile form of this symbiont and tentatively assigned it to the Desmomonodales, an order of primitive dinoflagellates. They caution the observer to be aware of the green color of A. xanthogrammica as belieing the true nature of the included symbiont. Spoehr (1943) mentioned brown cells from this anemone, as those having the typical dinoflagellate pigment characteristics. My work has dealt with both brown and green A. xanthogrammica and indeed I did find brown cells which when analyzed with the two-dimensional paper method gave a chromatogram identical to that of a typical dinoflagellate Gymnodinium (Jeffrey, 1961). Further analyzed with thin-layer chromatography and a Beckman DB-G Spectrophotometer gave a makeup of pigments which was that of dinoflagellates. However, cells extracted from A. xanthogrammica of a green color produced a paper chromatogram totally unlike that of the brown, but one which matched very well that of a free-living green flagellate Dunaliella tertiolecta, but with two very faint spots which corresponded to chlorophyll c and peridinin, the two principal pigments found in dinoflagellates. Thin-layer chromatography and subsequent spectrophotometric analysis showed that extract of algal cells from A. xanthogrammica of the green color contained a complete

complement of pigments which are unique to green (Division Chlorophyta) algae. In addition to these there were traces of some of the dinoflagellate pigments.

Microscopic examination of these algal cells showed that the brown anemone contained almost 100% of large golden-brown cells with a few scattered green cells. The green anemone had mostly the smaller green cells, but a number of the brown cells. If the brown cells were responsible for the dinoflagellate pigment, traces of these pigments in the analysis of extract from the green anemone would result. The plastid structure of the brown cells was found to be of the typical dinoflagellate type (Hovasse, 1937) and that of the green cells to be of a typical zoochlorella type (Oltmanns, 1922). The intermediate colorations would be varying mixtures of these two different algal types.

McLaughlin and Zahl (1966) caution the observer not to be swayed by the green color of the anemone because: (1) they have only been able to culture the dinoflagellate symbiont out of extracts from the anemone, (2) Spoehr's analysis of these pigments must have been from anemones which contained almost 100% of the brown cells. Apparently in culturing the algal symbiont from A. xanthogrammica (McLaughlin and Zahl, 1959), the extract taken from the animal contained both kinds of algal cells. However in every instance the

dinoflagellate was able to take over the culture. Or perhaps the culture media were not suited for the green form and it died out.

SUMMARY AND CONCLUSIONS

1. Anthopleura xanthogrammica collected in dark caves and in sunlight from three localities along the Oregon Coast were examined for cells producing the bright green and the muddy brown colorations.
2. The endozoic cells were removed and separated from the anemone tissue.
3. Some of the cells were introduced into white cave-dwelling A. xanthogrammica either by inoculation into the tissues or by ingestion in the food or in the respiratory currents.
4. Another part of the separated cells were extracted and the pigments analyzed by two-dimensional paper chromatography, thin layer chromatography and spectrophotometric analysis.
5. A. xanthogrammica found living naturally under nearly completely darkened conditions in caves did not contain any trace of the algal symbionts found in those forms exposed to sunlight.
6. Adult forms of A. xanthogrammica which do not contain any trace of the algal symbionts cannot become infected through ingestion or injection of cells removed from forms

naturally infected.

7. A. xanthogrammica contains within its tissues two different kinds of algal symbionts: an incompletely described dinoflagellate, and a green form which appears to be a zoochlorella. Differing ratios of these two symbionts produce variations in the color from bright emerald green to a muddy brown (see Figure 5).

8. Analysis by two-dimensional paper, and thin layer chromatography and spectrophotometry indicate that the green symbiont contained the following pigments: neoxanthin, violaxanthin, lutein, chlorophyll b, chlorophyll a, and mixed carotenes. The brown symbiont contained in contrast the following pigments: chlorophyll c, peridinin, diadinoxanthin, chlorophyll a and mixed carotenes.

9. Judging from the combination of pigments, the green anemone harbored an algae belonging to the Division Chlorophyta, and which appeared to be a zoochlorella. The brown anemone had as a symbiont a dinoflagellate, or at least a member of some closely related group.

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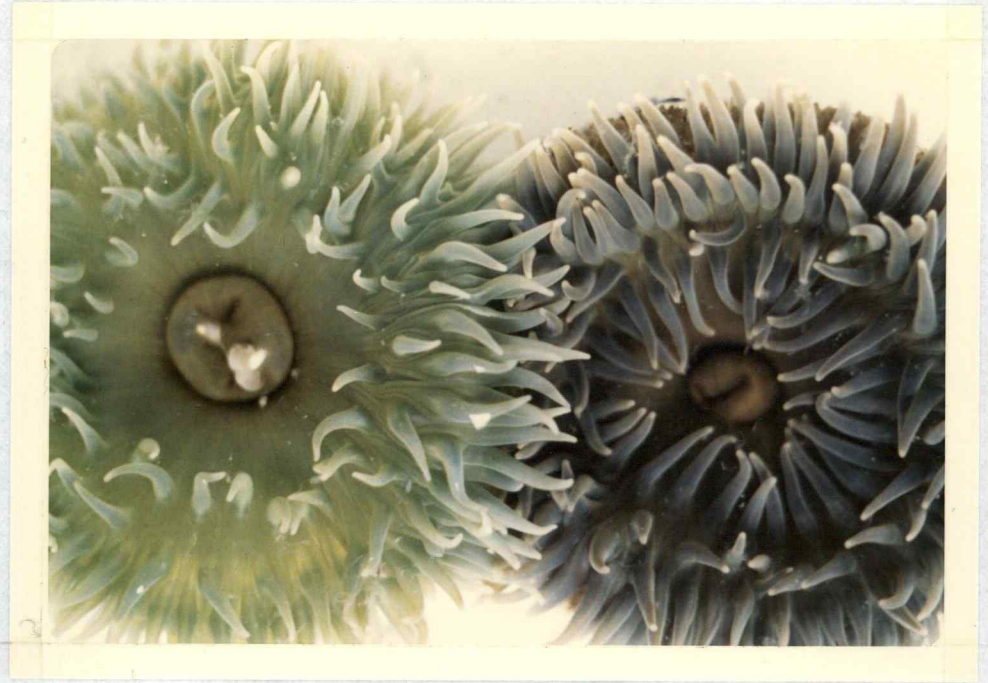
Figure 5. Two specimens of Anthopleura xanthogrammica showing the two extremes in the green-brown color variation.

Figure 6. Symbiotic algal cells from A. xanthogrammica. The larger golden-brown cells are those which have dinoflagellate characteristics and impart the brown color to the anemone. The smaller green cells are responsible for the green color in the anemone and have characteristics of green algae. (100X).



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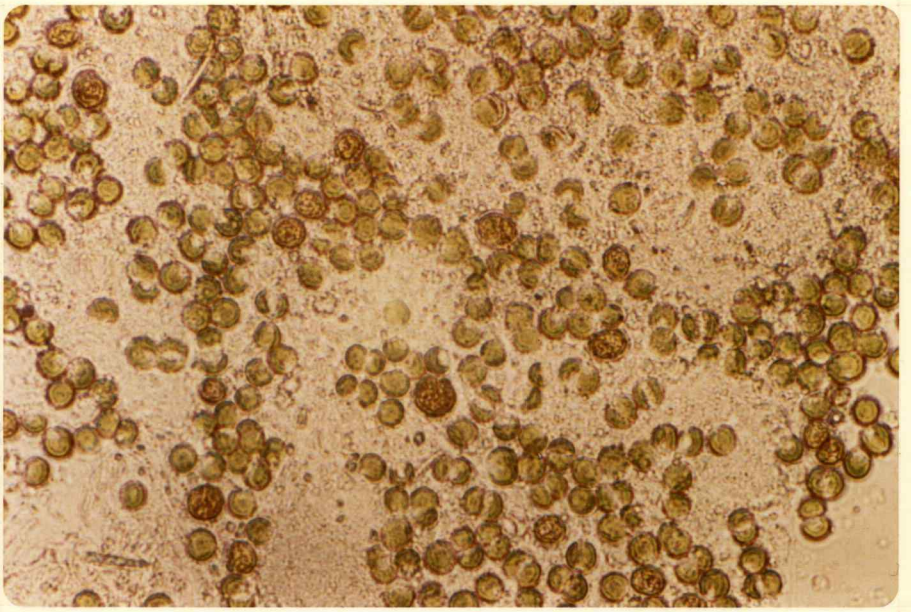

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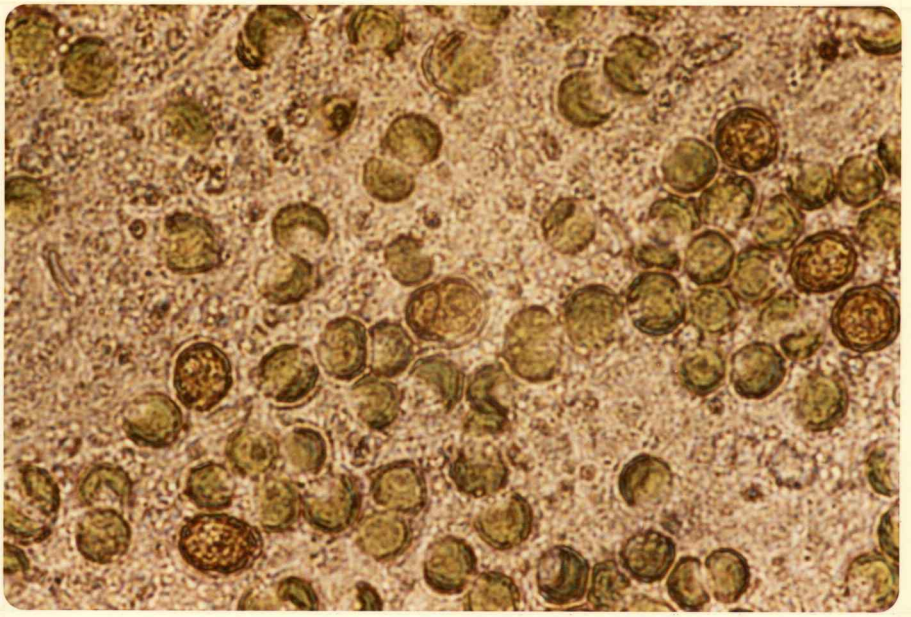
Figure 7. Same as Figure 6 (210X).

Figure 8. Same as Figure 6 (470X).



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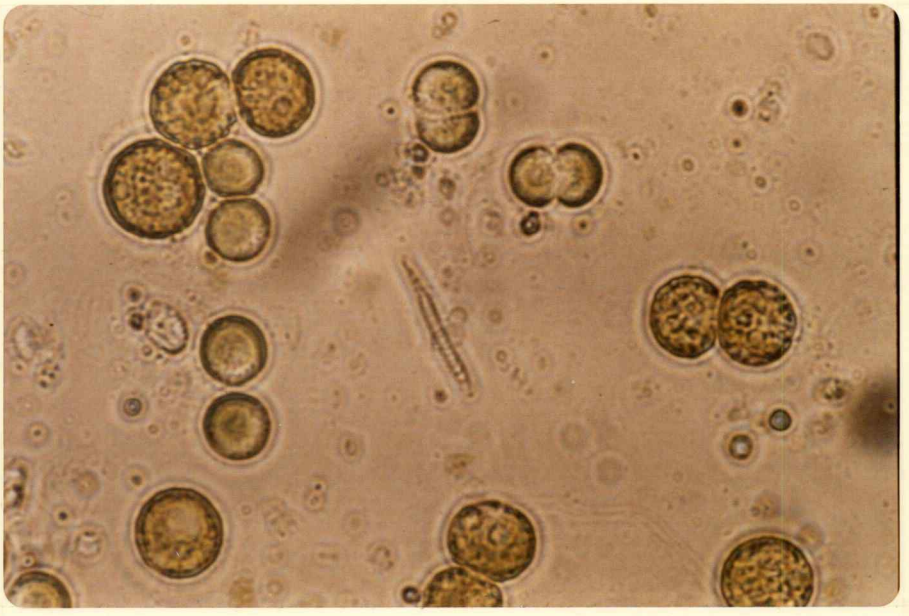

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