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Prostaglandins from *Plexaura homomalla*: Ecology, Utilization and Conservation of a Major Medical Marine Resource

A Symposium

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Foreword

The discovery of prostaglandins in the gorgonian coral *Plexaura homomalla* is one of the more exciting events of recent years. These substances are of wide medical interest, and the demand for them for research and clinical applications has already outstripped the usual sources of supply. Their occurrence in a common, readily accessible marine animal has, very naturally, attracted the attention of pharmaceutical manufacturers, the foremost of which is The Upjohn Company—a pioneer in research on prostaglandins. When the feasibility of using *Plexaura homomalla* as a source of prostaglandins was established, The Upjohn Company embarked upon a comprehensive investigation of the biology of this animal and of the ways that it might be exploited without damage to the reef environment. Then, having reached some conclusions and estimated short-term needs for *Plexaura homomalla* to meet research demands until practical techniques of synthesis could be developed, The Upjohn Company felt the need to bring their findings to the attention of the scientific community most directly concerned, and to obtain from the scientists themselves the soundest possible advice for the wise utilization of the natural stocks of the coral in certain Caribbean localities. Toward this end, the Company sponsored a symposium entitled *Prostaglandins from* Plexaura homomalla: *Ecology, Utilization and Conservation of a Major Medical Marine Resource* at the Rosenstiel School of Marine and Atmospheric Sciences on May 22 and 23, 1972, bringing together marine scientists from three continents and the islands of the Caribbean. The papers delivered at that symposium, and the results achieved, are published in this volume of *Studies in Tropical Oceanography*.

This volume is presented to provide some background about *Plexaura homomalla* for those whose primary interests lie in the fields of biochemistry, pharmacology and medicine, some background about prostaglandins for the zoologists and ecologists whose primary interests are in the reef environment and its inhabitants, and to disseminate the information so far gained about the biology, ecology, culture, utilization and conservation of *Plexaura homomalla*. It clearly demonstrates how little is known about this common and wide-spread coral that inhabits virtually every reef from Bermuda to Curaçao, how difficult it is to predict the effect of human activity on the marine environment and to advise the best ways to keeping such effects within acceptable limits, and how much there remains to be learned with simple techniques and modest resources provided the incentive is present.

Studies in Tropical Oceanography is a series established to accommodate large papers, or groups of papers on special subjects in the marine sciences. Its format is similar in most respects to that of the *Bulletin of Marine Science,* but it does not appear on a regular schedule. The present volume departs stylistically from preceding issues, especially in regard to bibliographic references, because several of the authors involved are chemists and have employed the style traditional for their discipline, and because their readers will find that style more familiar.

The Editors

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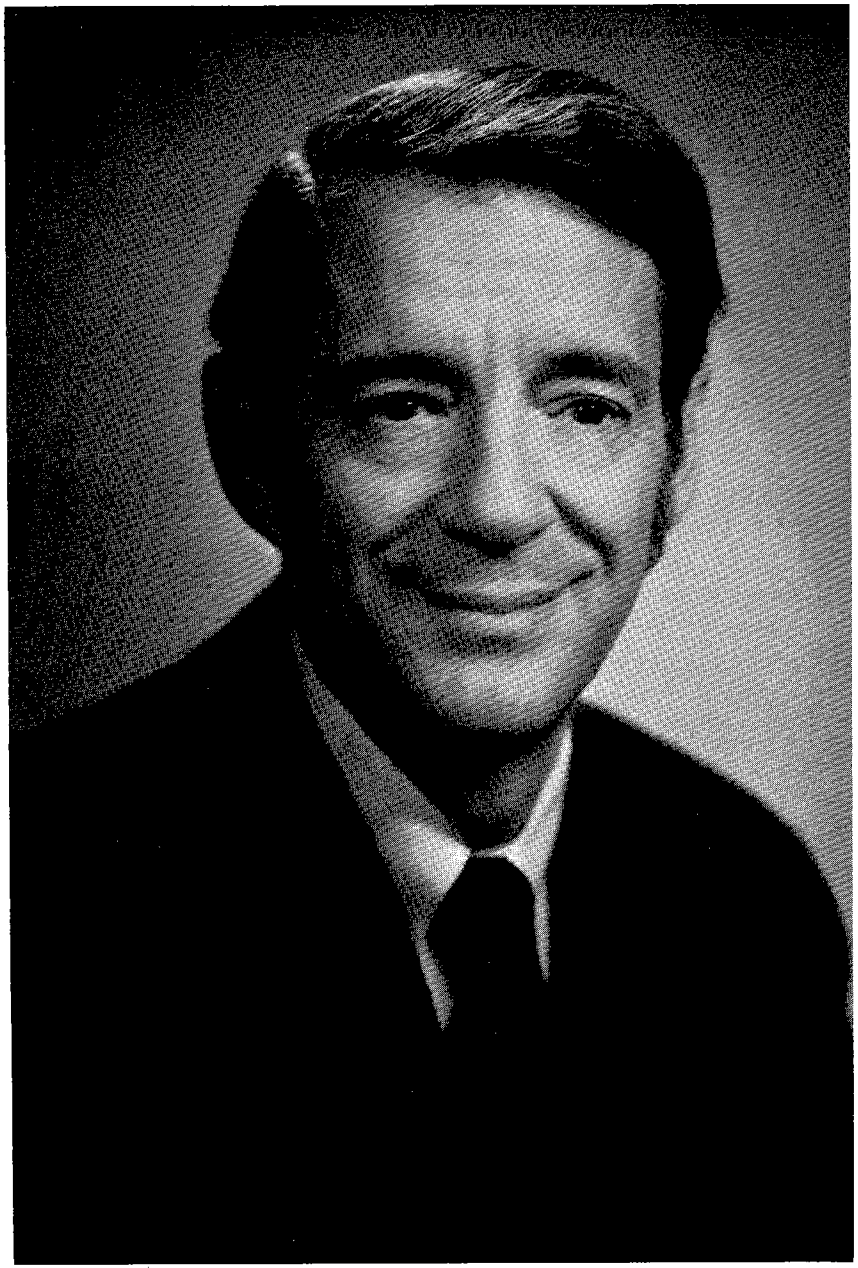

LIONEL EDWARD RHULAND
Mahone Bay, Nova Scotia, September 26, 1920
Kalamazoo, Michigan, October 7, 1972

Lionel E. Rhuland

The proceedings of this symposium are dedicated to the memory of one of its participants, Dr. Lionel E. Rhuland. Dr. Rhuland, at the time of his sudden death on October 7, 1972, was Manager of Experimental Biology Research at The Upjohn Company in Kalamazoo, Michigan. He was 52 years of age.

Born in Nova Scotia and reared in New Hampshire, Lionel served with the Army Combat Engineers in the European theater during World War II. He married a New Hampshirite, Gloria Blackburn, in 1943. After the war he completed his B.S. degree at the University of New Hampshire (1947) and subsequently obtained his M.S. and Ph.D. degrees in bacteriology at Indiana University (1947-1951). Lionel and Gloria, with their two sons, William and Jeffrey, moved to Kalamazoo in 1951, where a third son, Scott, was born in 1953.

As a Research Associate at The Upjohn Company, Lionel was involved with antibiotic developments. He also made important contributions toward understanding the metabolism of the bacterial cell wall and was considered one of the leading authorities in this field (e.g., Nature *185:* 224-228, 1960). In 1954 he became a Section Head and in 1961 Manager of the Department of Infectious Diseases Research. In a managerial capacity he directed basic and product-oriented research in the sciences of bacteriology and virology. Dr. Rhuland's advancement at The Upjohn Company was based upon significant contributions by him and by the scientists under his leadership. To cite one example, he provided major impetus for development of cytosine arabinoside as an antiviral agent. The importance of this compound is yet to be fully assessed, as it finds increasing use in treatment of certain systemic virus infections and leukemias.

In early 1970 Dr. Rhuland became manager of a newly organized unit doing prostaglandin research. As co-discoverer of the occurrence of natural prostaglandins in the S-variant of *P. homomalla,* one of his first concerns was with the preservation of these corals. In a company memorandum of February 8, 1971, he wrote, "I am personally convinced that The Upjohn Company has a moral obligation to insure that sound ecological practices are followed in our endeavor to produce prostaglandin from Gorgonians." It was in this spirit that he sought out the advice and assistance of specialists in marine science, Prof. F. M. Bayer and his colleagues, with whom he subsequently collaborated in basic studies of *Plexaura homomalla.*

Brief description of a man's accomplishments does not always say enough about the character of the man. Dr. Rhuland was deeply dedicated to the humanitarian goals he felt could be accomplished through science. As a scientist, and in his capacity as a critic of other scientists, he was keenly analytical and astute. In his judgment of people he was extremely fair. On the ball field, the golf course, and at his job he was fiercely competitive. He stood by his friends in their times of need, and he maintained, during his own tribulations, a style of humor that was uniquely his own. In short, he was a man who is dearly missed.

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PLEXAURA HOMOMALLA: BRIEF HISTORICAL BACKGROUND*

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The first chapter of this story begins in 1792, when Eugenius Johann Christoph Esper published the first description and figure of *Gorgonia homomalla* (Fig. 1). This appeared in the first comprehensive work ever written about corals and coral-like animals—*Die Pflanzenthiere in Abbildungen nach der Natur mit Farben erleuchtet, nebst Beschreibungen*—published in Nuremburg [2]. Even though Esper was not sure where his specimen had been collected, and thought it problably was from the Mediterranean, the hand-colored picture that he showed on Gorgonia Plate 29 is so well done and so characteristic of *Plexaura homomalla* that it could hardly be anything else (Fig. 2). Moreover, Esper's collection was safely preserved in the zoological museum at Erlangen, and Professor A.E. Verrill of Yale many years ago examined a preparation of spicules from the type specimen, sent to him by Professor Kölliker of Würzburg. I have seen that slide, in the Museum of Comparative Zoology, and though it is badly discolored with age, there is no doubt of the identity of the spicules with those of the gorgonian we now call *Plexaura homomalla.*

When Linnaeus first established the genus *Gorgonia* in 1758, it included all the flexible corals with a proteinaceous, horn-like central axis [8]. Pallas in 1766 recognized the distinctness of several kinds that have a rather thorny surface not covered by an outer bark-like layer of spicules, which he named as a separate genus, *Antipathes*—the true "black corals" or "thorny corals" [10]. All the rest remained in *Gorgonia* until Lamouroux began subdividing them into special genera based on gross external differences [5, 6, 7], *Plexaura* in 1812, *Eunicea* in 1816, and *Muricea* in 1821. In that era, characters such as colonial form, branching, surface texture and prominence of calices were the only bases for subdivision. Even though the optical instruments of that period certainly were adequate to see structures as large as gorgonian spicules, no one thought to use them as systematic characters until Valenciennes in 1855 published an abstract of a monograph based upon spicules, which unfortunately he never published in full [12].

Following the lead established by Valenciennes, Professor Kölliker studied many species of gorgonians and published three plates of hand-colored engravings of the various kinds of spicules that he found [3]. Those

*Contribution No. 1712 from the Rosenstiel School of Marine and Atmospheric Sciences, University of Miami.

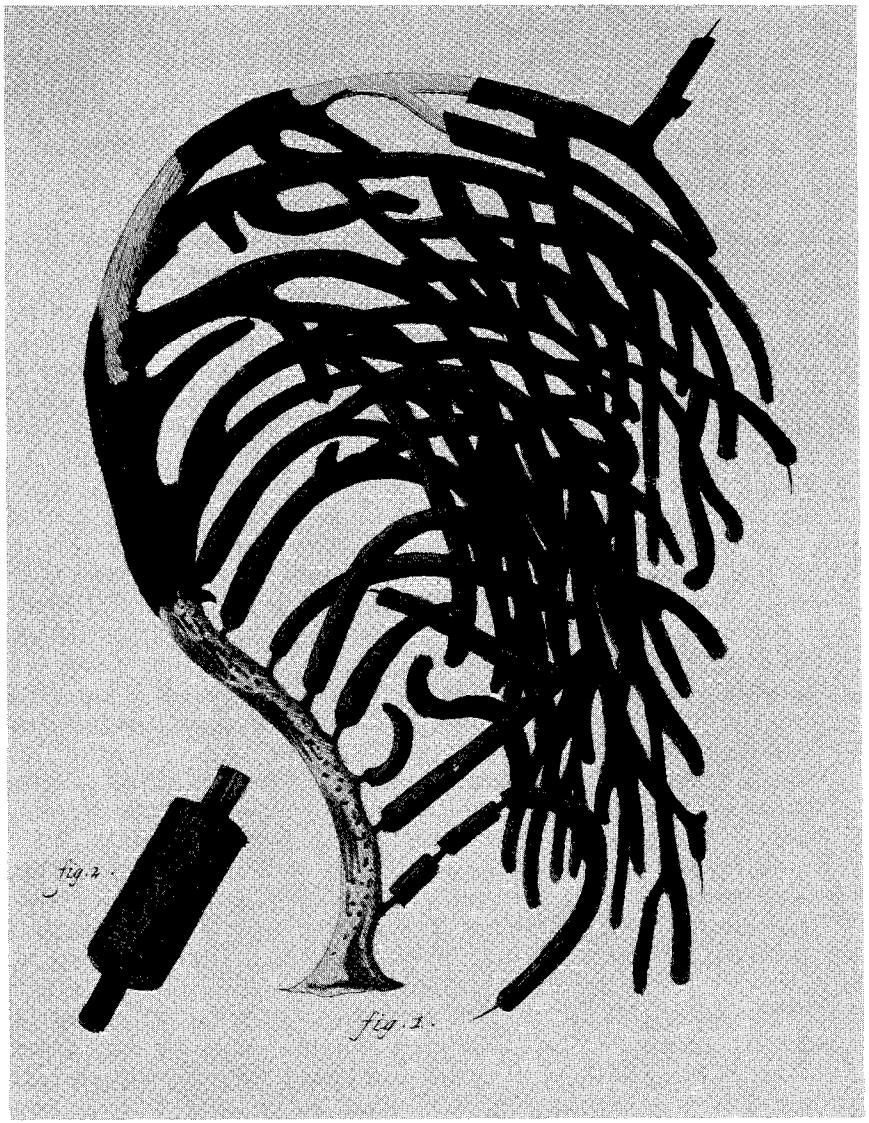


FIGURE 1. The original figure of *Plexaura homomalla* (Esper).

pictures in the classic *Icones Histiologicae* of 1865 are still equal in quality to any that have been published since. We can consider this the beginning of the second chapter of our story, and the beginning of our troubles.

Professor Verrill of Yale began publishing on gorgonians at about that time, and promptly adopted Kölliker's system based upon spicules. From Kölliker he obtained spicules of many old species that enabled him to

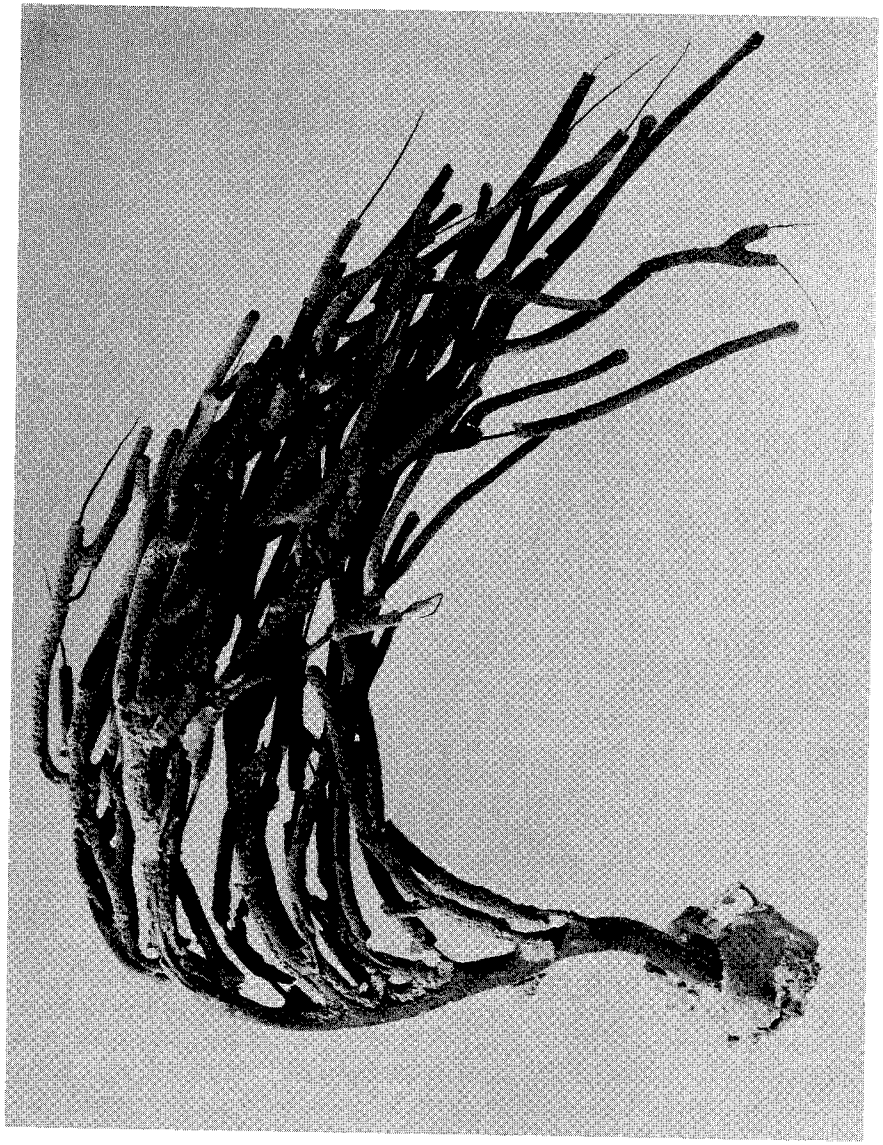


FIGURE 2. Dried specimen of *Plexaura homomalla* (Esper) from Florida.

identify some of the common forms in our American fauna. Naturally, he found many that did not agree with any of the old species, and during some 45 years he described a host of new ones.

About the turn of the century, Professor Willy Kükenthal in Breslau began working on octocorals, and stimulated the production of several theses on their taxonomy by his students. Kükenthal was a prolific worker

who published numerous papers on this subject, based upon collections from many parts of the world. However, his students for the most part dropped the study of octocorals after one or a few papers, although a couple of them are important from our special viewpoint today. Kunze in 1916 published an important paper on *Eunicea* from West Indian waters [4], and Moser published an outline revision of *Plexaura* including some new species in 1921 [9].

The most recent revisionary work dealing with plexaurids is a paper by Gustav Stiasny, published in the reports of the Dutch Siboga Expedition in 1935. Although based largely on East Indian material, that paper includes a number of West Indian plexuarids, among which is the new species *Plexauropsis tricolor* [11]. The original specimens came from Bermuda, and when I examined the type in the Leiden Museum, it proved to be nothing more than *Plexaura homomalla.* Even the name *tricolor* is a clue—it referred to the purple inner spicules, the white outer ones, and those that appeared yellowish brown because they were still coated with organic material, just as we find in preparations of *homomalla.* The genus *Plexauropsis,* to which Stiasny assigned his new species, had been established by Verrill in 1907 for a species he called *Plexauropsis bicolor* [13], which in turn, is nothing but the common *Pseudoplexaura crassa*—or *Pseudoplexaura porosa* as we now call it.

In my paper of 1961 on West Indian octocorals, I attempted to sort out the many nominal species that had been established in the family Plexauridae, and to assign them to genera that are clearly characterized [1]. Numerous obstacles stood in the way of that goal and the results were not wholly successful. It is impossible to be certain about the identity of many of the old species because the descriptions were short and lacked illustrations, and most of the original material is now destroyed or untraceable. Even the species described more recently, established within the framework of much sounder knowledge and fuller understanding of systematic problems among coelenterates, were so superficially defined that many of them are not recognizable without reference to the original type-specimens —and a large number of those were victims of World War II. This leaves us in a very difficult position with respect to many plexaurid species that are validly established in the literature, and it is likely that a good number of them will remain unrecognizable indefinitely.

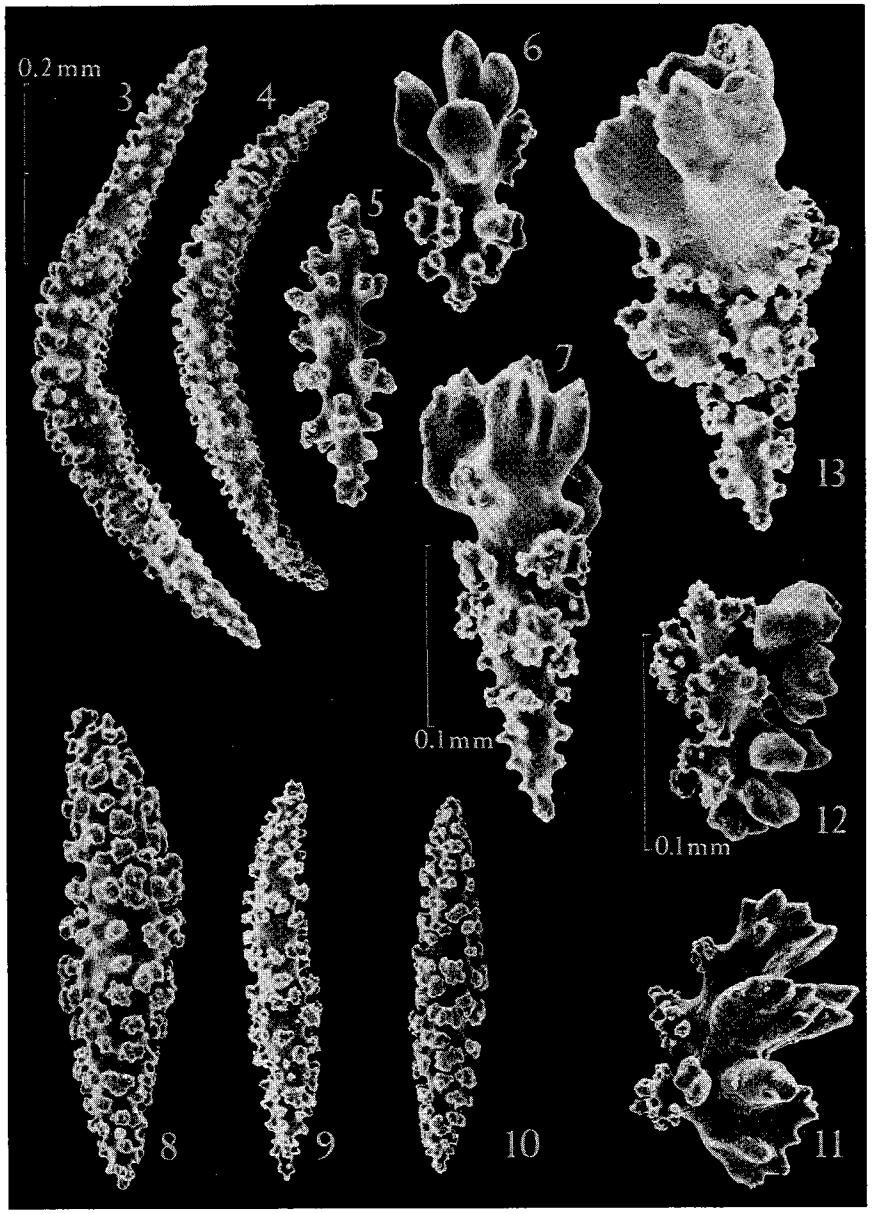
The difficulty in recognizing and classifying plexaurid genera and species arises primarily from the variability of the characters used, and secondarily from the difficulty of quantifying and expressing these characters in a meaningful way. Although the growth-form—i.e., the general plan of branching, the length and girth of branches and twigs—is reasonably constant within the genera and to some extent even in species, this character is under the influence of the environment and the extent to which it may vary under different conditions of current, turbulence, depth and illumination is not well known and rarely has been taken into account in discriminating between species.

The calcareous mesogloeal spicules, the most important single character in the classification of the octocorals, are constant only within broad limits of variation in both form and size. No two spicules are exactly alike. Those lying near the surface of the mesogloea are subject to external influences to an unknown degree, and each different type is present in all stages of development. However, each of the various types of spicules that characterize each species has its own particular location, and this distribution is constant. For example, the retractile part of the polyp in *P. homomalla* has a special type of spicule arranged in a very definite way to form the so-called "crown" or "operculum," and that type is not found elsewhere (Figs. 3, 4). The deep inner layer of mesogloea surrounding the proteinaceous axis also has its own characteristic type of spicule which also are differently colored from the rest (Fig. 5); the outermost layer of cortex contains club-shaped spicules with a torch-like head composed of several flattened projections (Figs. 6-7); and in between, in the intermediate layer of the cortex, the spicules are typically fusiform (Figs. 8-10), but those lying near the outer surface are unilaterally foliate (Figs. 11-12) or obliquely torch-like (Fig. 13), whereas those lying nearer the axis are not modified in form but tend to be colored pinkish or purplish though paler than the deep purple innermost spicules. These characters are difficult to evaluate objectively, and the language we use to express them is very easily misinterpreted. Therefore, the layman, or even the trained scientist in another field, may become thoroughly confused and frustrated when he tries to identify specimens on the basis of keys and descriptions, and his results often prove wrong.

Fortunately, we now have a new and promising tool in the scanning electron microscope, which enables us to see gorgonian spicules with a clarity and accuracy of detail heretofore impossible, and to record them photographically. Although the scanning microscope does nothing to help quantify data about spicules, it does bring out differences that were indistinct or overlooked with light optics and it greatly enhances direct visual comparison.

Another tool that holds great promise in solving some—certainly not all—of the vexing taxonomic problems in the octocorals is biochemistry. Dr. Weinheimer showed me several years ago how the TLC patterns of the species he studied were suggestively specific and might therefore be useful as a "fingerprinting" technique. It think that this method might well help in sorting out some of the closely similar plexaurid species, and possibly in aligning them generically. Dr. Weinheimer's biochemical research revealed the presence of prostaglandins in *Plexaura homomalla* but not in the related *Plexaura flexuosa,* nor in any of the species of *Eunicea.* I have always been uneasy about the generic relationship of *P. flexuosa* with *homomalla,* and now I am even more so.

Although Moser in his preliminary paper allocated 19 species to the genus *Plexaura* [9], they included various species of *Eunicea, Pseudoplexaura* and *Muriceopsis* because his idea of generic limits differed con-



FIGURES 3-13. Spicules of *Plexaura homomalla* (Esper): 3, 4, opercular; 5, axial sheath; 6, 7, outer cortical torches; 8-10, cortical spindles; 11, 12, unilaterally foliate; 13, oblique torch.

siderably from those of the present day. Thus, in my study [1], I reduced the number of species to three: *Plexaura homomalla,* the type-species; *Plexaura flexuosa* Lamouroux, and one from deep water in the Tongue of the Ocean which Miss Deichmann and I called *Plexaura nina.* I also considered some slender, shallow water colonies to be a growth-form of *homomalla* distinct from the typical form as originally illustrated by Esper. This form seems to intergrade with the typical *homomalla* in respect to both branching and spicules, and I gave up in despair trying to find ways in which they differ consistently. It seems certain that Dr. Weinheimer's investigations included both typical *homomalla* and the slimmer *kükenthali* form, and it also seems likely that subsequent studies of prostaglandins have done likewise. I now begin to wonder if the distribution of the R and S configurations could reflect a biochemical difference between these two so-called growth forms, and I wonder also if the prostaglandins might not link the *kükenthali* form with the deeper-dwelling *Plexaura nina* instead of with *homomalla* as I originally thought. A series of about 150 SEM photos made at the Smithsonian from six samples of *homomalla* supplied by Dr. Rhuland showed no differences that I could consider significant. However, the samples from which I worked were not large enough to distinguish differences in colonial form.

Now a selected batch of the *kükenthali* form should be investigated to determine whether the prostaglandins are exclusively of either R or S configuration, or are a mixture. If of one kind only, then a detailed evaluation of skeletal characters may warrant a different conclusion than was reached previously.

Thus, the discovery of prostaglandins in *Plexaura homomalla,* exciting as it is in ts own right, valuable as it may be to mankind through its medical and research applications, has still another significance. It has opened Chapter 3 of this story. It may be the key to one of the many unsolved problems in the systematics of gorgonians.

REFERENCES

1. BAYER, FREDERICK M., 1961. *The shallow-water Octocorallia of the West Indian Region. A manual for marine biologists.* Martinus Nijhoff, The Hague. 373 pp., 101 text figs., 27 pls.
2. ESPER, EUGENIUS JOHANN CHRISTOPH, 1788-1830. *Die Pflanzenthiere in Abbildungen nach der Natur mit Farben erleuchtet nebst Beschreibungen,* and *Fortsetzungen der Pflanzenthiere.* Nürnberg. 5 vols., pp. xii+320, 220, 285+, 230, 48; 428 pls.
3. KÖLLIKER, RUDOLPH ALBERT, 1865. Die Bindesubstanz der Coelenteraten, *in:* Icones histiologicae oder Atlas der vergleichenden Gewebelehre, *2*(1): 87-181, figs. 16-28, A & B, pls. 10-19.
4. KUNZE, G., 1916. Die Gorgonarien Westindiens, 4. Die Gattung Eunicea Lamouroux. *Zool. Jahrb. Suppl. 11*(4):505-552, pls. 24-26.
5. LAMOUROUX, J. V. F., 1812. Sur la classification des Polypiers coralligènes non entièrement pierreux. *Nouv. Bull. Sci. Soc. Philom. 3:* 181-188.
6. LAMOUROUX, J. V. F., 1816. *Histoire des Polypiers coralligènes flexibles, vulgairement nommés Zoophytes.* Caen. lxxxiv+560 pp., 19 pls.

7. LAMOUROUX, J. V. F., 1821. *Exposition méthodique des genres de l'ordre des Polypiers, avec leur description et celles des principales espèces, figurées dans 84 planches; les 63 premieres appartenant a l'Histoire naturelle des Zoophytes d'Ellis et Solander.* Paris. viii+115 pp., 84 pls.
8. LINNAEUS, CAROLUS, 1758. *Systema naturae. Editio decima, reformata. 1.* Holmiae. 824 pp.
9. MOSER, JOHANNES, 1921. Ergebnisse einer Revision der Gattung *Plexaura* Lamouroux. *Zool. Anz. 53:* 110-118.
10. PALLAS, PETER SIMON, 1766. *Elenchus zoophytorum sistens generum adumbrationes generaliores et specierum cognitarum succinctas descriptiones cum selectis auctorum synonymis.* Hagae-Comitum. xvi+28+451 pp.
11. STIASNY, GUSTAV, 1935. Revision der Plexauridae. Die Gorgonacea der Siboga-Expedition, Supplement 1. *Siboga-Exped. Monogr. 13b[7]:* vi+106 pp., 7 pls.
12. VALENCIENNES, ACHILLE, 1855. Extrait d'une monographie de la famille des Gorgonidés de la classe des Polypes. *C. R. Acad. Sci. Paris 41:* 7-15.
13. VERRILL, ADDISON EMERY, 1907. The Bermuda Islands, Part 5. Characteristic life of the Bermuda coral reefs. *Trans. Conn. Acad. Arts Sci. 12:* 204-348, 413-418, pls. 28-40.

THE PROSTAGLANDINS: THEIR HISTORY, DEVELOPMENT AND POTENTIAL

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ABSTRACT

This paper reviews the history of prostaglandins, and summarizes their chemical nature, origin and distribution. Their biological and chemical properties are discussed, and their practical and research applications are considered. The original discovery of prostaglandins in the gorgonian coral *Plexaura homomalla* (Esper) is recalled, and additional discoveries made by Upjohn scientists are described. The practical use of *P. homomalla* as a source of prostaglandins is discussed.

Prostaglandins were first discovered as unusual vasodepressor smooth-muscle-stimulating substances in human semen and in the semen and vesicular glands of sheep. Early work in the 1930's by Goldblatt and von Euler established that extracts from accessory sex glands yielded a pharmacologically active substance with novel properties. von Euler named this material prostaglandin [1], believing that it was synthesized by the prostate gland. Although prostaglandins are now known to be produced by many tissues and cells, the name has been firmly established by use. The present day interest in prostaglandins stems primarily from the determination of their chemical structure by Bergström and his associates who, in 1947, started an investigation of a concentrate prepared from thousands of Icelandic sheep glands. Using every careful chromatographic technique, they were able to isolate milligram quantities of two crystalline prostaglandins, and a few years later, following some brilliant structural elucidation studies, they were able to announce in 1962 the chemical structure of the new prostaglandins [2]. Subsequently, additional natural prostaglandins were isolated and their structures determined, and the prostaglandins were shown to be a family of closely related chemical structures [3] (Fig. 1).

All the prostaglandins were found to be unsaturated, hydroxy acids with a five-membered ring on a 20-carbon skeleton and are described by number and letter. Prostaglandins of the 1, 2, and 3 series have 1, 2, and 3 double bonds respectively with the position and stereo-chemistry indicated (Fig. 2). The F prostaglandins have an alpha-hydroxy on the ring at carbon 9. E prostaglandins have a ketone instead. The A prostaglandins are dehydrated derivates of the E compounds in which there is a double bond in the ring between carbons 10 and 11 (Figure 3).

Biological studies showed that prostaglandins are formed enzymatically from certain polyunsaturated fatty acids by cyclization to give a five-

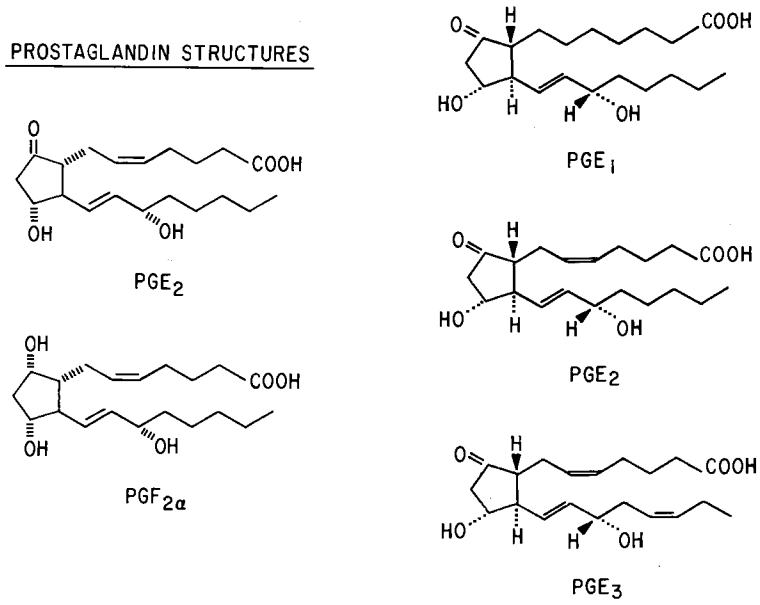


FIGURE 1 (left) AND 2 (right)

membered ring and the incorporation of three oxygen atoms in certain positions [4] (Figure 4). Enzymes involved in their synthesis are found to be widely distributed in a variety of tissues [5]. As an example, prostaglandin E_2, and prostaglandin $F_2\alpha$, are formed from the precusor arachidonic acid. Tissue arachidonic acid exists primarily in phospholipids and in different situations following particular stimuli, prostaglandins are formed by the enzymatic hydrolysis of the phospholipid to release precursor arachidonic acid which is then converted to prostaglandins [6]. Tissue stores of prostaglandins are thought to be unlikely and the level of prostaglandins in particular tissues is thought only to represent the capability of the tissue to synthesize prostaglandin in the interval between removal and preparation for extration. For many years a laboratory scale-up of this biosynthesis using substrate-polyunsaturated fatty acids and the enzyme preparation from sheep seminal vesicular glands was used to obtain prostaglandins for biological evaluation [7]. Using this technique gram-quantities of prostaglandins were avaible for the first time in 1965.

In addition to the problems associated with the low levels (microgram/gram wet tissue weight) of prostaglandins which could be obtained by extraction, it was also established that the prostaglandins were very rapidly metabolized [8], especially by the lungs [9]. A variety of enzymatic steps have been identified which degrade the prostaglandin molecule, particularly by oxidation of the C-15 hydroxyl group and by oxidative cleavage of the carboxy side chain. The ultimate urinary metabolites of the prostaglandins reflect the overall result of the catabolic enzymes [10].

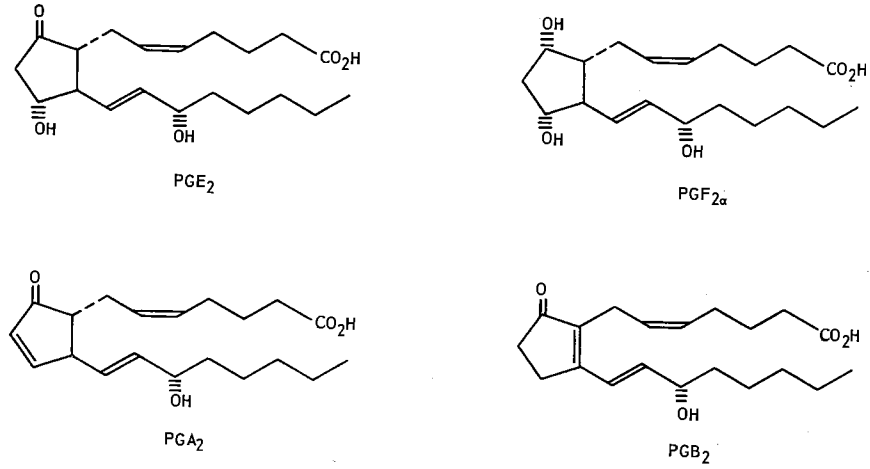


FIGURE 3.

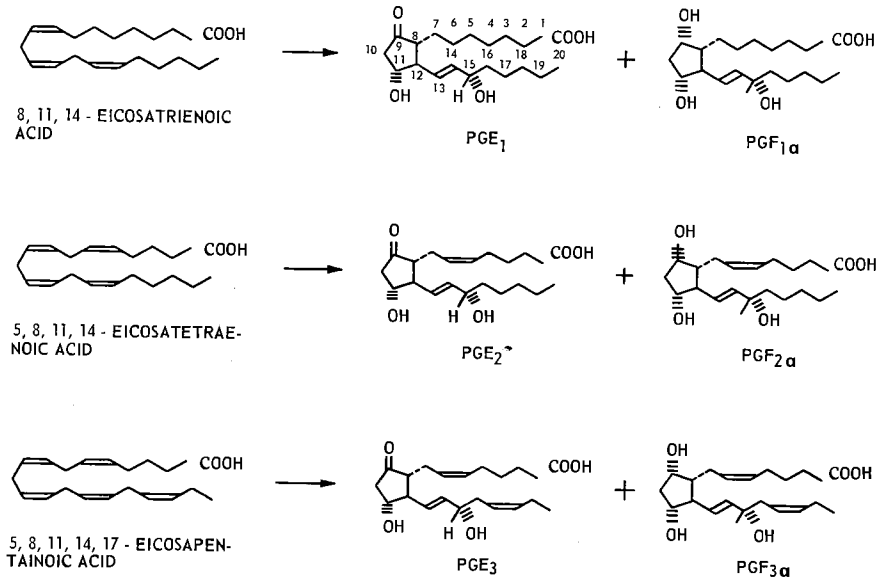
The principal interest in the prostaglandins has focused on their remarkable biological versatility and the wide range of their effects [11]. The effects themselves may be quite specific and the different prostaglandins may have quite unique actions. For example, one prostaglandin, PGE_2, lowers blood pressure when administered to a test animal, whereas $PGF_2\alpha$, a closely related member of the family, raises blood pressure. Also, the prostaglandins produce their effects in very small concentrations. For example, *in vitro* effects of prostaglandins can be seen with ng/ml concentrations. In clinical situations, several milligrams may be sufficient to cause a response [12]. Indeed, the prostaglandins are among the most potent biological materials known. In general, the biological properties of the prostaglandin family are based on certain broad powers: regulation of the activity in smooth muscles, of secretion, including endocrine gland secretions, or of blood flow. Over the past ten years basic research investigation of the effects of prostaglandins have suggested several potential applications of the prostaglandins in cilincal medicine. The dramatic proliferation of publications in the prostaglandin area which have been made possible by the increased availability of these materials for study grows exponentially, and one would anticipate that additional uses for these materials may become evident in future years. Another important aspect has been the inhibition of the production of prostaglandins in the body, and Vane and his coworkers recently obtained results suggesting that the anti-inflammatory action of aspirin and certain other agents may be explainable on the basis that they block the synthesis of prostaglandins [13].

Some of the most striking effects of the prostaglandins have been on the female reproductive system [15, 16]. Intravenous injections of very low doses of PGE_2 or $PGF_2\alpha$ have been shown to stimulate uterine con-

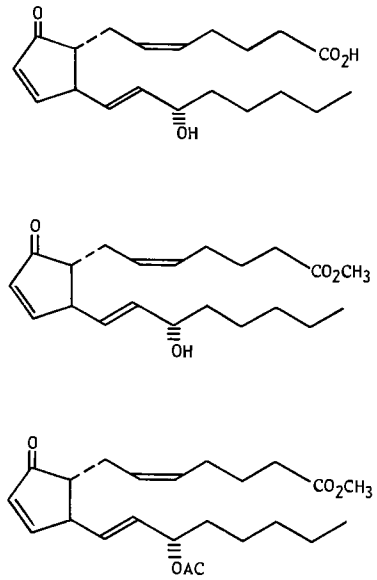
tractions [17, 18]. These materials have been used to facilitate child-bearing labor in several thousand women. Recently, oral administration of PGE_2 has also been reported to be effective for induction of labor [19] and further studies may lay the basis for the wide adoption of a prostaglandin for this purpose. Additionally, prostaglandins have been widely studied as agents for therapeutic abortion [20] and for inducing menstruation in case of menstrual failure [21]. There seems also to be a strong possibility that prostaglandins or perhaps synthetic analogs may become important agents in controlling population growth.

With the anticipated need that prostaglandins would become medically useful agents, it was necessary to devise efficient methods for their synthesis on a larger scale. Clearly, the known natural sources were not suitable. The scale-up of the the biosynthesis was not thought to offer the potential for larger-scale operation nor the flexibility necessary to produce a wide range of different prostaglandins. Starting at the time that the first prostaglandin structures were known, chemical total synthesis has been investigated as one of the routes to obtain these harmone-like lipids. Several routes have been developed, particularly at Upjohn and by Corey and his coworkers at Harvard, for the total chemical synthesis of prostaglandins [22, 23]. These chemical total syntheses characteristically involved perhaps 16 distinct chemical operations. This complexity was necessitated by the stereochemical problems associated with the prostaglandin molecule. For example, in the case of the prostaglandin $F_{2\alpha}$ there are 5 asymmetric centers and 6 distinct functional entities. That a molecule of this complexity could be efficiently synthesized is a remarkable demonstration of the power of modern synthetic organic chemistry. For many years no alternative to a totally chemical approach seemed feasible. However, in 1969, Weinheimer and Spraggins reported the isolation of several prostaglandins from the gorgonian *Plexaura homomalla* obtained from Florida coastal waters [24]. A remarkable feature of their discovery was that they were able to obtain from the air-dried cortex a yield of between 1 and 2 per cent of certain prostaglandin isomers, a remarkably high concentration. In particular, they showed the presence of 15-epi-PGA_2-15-acetate, methyl esters. Unfortunately, this prostaglandin did not have the typical biological properties associated with the known mammalian prostaglandins [25]. It was possible to convert this isomer at 15 by chemical routes to the 15(S) or natural prostaglandins [26]. Still, the amounts found in the gorgonian remained quite startling compared, for example, to the concentration of 300 micrograms/ml of a mixture of prostaglandins found in the richest mammalian source (human seminal plasma). Considerable effort was expended to find efficient routes to make the two most widely studied prostaglandins, PGE_2 and $PGF_{2\alpha}$, from these stereochemically unnatural 15(R) prostaglandins.

However, to our surprise, during the processing of *Plexaura homomalla* from other areas in the Caribbean, there were obtained the derivatives of 15(S)-PGA_2 and PGE_2 which were indentical with the prostaglandins derived from mammalian sources [27] (Figure 5). Also, in certain single



[5] –CORAL DERIVED PROSTAGLANDINS



FIGURES 4 (top) AND 5 (bottom).

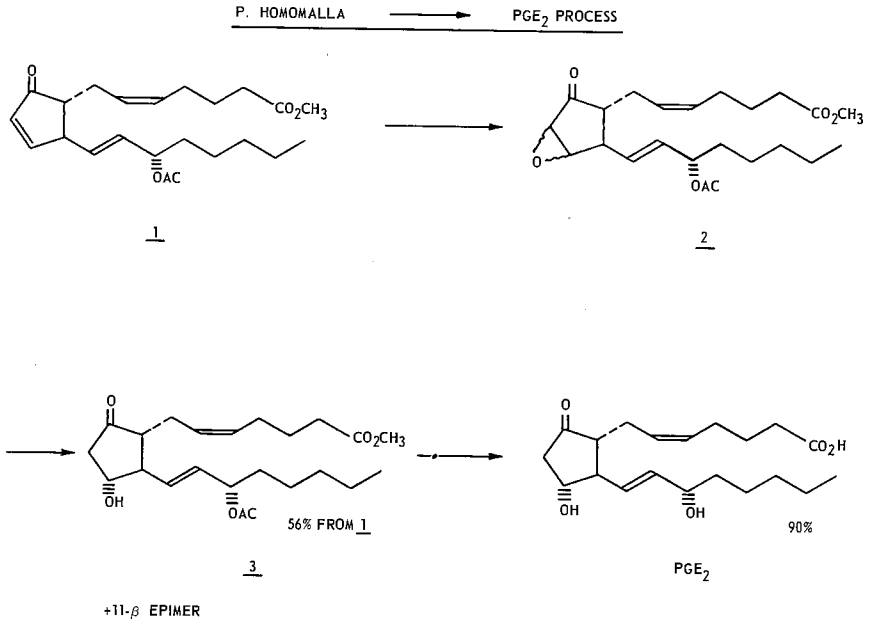


FIGURE 6.

specimens of the gorgonian, both 15(R) and 15(S) prostaglandins were shown to occur. This finding clearly made the investigation of the *Plexaura homomalla* an even more important task, and recently it has been possible to convert the prostaglandins obtained from the S-variety *Plexaura homomalla,* in three chemical steps to PGE_2 and in four steps to $PGF_{2\alpha}$ [28] (Figure 6). Additionally, it was possible to isolate directly a low yield (.06 per cent) of crystalline PGE_2 itself. During the chromatographic purification of the 15(S)-PGA_2 from *Plexaura homomalla,* an additional new natural prostaglandin was detected, isolated, purified and its structure shown to be the 5-*trans* isomer of PGA_2 [29] (Figure 7). This occurred as a minor component (5-15 per cent) of the PGA_2 isomer. This extension at Upjohn of the original Weinheimer and Spraggins discovery has focused additional interest on *Plexaura homomalla* as a starting material for obtaining prostaglandins for practical purposes. This symposium reflects, in part, that expanded interest.

In any practical considerations, it is vital to know whether harvesting or farming *Plexaura homomalla* is possible, bearing in mind any possible dangers in upsetting the ecology of reefs. It is also to be hoped that efforts may be found to study *Plexaura homomalla* under laboratory conditions, to permit an understanding of why this particular gorgonian should produce such remarkably high concentration of these compounds. In considering the merits of obtaining a drug from the sea, by extracting material from

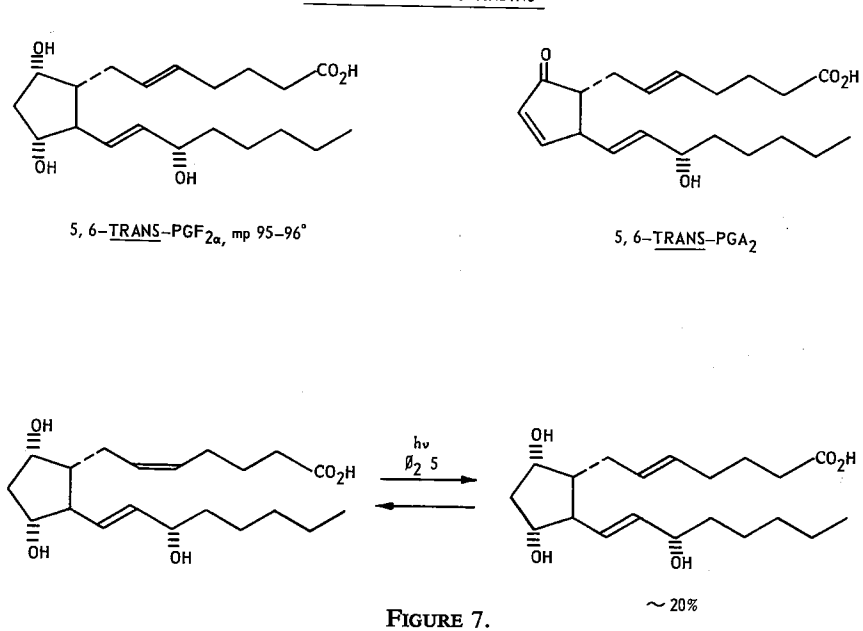


FIGURE 7.

harvested *Plexaura homomalla,* it is perhaps worth weighing the ecological factors against the potential benefit in using prostaglandins as agents for reducing or controlling population growth. Since a major contributing factor to environmental problems in general, is uncontrolled population expansion, the wise use of the marine environment to obtain these drugs potentially valuable in the control of fertility, deserves careful study.

REFERENCES

1. EULER, U. S. VON, 1935. Über die spezifische blutdrucksenkende Substanz des menschlichen Prostata- und Samenblasensekretes, *Klin. Wschr. 14:* 1182-1183.
2. BERGSTRÖM, S., R. RYHAGE, B. SAMUELSSON AND J. SJÖVALL, 1962. The structure of prostaglandin E, F_1 and F_2, *Acta chem. scand. 16:* 501-502.
3. BERGSTRÖM, S. AND B. SAMUELSSON, 1965. Prostaglandins, *A. Rev. Biochem. 34:* 101-108.
4. SAMUELSSON, B., 1969. Biosynthesis of prostaglandins, *Prog. biochem. Pharmac. 5:* 109-128.
5. DORP, D. A. VAN, 1969. Essentielle Fettsauren und Prostaglandine, *Naturwissenschaften 56:* 124-130.
6. LANDS, W., R. LEE AND W. SMITH, 1971. Factors regulating the biosynthesis of various prostaglandins, *Ann. N. Y. Acad. Sci. 180:* 107-122.
7. DANIELS, E. G. AND J. E. PIKE, 1968. Isolation of prostaglandins, *Prostaglandin Symposium of the Worcester Foundation for Exp. Biol.,* Ramwell, P. W. and J. E. Shaw, Eds., pp. 379-387, Inter-science, New York.

8. SAMUELSSON, B., E. GRANSTRÖM, K. GRÉEN AND M. HAMBERG, 1971. *Ann. N. Y. Acad. Sci. 180:* 138-163.
9. ANGGÁRD, E. AND B. SAMUELSSON, 1966. Metabolism of prostaglandins in the lung, *Acta physiol scand. 68,* suppl. 277: 232.
10. HAMBERG, M. AND B. SAMUELSSON, 1969. The structure of the major urinary metabolite of prostaglandin E_2 in man, *J. Am. chem. Soc. 91:* 2177-2178.
11. BERGSTRÖM, S., L. A. CARLSON AND J. R. WEEKS, 1968. The prostaglandins: A family of biologically active lipids, *Pharmac. Rev. 20:* 1-48.
12. HINMAN, J. W., 1970. Prostaglandins: A report on early clinical studies, *Postgrad. med. J. 46:* 562-575.
13. VANE, J. R., 1971. Inhibition of prostaglandin synthesis as a mechanism of action of aspirin-like drugs, *Nature New Biol. 231:* 232-235.
14. VANE, J. R., 1972. Prostaglandins and the aspirin-like drugs, *Hosp. Prac. 7*(3): 61-71.
15. KARIM, S. M. M. AND R. R. TRUSSELL, 1971. The use of prostaglandins in obstetrics, *E. Afr. med. J. 48:* 1-12.
16. BERGSTRÖM, S., E. DICZFALUSY, U. BORELL, S. KARIM, B. SAMUELSSON, B. UVNAS, N. WIQVIST AND M. BYGDEMAN, 1972. Prostaglandins in fertility control (meeting summary), *Science, N. Y. 175:* 1280-1287.
17. KARIM, S., 1971. Action of prostaglandin in the pregnant woman, *Ann. N. Y. Acad. Sci. 180:* 483-498.
18. BYGDEMAN, M., 1971. Prostaglandiner och uterus. Stimul rande effekt pa uterus' kontraktilitet (Prostaglandins and the uterus. Stimulating effect on contractility of the uterus) (in Swedish), *Nord. Med. 85:* 436.
19. KARIM, S. M. M. AND S. D. SHARMA, 1971. Oral administration of prostaglandins for the induction of labour, *Br. med. J. 1:* 260-262.
20. KARIM, S. M. M. AND G. M. FILSHIE, 1970. Use of prostaglandin E_2 for therapeutic abortion, *Br. med. J. 3:* 198-200.
21. KARIM, S. M. M., 1971. Once-a-month vaginal administration of prostaglandins E_2 and $F_{2\alpha}$ for fertility control, *Contraception 3:* 173-183.
22. COREY, E. J., 1971. Studies on the total synthesis of prostaglandins, *Ann. N. Y. Acad. Sci. 180:* 24-37.
23. AXEN, U. F., J. E. PIKE AND W. P. SCHNEIDER, 1972. Prostaglandins, In: *Total Synthesis of Natural Products,* Vol. 3, ApSimon, J. W., Ed., Wiley, New York.
24. WEINHEIMER, A. J. AND R. L. SPRAGGINS, 1969. *Tetrahedron Lett.* 5183; presented at "Drugs from the Sea" Conference, University of Rhode Island, Kingston, Rhode Island, August 24-27, 1969.
25. NAKANO, J., 1969. Cardiovascular effect of a prostaglandin isolated from a gorgonian *Plexaura homomalla, J. Pharm. Pharmac. 21:* 782-783.
26. BUNDY, G., F. LINCOLN, N. NELSON, J. PIKE AND W. SCHNEIDER, 1971. Novel prostaglandin syntheses, *Ann. N. Y. Acad. Sci. 180:* 76-90.
27. SCHNEIDER, W. P., R. D. HAMILTON AND L. E. RHULAND, 1972. *J. Am. Chem. Soc. 94:* 2122.
28. BUNDY, G. L., W. P. SCHNEIDER, F. H. LINCOLN AND J. E. PIKE, 1972. *J. Am. Chem. Soc. 94:* 2123.
29. BUNDY, G. L., E. G. DANIELS, F. H. LINCOLN AND J. E. PIKE, 1972. *J. Am. Chem. Soc. 94:* 2124.

THE DISCOVERY OF 15-EPI PGA_2 IN *PLEXAURA HOMOMALLA*

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ABSTRACT

A brief review of the history of the marine natural product program at the University of Oklahoma is presented. The nature of some of the unusual compounds encountered previously in gorgonians is surveyed as a background for the discovery of 15-epi prostaglandins in the gorgonian *Plexaura homomalla.*

The theme of this symposium, prostaglandins from *Plexaura homomalla,* follows from a discovery that had its innocent beginnings in a highly unlikely source—the marine natural products program at the University of Oklahoma.

In the latter half of the 1960's, when prostaglandins were still in extremely short supply, lower marine invertebrates could hardly have been considered a promising hunting ground in the search for additional sources of these typically mammalian compounds. For that matter, the reasons for the presence of large quantities of PGA_2 derivatives in the gorgonian *Plexaura homomalla* still remain obscure and enigmatic. Nor with the exception of this one gorgonian, have the prospects improved for finding other practical sources of prostaglandins among marine organisms of any kind. Since the initial report [1] intensive examination of large numbers of marine species of all kinds, searching specifically for prostaglandins and conducted primarily by others, has failed to turn up any additional leads to the presence of these compounds.

Also incongruous to most when first learning of it, is the existence of a strong marine natural products program in such an unlikely geographical location as the heart of the Great Plains. Suffice it to say that access to marine materials for chemical study is only one simple step more involved (*i.e.,* a brief airline flight) for us in Oklahoma than it is for those situated at coastal institutions.

Since its inception in the early 1950's by Professor Leon S. Ciereszko, the marine program at Oklahoma has gradually grown to include myself and Professor Francis J. Schmitz, both of the Chemistry Faculty, and Professor Pushkar N. Kaul of the Pharmacology Faculty. Our encounter with the first marine prostaglandins, though themselves possessed of no dramatic biological activity, fittingly occurred at that point in the evolution of our marine program at which we began to orient our studies toward the

systematic search for bioactive materials in marine organisms. The stimulus for this change of direction was the observation in our own work and that of others that marine organisms appeared to contain a high frequency of anticancer agents [2] as well as other bioactive materials. Our major emphasis currently is the isolation of potentially useful anticancer, cardiovascular and CNS agents from marine sources.

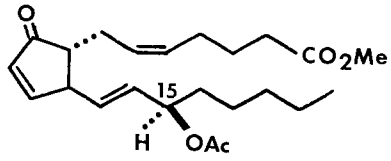
Returning to the prostaglandins, I might observe that it was inevitable that we would sooner or later come upon them as part of our systematic survey of the Caribbean gorgonians. These include the organisms commonly known as sea fans, sea rods and sea whips. Ciereszko had chosen the gorgonians as possibly the most convenient yet promising group of organisms upon which to base a fledgling marine natural products program. From the very practical standpoint of accessibility, these colonial organisms stand out as the most prominent members of the Caribbean reef community. They occur in quite shallow waters in great profusion, and since individual, multi-branch colonies normally range from one foot to four or more feet in overall height, it is a simple matter using only snorkeling equipment to collect the large quantities needed for thorough chemical studies. Chemically, these organisms possess the distinct advantage of high contents of extractable lipid material, varying with species of course, but not infrequently well in excess of 10 per cent on a dry weight basis. Even in the field, their promise of interesting chemical content is evidenced by their unusual and strong "bouquet," and by the thick juices which can be expressed from a wet colony by the fingers.

The gorgonians offered one further distinct advantage which simplified the logistics of collection and storage prior to laboratory work. The generally corky texture of the cortical material, which contains the bulk of the extractable material and which is supported by the internal structural proteinaceous axis, is ordinarily sufficiently porous to permit the organism to air-dry in a day or two, without the spoilage and rotting which usually attends attempts to dry fleshy, non-porous marine forms. Thus in our earlier work, it was a convenient routine to air dry our gorgonian collections prior to shipment and subsequent extractions. This prolonged exposure to air undoubtedly resulted in oxidation, polymerization and other modes of destruction of sensitive compounds, and it is now our practice to preserve specimen materials immediately in alcohol. It is interesting, though, to note that the quite sensitive prostaglandins were isolated from air-dried specimens.

In the earlier phases of our studies, individual species from a cross-section of several major gorgonian genera were examined for the presence of novel types of compounds. This search encountered a large proportion of quite ordinary lipid compounds which were largely ignored, but also was rewarded with the discovery of a wide variety of unusual natural products. These included a variety of sesquiterpene hydrocarbons from the genera *Eunicea, Pseudoplexaura, Pseudopterogorgia,* and *Plexaurella,* some of which represented new structures, but many of which were antipodal

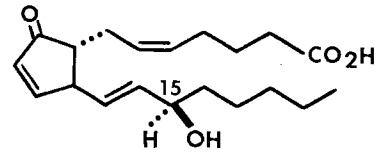
forms of well known compounds from terrestrial plant sources [3]. A series of diterpene lactones, cembranolides, which possess an unusual monocarbocyclic 14-membered ring system, were obtained from several species of *Eunicea* and *Pseudoplexaura* [3]. These lactones, in certain species, are present to the extent of as much as 2 per cent of the dry weight of the organism. A series of unusual C-22 compounds was encountered in the genus *Pterogorgia.* Based upon the bislactone ancepsenolide, these compounds possess a primarily straight chain carbon skeleton [4]. The presence of terpenoid compounds in these animal colonies is perhaps attributable to the symbiotic single-celled algae (zooxanthellae) that are intimately associated with the flesh of the coral polyps, and which are photosynthetically active in most gorgonians.

Even the sterols of gorgonians presented surprises. The C-30 sterol, gorgosterol [5], and the C-29 sterol, demethylgorgosterol [6], possess an unprecedented cyclopropane structure bridging the 22 and 23 positions of the side chain. *Pseudopterogorgia americana* also afforded the 9, 11-dihydroxy derivative of gorgosterol [7] in addition to a 9, 11-seco derivative [8] in which the C-ring was no longer intact.



I

15-epi PGA_2 methyl ester, acetate



II

15-epi PGA_2

It was against this background that Dr. Robert L. Spraggins had chosen to investigate *P. homomalla* for his dissertation work. Thus, we attributed no special significance to the presence of a large proportion of a spectrally unusual fatty acid derivative in its lipid fraction. However, I recall being mildly disappointed that the major component was not a new member of the interesting cembranolide group which we had suspected from its TLC behavior.

Attempts to purify this component by routine chromatographic methods quickly established that it was sensitive to degradation by the combined effects of various adsorbents and solvent systems. Had Dr. Spraggins heeded my suggestions to abandon this recalcitrant compound in favor of other more amenable materials awaiting study, it is likely that we would not be here today. Nevertheless, his persistence and superb experimental capabilities led finally to a straightforward purification procedure that provided the materials required for elucidation of structure.

The detailed structure finally adduced [1] for this major component is shown in (I), and is the methyl ester, acetate of 15-epi PGA_2. It com-

prised 1.3 per cent of the weight of the air dried gorgonian. The corresponding free hydroxy acid (II) was also present, to the extent of 0.2 per cent.

The gross structures of these compounds were of course recognized as prostaglandins considerably in advance of the establishment of the stereochemical detail at the three chiral centers and two double bonds, and a preliminary pharmacological evaluation was undertaken with (II). Dr. Jiro Nakano of the University of Oklahoma Health Sciences Center, who conducted the study, reported that (II) was almost completely ineffective in reducing blood pressure in the dog in side-by-side tests with authentic PGA_2 provided to him by the Upjohn Co.

This absence of a significant biological effect had to be attributable to a stereochemical difference between (II) and mammalian PGA_2 and we subsequently demonstrated that the only such difference resided in the configuration at position 15. This was accomplished by ozonolytic cleavage of the 13, 14-double bond and conversion of the side-chain fragment to alpha-hydroxy heptanoic acid which had the R configuration. Thus (II) was shown to possess the 15-R configuration, whereas the mammalian form has the 15-S configuration.

P. homomalla is one of the most widely distributed of the Caribbean gorgonians. Bayer's monograph [9] tabulates reports of its occurrence in practically every shallow water portion of the Caribbean region, including both islands and continental shores. Following our report, it was not surprising that a number of pharmaceutical companies undertook to evaluate the coral as a potential source of starting material for conversion to the mammalian forms of the scarce prostaglandins. The Upjohn group soon reported the transformation of the 15-epi derivatives to members of the normal series [10], and subsequently also reported the discovery of similar large quantities of the 15-S forms of PGA_2 derivatives in *P. homomalla* from other locations in the Caribbean [11]. The latter observation established the coral beyond any question as an attractive source of raw material.

In closing, I would like to observe how exceptionally appropriate it is that this Symposium is being held at the Rosenstiel School. It is the home base of Ted Bayer who has been very patient over the years in instructing various members of the Oklahoma group in the almost impossible art of identification of gorgonians, and of distinguishing one species from another. It is also the home base of Charles E. Lane who, during a site visit at our laboratories in 1964, extended a cordial invitation to our group to use the collecting facilities of the Rosenstiel School. It was Lane's enthusiasm that was largely responsible for the generous funding from NIH (HE-05675), which supported the work leading to the discovery that ultimately generated this Symposium. And finally, it was during one of our subsequent visits here that the collection of *P. homomalla* from which we isolated the prostaglandins was strung up to dry on the very spot on which this new building now stands.

REFERENCES

1. WEINHEIMER, A. J. AND R. L. SPRAGGINS, 1969. *Tetrahedron Letters* 5158.
2. SIGEL, M. M. ET AL., 1969. Proceedings of the Food-Drugs from the Sea Symposium, Marine Technology Society, 281; G. R. PETTIT ET AL., 1970. *Nature 227:* 962.
3. WEINHEIMER, A. J., F. J. SCHMITZ AND L. S. CIERESZKO, 1967. Transactions of the Drugs from the Sea Symposium, Marine Technology Society, 135.
4. SCHMITZ, F. J. ET AL., 1966. *Tetrahedron Letters* 97; *idem,* 1969. *J. Org. Chem. 34:* 1989; *idem,* 1971. *J. Org. Chem. 36:* 719.
5. HALE, R. L. ET AL., 1970. *J. Amer. Chem. Soc. 92:* 2179; N. C. LING ET AL., 1972. *Ibid. 92:* 5281.
6. SCHMITZ, F. J. AND T. PATTABHIRAMAN, 1970. *J. Amer. Chem. Soc. 92:* 6073.
7. WEINHEIMER, A. J. AND W. R. HAERTLE, unpublished work.
8. ENWALL, E. L. ET AL., 1972. *Chem. Commun.* 215.
9. BAYER, F. M., 1961. *The shallow-water Octocorallia of the West Indian region.* Martinus Nijhoff, The Hague.
10. BUNDY, G. ET AL., 1971. *Ann. N.Y. Acad. Sci. 180:* 76.
11. SCHNEIDER, W. P., R. D. HAMILTON AND L. E. RHULAND, 1972. *J. Amer. Chem. Soc. 94:* 2122.

PLEXAURA HOMOMALLA: THE BIOLOGY AND ECOLOGY OF A HARVESTABLE MARINE RESOURCE

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ABSTRACT

The biology of *Plexaura homomalla* is reviewed with respect to morphology, nutrition, ecology, predation, mortality, and reproduction. Field experiments to determine the practicability of using *P. homomalla* as a source of prostaglandins, and the possible ecological effects of commercial harvesting are described.

INTRODUCTION

The discovery of medically important prostaglandins from the gorgonian *Plexaura homomalla* [15] has raised some questions of a nature not frequently encountered by marine ecologists. The practical problems of securing a sufficient amount of material from a reef coelenterate, while developing a collecting policy that adequately safeguards the ecosystem, are almost unique. Furthermore, the problems of assuring a continued supply are intimately associated with questions about the biology of these organisms. This paper will first discuss what little is known about the biology and ecology of the West Indian reef gorgonians with emphasis on *Plexaura homomalla,* and then indicate how this information is being used to design and maintain an ecologically sound policy of harvesting.

GORGONIAN BIOLOGY

Morphology.—Gorgonians, like all other anthozoan coelenterates, are exclusively polypoid. The polyp consists of: (1) a tube-like portion containing the gastric cavity, the septa and, periodically, the gonads; and (2) eight pinnate tentacles surrounding an oral disc with a central slit-like mouth (Fig. 1). Like many of the Anthozoa, the gorgonians are colonial (Fig. 2). The gastric cavities of neighboring polyps are connected to each other by gastrodermal tubes called solenia. The polyps of a gorgonian colony are supported by a common skeleton and are embedded in tissue termed coenenchyme.

The gorgonian octocorals differ from the hard corals or scleractinians in that they do not have a solid stony skeleton. The polyps are surrounded not by calcareous material but by tissue. The skeleton system of the gorgonians differs from that of the scleractinians in that it consists of two elements. The central skeleton of the holaxonian gorgonians is composed of a proteinaceous material called gorgonin [6; p. 551]. This axial skeleton

is laid down by the axis epithelium and is in reality an exoskeleton, even though it is completely covered by living tissue. Gorgonians also possess a second skeletal element of calcite spicules that are produced by modified epithelial cells. These spicules lie in the mesogloea, cover parts of the surface of the colony, and in some cases support and protect the individual polyps. The morphology of these spicules and their location in the tissues are very important taxonomic characters [2, 3].

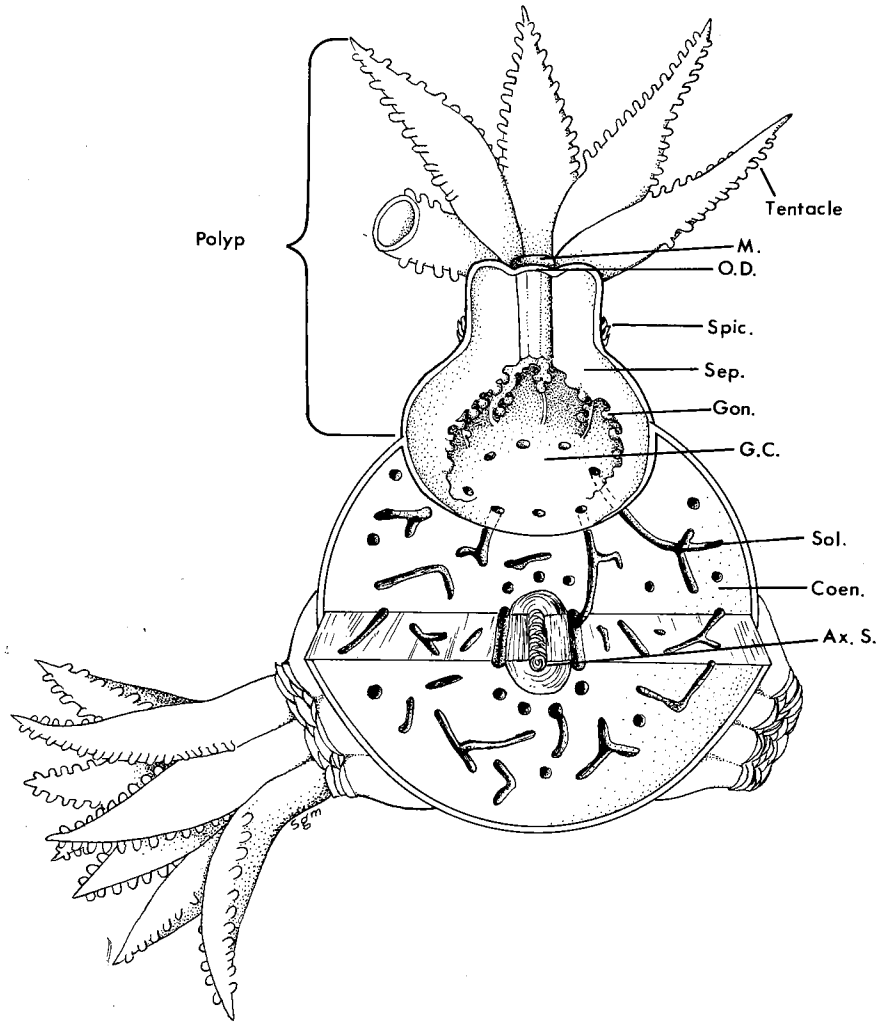

FIGURE 1. Diagramatic section through a gorgonian stem. Ax.S., axial skeleton; Coen., coenenchyme; G.C., gastric cavity; Gon., gonad; M., mouth; O.D., oral disc; Sep., septum; Sol., solenia; Spic., spicules.

Nutrition.—The exact mode of nutrition of gorgonians, like many of the coelenterates, is not well known. Feeding on particulate matter and small zooplankters, which is commonly assumed to be the primary source of food, is brought about by the action of the tentacles. The feeding action of the tentacles is assisted by the nematocysts, or stinging capsules, which lie in the epidermis. Food thus captured is brought to the mouth by the tentacles and is ingested into the gastric cavity where it is partially digested and taken up by the gastrodermal epithelium. In fact the ability of the gorgonians to capture and subdue actively swimming zooplankters varies greatly from species to species. Some are well equipped with nematocysts and active tentacles and can quickly catch and ingest small swimming

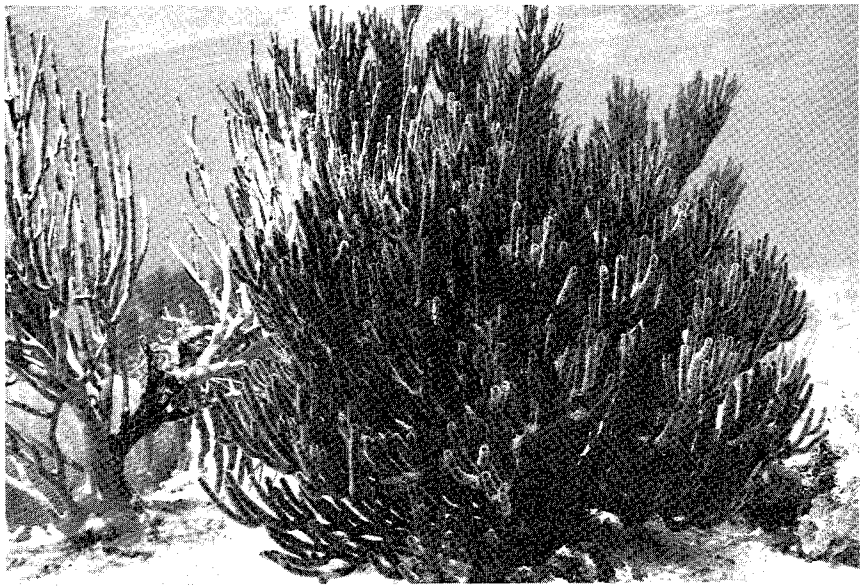

FIGURE 2. A colony of *Plexaura homomalla.* The white spots on the branches are expanded polyps. This colony is about 60 cm tall.

plankters. Others seem incapable of securing much food this way because of their few nematocysts and weak tentacular action. *Plexaura homomalla* falls about midway between these extremes. It has numerous nematocysts and moderately active polyps, but its small polyp size and the relatively slight elasticity of the mouth, which is characteristic of many of the gorgonians, limit the size and activity of the food it is able to capture.

Another important feature of many of the shallow water gorgonians of the West Indies is their symbiotic association with unicellular algae called zooxanthellae. These algae are endo-symbiotic dinoflagellates which are found in great numbers in the gastrodermis of the gorgonians [12]. The mode of infection, epidemiology and even the taxonomy of these algae

are unclear at present. Also the relationship between these algae and their hosts, while long a subject of investigation, has not been completely answered. It has been suggested that the algae play a part in the nutrition of the host [8, 14] as well as influencing the calcification [4, 9], waste removal [16] and recycling of nutrients within the algal-host system [10]. The ability of the algae to fix carbon photosynthetically is undoubtedly of importance to the host, but the degree of dependence on this source of energy has not been determined for any species. Most of the research on zooxanthellae has been done on scleractinian corals, and while the relationship may well be similar in gorgonians there is no reason to assume that it

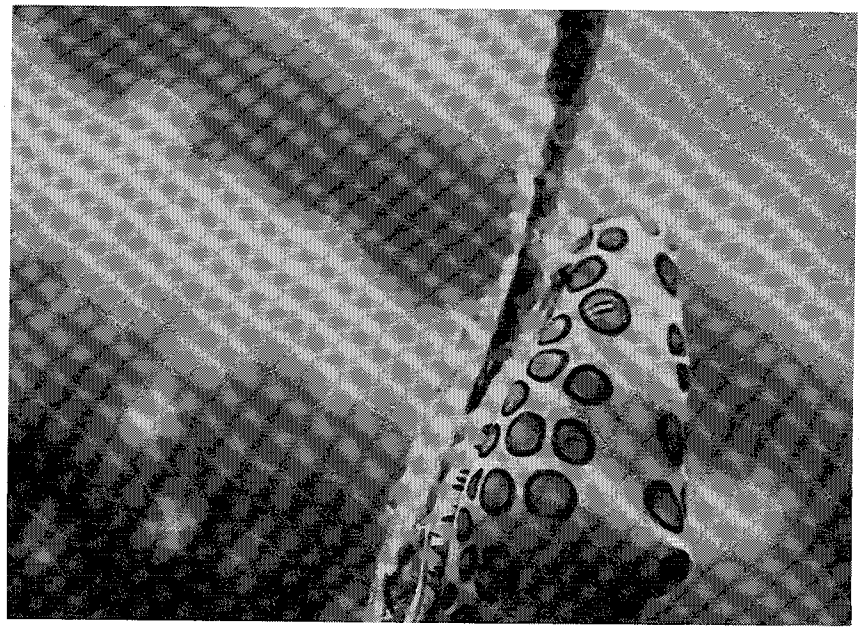

FIGURE 3. *Cyphoma gibbosum* eating a gorgonian. The spots are on the mantle of the snail. Note that most of the gorgonian tissue has been removed, exposing the axial skeleton.

is identical. In fact, some preliminary work has suggested that the symbiosis between gorgonians and their algae is substantially different than in the case in scleractinian corals [7; p. 32]. In any case, all the symbiotic gorgonians are to some extent dependent on light.

Predation.—Plexaura homomalla, like other shallow water West Indian gorgonians, is subject to predation by ovulid gastropods of the genera *Cyphoma, Simnia* and *Neosimnia* [1, 3, 7]. Of these, *Cyphoma gibbosum,* the flamingo tongue, is the most important on *Plexaura homomalla.* This gastropod crawls over the gorgonian colony rasping off tissue with its toothed radula (Fig. 3). The colony is usually scraped down to the axis

leaving it exposed and subject to settling and fouling by other organisms. Preliminary work suggests that under most situations the rate of regrowth of the damaged tissue by a colony is similar to the rate of removal of tissue by the snail. If this is true, the gorgonian would be able to provide food for the gastropods which would be harvesting only the production of regenerated tissue. However this symbiotic balance has not been unequivocally demonstrated in the field. In fact there have been cases observed with as many as 17 *Cyphoma* feeding on one colony instead of the usual one or two. In these cases, the entire colony was soon completely stripped and the "herd" of snails then moved on.

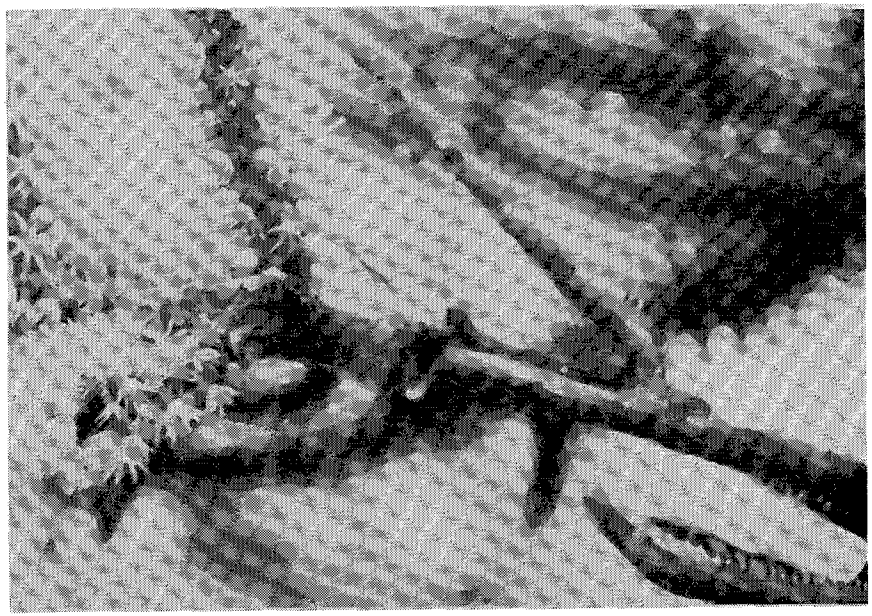

FIGURE 4. Photograph of a gorgonian that is being overgrown by *Millepora*. Note that the unaffected part of the gorgonian appears healthy. Also note the area of contact between the gorgonian and the *Millepora*.

The other main enemy of the shallow water gorgonians in the West Indies appears to be the hydrocoral *Millepora,* or "fire coral." When some injury exposes the axis of the gorgonian it becomes available as a settling site for other animals and plants. When *Millepora* thus gains a foothold, it is able over a period of time to kill the whole colony and entirely cover the axis (Fig. 4). This process may take months or even years depending on the size of the colony, but once *Millepora* has gained a foothold the fate of the gorgonian is sealed.

Algae also can attach to areas where the axis is exposed, and in some cases to the healthy colony as well.

Small labrid fishes are frequently seen nibbling at colonies of *Plexaura homomalla* and other shallow water gorgonians, but it is not known whether they are actually eating living gorgonian tissue, small algal filaments, or mucous secreted by the gorgonian [11]. Small hermit crabs, sometimes in large numbers, are frequently seen in the branches of shallow water gorgonians. Small coralliophilid gastropods are often found almost imbedded in the tissues of the basal portions of the colonies. The effect of these animals on the colonies is not known. Considering how little is known about even the most basic natural history of the gorgonians, it would not be surprising if there were other predators or parasites of *Plexaura homomalla,* but knowledge of them must await further investigations.

Mortality.—The main cause of mortality of the shallow water gorgonians in the West Indies appears to be toppling of the colonies due to the action of storms and strong water movement [7]. As the colonies grow larger they are more and more influenced by the motion of passing water and must be able to resist increasingly greater total pressure. The destructive effect of this water movement is enhanced by the destruction of the calcareous substratum that is the result of the biodegradation by boring organisms, principally sponges [5]. The substratum thus weakened is not able to support the stresses placed on it by the attachment of the gorgonian and it breaks off. Most toppled gorgonians still have the holdfast attached to a piece of calcareous rock. This attests to strength of the attachment of the gorgonian and the impermanence of the bottom.

Another source of mortality of gorgonians, especially smaller colonies, is that due to smothering of the colonies by algal overgrowth. Algal mats up to 5 cm in thickness appear periodically in shallow water. Their growth appears to be very rapid relative to the growth of the small gorgonian colonies, which are thus simply overgrown by the algae and cut off from free water movement and light. In the laboratory, single-polyp stages of some gorgonians have been observed to be strangled by a single algal filament. Presumably this could be an important source of mortality to newly settled polyps but it would be very difficult to verify in the field.

Gorgonians also can be smothered by silt or sand. If the bottom is constantly silty the gorgonians cannot settle, since they require a firm substratum for attachment. If the siltation is periodic, a colony might settle and begin to grow only to be covered soon afterward.

Ecological Requirements.—The basic requirements of the symbiotic gorgonians in the West Indies, are shallow (0-15 m), warm (18-30° C) clear tropical waters with a moderate amout of water movement. A further requirement is a hard bottom that is free from extensive siltation and sand scouring. *Plexaura homomalla* is generally found in water ranging from less than one meter in depth to about 20 m. However, it has been found as deep as 30 m, and transplanted colonies will survive at 40 m. *Plexaura homomalla* appears to require more protection from strong wave action than some other species such as *Gorgonia flabellum* and *Plexaura flexuosa,* but

it is not common in areas of very quiet water. In places where there are significant amounts of sand, *Plexaura homomalla* appears to be concentrated on small rises and ridges which presumably represent the areas of survival of newly settled planulae (Fig. 5).

Plexaura homomalla has been found throughout the tropical West Atlantic from Bermuda to Curaçao and from Mexico to the windward Lesser Antilles.

Growth.—Once a colony has been successfully established, growth occurs by elongation of branches by growth at the tip, and by branching. The tip

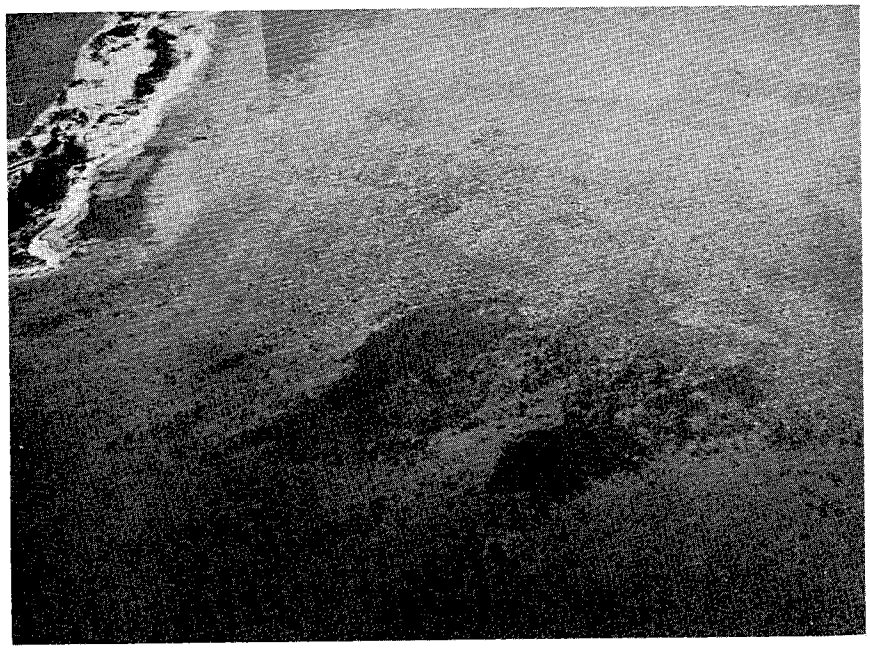

FIGURE 5. Aerial photograph of North Sound, Grand Cayman. The long, dark lines mark ridges on the bottom of the sound. These ridges have a rich growth of *Plexaura homomalla*.

growth occurs by elongation of the axial skeleton and with it growth of the coenenchyme surrounding and secreting it. The density of the polyps near the tips is not very different from that lower down the branches, suggesting that the asexual budding of new polyps is in balance with the rate of elongation of the tips.

Attempts to estimate the growth rate of gorgonians is difficult due to the irregular shape of the colonies and the rather slow rate of growth. In an attempt to standardize some of these problems, selected colonies were placed in PVC tubes which had been embedded in concrete blocks. The transplants were secured in the tubes by nylon screws (Fig. 6). The lack

FIGURE 6. Concrete transplant blocks that were used in the transplant experiments. Note that the colony of *Plexaura homomalla* in the foreground has grown down over the top part of the PVC pipe and has covered the nylon holding screw. The scale is in centimeters.

of toxicity of this transplant system is demonstrated by the spreading of the basal part of some of the colonies down over the tube and the screw. In fact in some instances branching has begun from the screw.

Growth rates were measured as increase in total height of the colony. This alone is not a very meaningful measure since the increase in biomass is greatly influenced by the bushiness of the colony. As a measure of bushiness, the increase in the number of tips also was measured. These data are given in Table 1. Great care must be exercised in interpreting these data.

TABLE 1

GROWTH RATES OF *Plexaura homomalla*

Colony*	Start			Finish			Growth Rates		
K2a	6/11/69	10.25cm	4 tips	7/19/71	17.2cm	34 tips	.278cm/mo.	1.2	tips/mo.
K3c	6/11/69	25.7cm	6 tips	8/4/72	26.1cm	120 tips	.011cm/mo.	3.0	tips/mo.
K4d	6/11/69	23.5cm	16 tips	12/30/69	25.6cm	32 tips	.350cm/mo.	2.7	tips/mo.
K7a	6/11/69	25.3cm	54 tips	8/4/72	32.0cm	74 tips	.176cm/mo.	.53	tips/mo.
C3b	11/6/69	11.6cm	13 tips	8/4/72	15.4cm	24 tips	.115cm/mo.	.33	tips/mo.
C4b	6/6/69	15.2cm	38 tips	8/4/72	23.1cm	70 tips	.208cm/mo.	.97	tips/mo.
C9b	9/5/69	3.0cm	1 tip	8/4/72	7.0cm	5 tips	.114cm/mo.	.114	tips/mo.
C11b	9/5/69	11.2cm	4 tips	8/4/72	14.0cm	24 tips	.080cm/mo.	.57	tips/mo.
						$\bar{X}$	0.166cm/mo.	1.18	tips/mo.

Average annual growth rates: 2 cm/year, range 0.13-4.2 cm/year; 14.2 tips/year, range 0.14-24 tips/year.

*Colonies in the K series were at a depth of 1 m; those of the C series were at a depth of 16m.

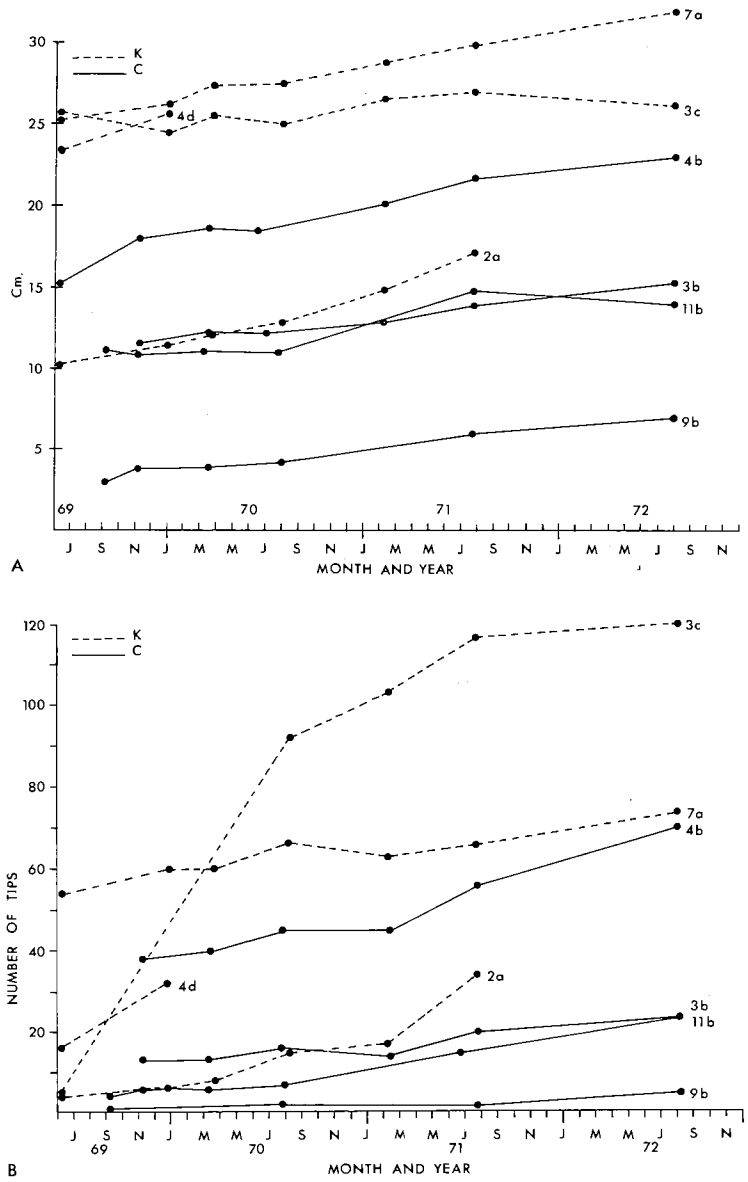

FIGURE 7. A, Growth rates of the eight colonies of *Plexaura homomalla* listed in Table 1. The C series were at a depth of 16 meters and the K series were one meter deep. Growth is measured as maximum height of the colony in cm. B, Growth rates of the same eight colonies. Growth measured as number of tips. (There is a possibility of error in the count of tips for colony K3C on 6/11/69. However, two independent records show that this colony had only 6 tips at that time.)

The variation is great both between colonies and even in the same colony during different time periods (Fig. 7). It is likely that much of this variation is environmentally induced, but the exact causes are unknown.

Good data exist for eight colonies of *Plexaura homomalla:* four from water one meter deep and four from a depth of 16 m. Most of these measurements were initiated in June of 1969. The most recent measurements were made in August 1972. The average growth rate taken over the maximum period of time of measurement for each colony is two cm/year, with a range of 0.1 to 4 cm/year. The average increase in number of tips is 14 tips/year with a range of 0.1 to 24 new tips/year. Note that the colony that added 24 new tips per year (No. K3c) showed an annual increase in height of only 0.13 cm. This indicates why caution must be used in interpreting simple growth data for gorgonians.

Reproduction.—In addition to increase in biomass by growth of existing colonies, potential new material also is added to the total on the reef by the settlement of new colonies. Whereas the increase in number of polyps in any existing colony is asexual, the increase in the number of colonies involves sexual reproduction. There is very little information available about the reproduction of reef coelenterates, and *Plexaura homomalla* is no exception. It is not known whether colonies are monoecious or dioecious,

FIGURE 8. Release of sperm by a colony of *Plexaura homomalla.* This colony was being shaken when the photograph was made, in order to produce a visible amount of sperm.

whether the sex of the colony remains constant throughout the life of the colony or even whether all of the polyps are sexually active at the same time. There is some suggestion that at any given time a colony is either male or female and that many of the polyps are at about the same stage of sexual maturity. It is likely that sperm are released from many colonies in more or less synchronous fashion (Fig. 8) and that fertilization is internal (Fig. 9). The time of development of the planula larvae in the polyp before release is not known, and the length of larval life is only approximately known to range from a few hours to some two or three weeks [7,13].

A little information has been accumulated which suggests that late summer is the period of reproduction for *Plexaura homomalla* as shown in this symposium. The only other Caribbean gorgonian whose reproduction is known is *Pseudopterogorgia bipinnata,* which planulates in late winter [7]. This suggests that biological parameters may have at least as much to do with seasonality of reproduction as physical ones.

After the larval period, the planulae settle and within a few days the asexual budding that will continue throughout the life of the colony has produced two or three new polyps (Fig. 10). Almost nothing is known about the growth rate or the mortality of these very early stages. There are no estimates of success of settling or recruitment.

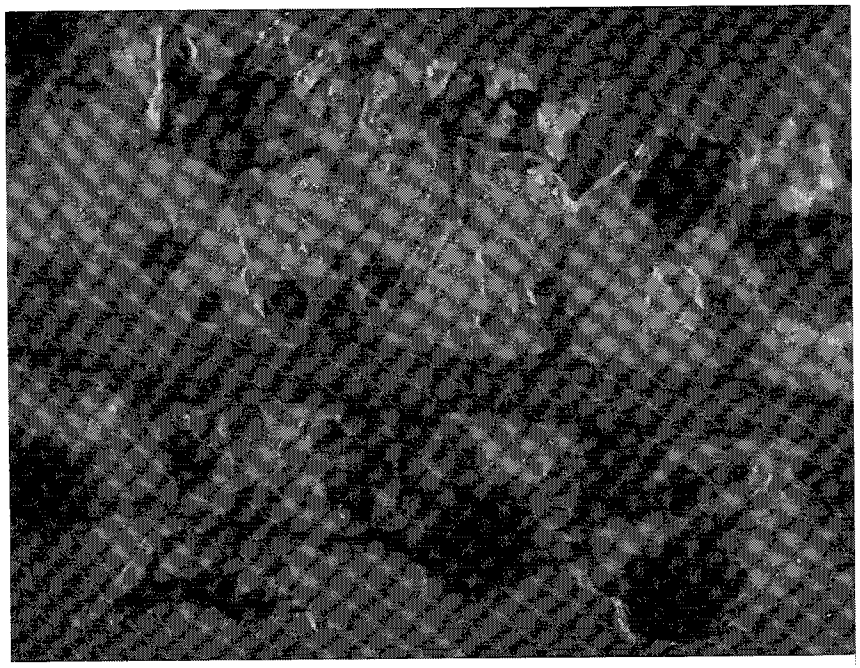

FIGURE 9. Fixed and cleared branch of a gorgonian. Note the eggs attached to the septa.

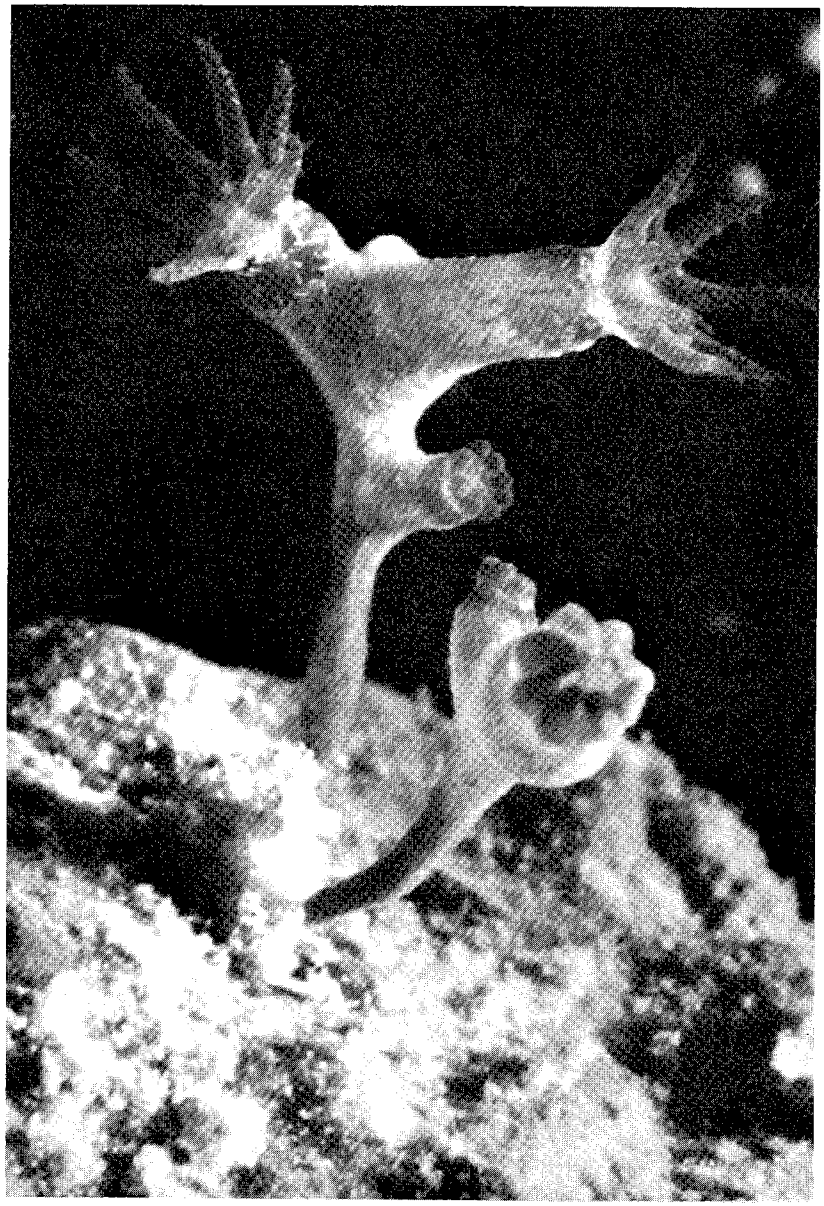

FIGURE 10. Photograph of two newly settled gorgonians. Note the form that the branching takes. Colonies about 2 weeks old. (Photo by T. F. Goreau.)

Harvesting of *Plexaura homomalla*

Given this knowledge of the biology and ecology of *Plexaura homomalla,* scant as it is, the problem was to determine the most efficient way to harvest reasonable amounts of gorgonian material without disturbing the situation on the reef which gave rise to the flourishing *Plexaura homomalla* populations, and without diminishing the continuing use of this resource.

It was evident from the beginning that harvesting entire colonies and waiting for sexual production, settling, and growth to replace the amount removed was not suitable for the following reasons.

1. Removal of a colony would reduce the reproductive potential of that population by an amount equal to the contribution of each individual taken.
2. Reproduction is at best annual, and for any individual colony perhaps less frequent.
3. The mortality of the newly settled polyps is high due to:
   a, overgrowth by algae;
   b, smothering by siltation, and sand scouring;
   c, predation by fishes and invertebrates.
4. The growth rate of young colonies, while not known, is certainly slow and apparently not much different from that of the adults (No. C9b, Table 1).
5. The changes in the ecology of the reef that would be brought about by the removal of a sizeable number of *Plexaura homomalla* colonies are unknown, but they might well reduce the production of planulae in the area. Harvesting of entire colonies could well alter the conditions which allowed the success of the *Plexaura homomalla* population in that area.

For these reasons it appeared that harvesting material by the removal of only part of the colony by cutting or pruning might be a better procedure. This method would utilize the ability of *Plexaura homomalla* to regenerate tissue removed, as demonstrated in the case of predation by *Cyphoma gibbosum.*

The advantages of this method are:

1. There is no need to wait for sexual reproduction to initiate the production of new gorgonian tissue since existing colonies simply regenerate tissue.
2. The losses due to mortality of the larvae and the newly settled colonies are eliminated.

3. The number of potential parent colonies is not reduced since substantial parts of the harvested colonies remain after harvest. (It should be noted however that the number of potential parent polyps is reduced.)
4. The ecology of the area would be less disturbed by a pruning type of harvest rather than by removing entire colonies.
5. The reduction in size by pruning might reduce the effects of storms that could otherwise topple these large colonies.

Thus, as a result of a suggestion provided by the apparent symbiosis between *Cyphoma* and *Plexaura homomalla,* the technique of harvesting a portion of each large colony in an area and allowing the regenerative abilities of the colony to replace the tissue removed was adopted.

Harvesting Technique.—One of the questions that had to be answered was: what is the optimal amount that should be removed from each colony? Part of this question is related to the size of the axis relative to the thickness of the coenenchyme (Fig. 11). If the colony is pruned too near the base, the axis makes cuttings difficult, tears the remaining parts of the colony, leaves a larger area for epibionts to settle on and takes longer to heal over. Furthermore, since the coenenchyme is thickest and the axis thinnest at the branch tips, these parts are most efficient for recovery of material. In addition, if the cuts are made higher up the colony, there are more tips from which possible regrowth could initiate. However, if the cuts are made too high the many small cut tips that result are difficult to retrieve from the bottom where they fall after cutting.

On empirical grounds it was determined to remove from 50 to 75 per cent of each large (over 30 cm) colony depending on the shape and bushiness of the colony. The largest area of axis exposed by this technique was not more than about 1.5 cm in diameter. The covering of these exposed ends is remarkably rapid. Most tips are sealed over in 3 to 4 days and healing of the largest cuts is complete within less than a week. A small number of the stumps were infected with filamentous algae which prevented regeneration. New growing tips were visible (often two per cut branch) within about 6 weeks (Fig. 12). A number of colonies thus harvested are currently being observed to determine:

1. The rate of regrowth of the new tips relative to the rate of growth of normal colonies.
2. The prostaglandin content of the regenerated tissue.
3. The reproductive potential of these new tips, and the untouched part of the harvested colonies relative to unharvested colonies.

This information will be used to answer questions about the suitability of *Plexaura homomalla* as a long-term source of prostaglandins. These questions are: What is the maximum sustainable yield from a population

of *Plexaura homomalla,* and what is a reasonable estimate of the potential production of prostaglandins from this source in the West Indies?

To answer this second question, as well as to obtain information about the yield of different reef areas, the measurement of the abundance of

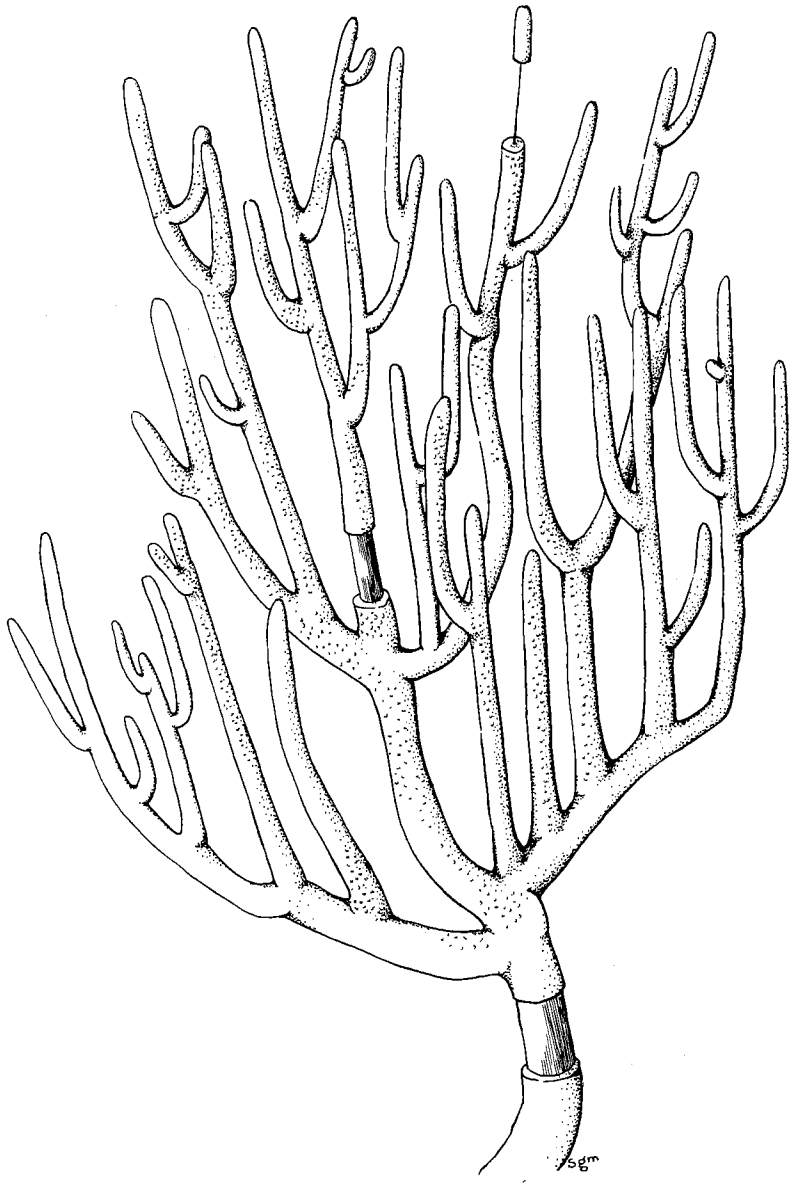

FIGURE 11. Diagram of a colony of *Plexaura homomalla* showing the relative proportions of axial skeleton and coenenchyme in different parts of the colony.

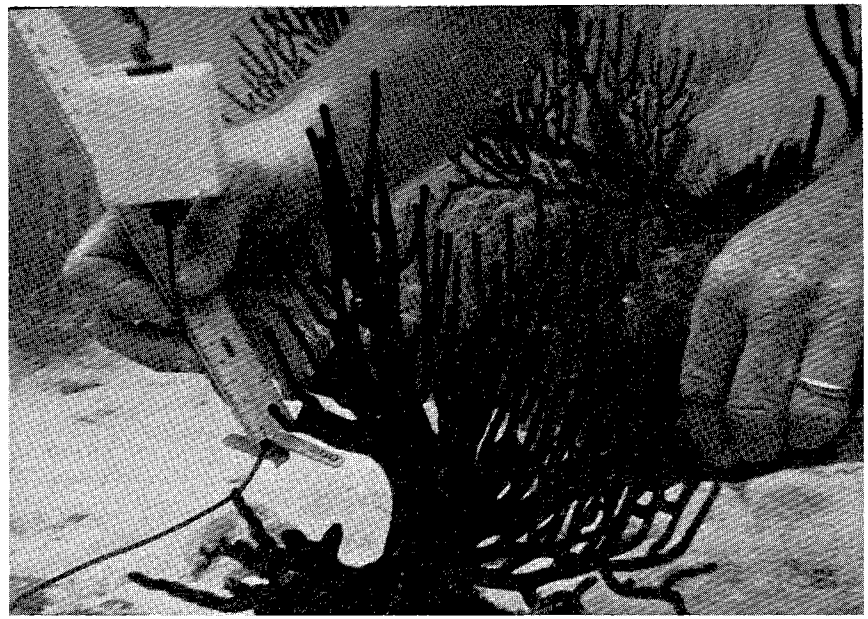

FIGURE 12. Photograph of a harvested colony of *Plexaura homomalla.* Note the two new tips growing from the cut branch end. This photograph was taken 6 months after the colony was harvested.

Plexaura homomalla is being undertaken along with the harvesting and regrowth studies. It has been found that *Plexaura homomalla* is not distributed randomly or evenly in the reef zones where it is common, but rather it is markedly clumped into patches. These patches, which are visible from the air, reflect to some extent areas of slightly shallower bottom (Fig. 5). The area of these patches spotted from the air is determined and an estimate of the amount of gorgonian material available on an areal basis is made from small sample harvests in selected patches. This information, along with estimates of the area and number of patches, gives some estimate of the amount of *Plexaura homomalla* available along a stretch of reef. These studies are currently underway along the reefs across the mouth of North Sound in Grand Cayman where some harvesting has been initiated, as reported in this symposium. Preliminary surveys have also been started along parts of the Andros reef in the Bahamas. By these surveys, areas suitable for harvest can be pinpointed and further investigation at the site can be undertaken.

It can be seen from the preceeding description of the harvest technique, that the situation at the present is essentially a fishery operation with the production of the standing crop being balanced against the harvested yield. While this is not at all a new idea to marine biologists, its application to a reef invertebrate, especially a coral, is unique and opens up areas for many questions that have not been asked about reef biology. For instance:

Does the reef represent a climax community? If so, can it sustain a yield over a long period of time? What factors regulate the periods of reproduction of reef animals and plants? Will selective predation (the harvesting) alter the diversity of the gorgonian fauna?

A further possibility of a method more sophisticated than a harvest-fishery is that of actual farming of gorgonians (see Schroeder, this symposium). The problems raised by an approach of this kind are even more complex than those raised here, but they are by no means insolvable and in attempting to find the solutions many other secrets of the reef will be brought to the light.

REFERENCES

1. ADAMS, R. O., 1968. *The color variation of Neosimnia (Mollusca, Gastropoda) with notes on the natural history.* M. S. Thesis, Florida State Univ.
2. BAYER, F. M., 1956. Octocorallia. In: *Treatise on Invertebrate Paleontology.* Ed. R. C. Moore. Univ. Kansas Press. xx + 498 pp.
3. BAYER, F. M., 1961. *The Shallow Water Octocorallia of the West Indian Region.* Martinus Nijhoff, The Hague. 373 pp., 27 pls.
4. GOREAU, T. F. AND N. I. GOREAU, 1959. The physiology of skeleton formation in corals, II: Calcium deposition by hermatypic corals under various conditions in the reef. *Biol. Bull. 117:* 239-250.
5. GOREAU, T. F. AND W. D. HARTMAN, 1963. Boring sponges as controlling factors in the formation and maintenance of coral reefs. In: *Mechanisms of Hard Tissue Destruction.* Publ. 75 A.A.A.S.
6. HYMAN, L. H., 1940. *The Invertebrates: Protozoa through Ctenophora.* McGraw-Hill, New York. vii + 726 pp.
7. KINZIE, R. A., 1970. *The ecology of the gorgonians (Cnidaria, Octocorallia) of Discovery Bay, Jamaica.* Ph.D. Thesis, Yale University.
8. MUSCATINE, L., 1967. Glycerol excretion by symbiotic algae from corals and *Tridacna* and its control by the host. *Science 156:* 516-519.
9. PEARSE, V. B. AND L. MUSCATINE, 1971. Role of symbiotic algae (Zooxanthellae) in coral calcification. *Biol. Bull. 141:* 352-363.
10. POMEROY, L. R. AND E. J. KUENZLER, 1969. Phosphorus turnover by coral reef animals. In: *Proc. 2nd Symb. Radioecol. U.S. At. Energy Comm.* TID4500, Ed. D. J. Nelson, F. C. Evans, pp. 474-482.
11. RANDALL, J. E., 1967. Food habits of Reef fishes of the West Indies. *Stud. Trop. Oceanogr. Miami 5:* 665-847.
12. TAYLOR, D., 1972. In press.
13. THEODOR, J., 1967. Contribution à l'étude des Gorgones VII: Ecologie et comportement de la planula. *Vie et Milieu 18:* 291-301.
14. TRENCH, R. K., 1971. The physiology and biochemistry of zooxanthellae symbiotic with marine coelenterates. I. The assimilation of photosynthetic products of zooxanthellae by two marine coelenterates. *Proc. Roy. Soc. Lond.* B *177:* 225-235.
15. WEINHEIMER, A. J. AND R. L. SPRAGGINS, 1969. Abstracts of the American Chemical Society Meeting. New York 1969.
16. YONGE, C. M., 1963. The biology of reef corals. *Adv. Mar. Biol. 1:* 209-260.

STUDIES ON EXPERIMENTAL HARVESTING AND REGROWTH OF *PLEXAURA HOMOMALLA* IN GRAND CAYMAN WATERS

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ABSTRACT

Increasing demands for prostaglandins for research and chemical testing have made it desirable to consider the feasibility of using the gorgonian *Plexaura homomalla* as a source of these substances. Studies were made to determine (1) the quantity of *P. homomalla* present in the experimental area, (2) ecologically acceptable methods of harvesting, (3) feasibility of artificial culture, (4) impact of harvesting upon the ecology of the reef, and (5) growth rates and reproduction.

INTRODUCTION

By 1969 when we learned of the exciting discovery [1, 2] of the occurrence of substantial quantities of A-type prostaglandins in *Plexaura homomalla,* we were faced with the problem of producting sufficient supplies of various prostaglandins to meet ever increasing research and clinical testing needs. The enzymatic bioconversion method had been scaled up to the point that we were using close to the world's total supply of sheep seminal vesicles. Even at that time we could forecast with confidence that the world's research needs alone could scarcely be met by utilization of this production method. Furthermore, while the cost of prostaglandins prepared by this method could be tolerated temporarily for research needs, it was clearly exorbitant for marketing. Total chemical synthesis of a number of the naturally occurring prostaglandins had been achieved by that time (1969) but the methods involved 15 to 20 steps, and a great deal of development research was needed to learn how to deal with this new and complex chemisty before large scale production by this route could be economically feasible. In addition, we considered it scientifically important to prepare modified structures of the naturally occurring prostaglandins so their properties could be evaluated. Although prostaglandins proved to be exceedingly potent, the parallel tasks of chemical research and chemical

production to meet biological and clinical needs taxed severely our available resources. With this background, you can understand why the discovery of Weinheimer and Spraggins generated a great amount of interest in our laboratories, because it presented the possibility of a third method for the preparation of prostaglandins.

Our decision to investigate this possibility was tempered, however, by two considerations: (1) We knew nothing about gorgonians or *P. homomalla* and we had no marine biologists among our research staff; and (2) We were totally committed as a corporation to a greater priority for ecological safety than for commercial opportunity. To be advised, we turned to experts in the field, and, at the suggestion of Dr. Weinheimer, we consulted with Dr. Bayer. We read all the literature on gorgonians and related species we could find and soon learned that Bayer's "The Shallow-Water Octocorallia of the West Indian Region" was the most valuable reference work available [3]. We also met Dr. Robert Kinzie, who at that time was just completing research for his Ph.D. degree at Yale on various aspects of the biology of *P. homomalla*. Serious discussions with these specialists soon convinced us that a careful investigation could safely be undertaken. With the assistance of these scientists we were able to obtain both fresh and preserved specimens of *P. homomalla* from a number of locations in the south Atlantic and the Caribbean Sea. Analytical methods for determination of the prostaglandins present in these samples were quickly worked out in our laboratory and a systematic investigation was soon under way. Then one day the unexpected happened: a sample of *P. homomalla* yielded PGA_2 with the 15S, or mammalian, configuration! This made the sea-whip intermediate even more important because the clinically useful PGE_2 and $PGF_{2\alpha}$ could be prepared with only 3 or 4 chemical steps. Soon after this we obtained through Dr. Kinzie a sample of *P. homomalla* from Grand Cayman Island and it also provided 15S-PGA_2.

In October 1970 Dr. W. J. H. Stone, Manager of Upjohn's Delray Beach facility, who had assisted us greatly in collecting samples from the Florida coast, collected several kilograms of *P. homomalla* from Grand Cayman. This material proved to be sufficiently interesting that we decided to organize an in-depth research project to study the economic and ecological feasibility of harvesting *P. homomalla* in Cayman Island waters provided we could get permission from the government to do so. After some preliminary correspondence, we went to Grand Cayman in December 1970 to discuss our proposed research project first with the Honorable Desmond Watler, Deputy Administrator, and then with His Excellency, A. C. E. Long, Administrator.* Permission was granted to initiate a conservative study with the understanding that ecological audits would be obtained and full reports made periodically to the government. Having secured this permission, we set about with the able help of my co-authors to carry out the experiments we wish to report today.

*In the fall of 1971, A. C. E. Long was promoted to Governor and Mr. Watler's title changed to Chief Secretary. Shortly thereafter, His Excellency, Roy Crook was appointed Governor of the Cayman Islands.

Objectives

Our overall objective was to determine, if possible, the economic and ecological feasibility of harvesting *P. homomalla* for large scale production of prostaglandins. It seemed to us that this would require the following:

1. Estimation of the quantities of *P. homomalla* available.
2. Development of methods for harvesting which would be ecologically acceptable and would provide maximum opportunity for replenishing the natural supply.
3. Determination of whether artificial cultivation of *P. homomalla* is possible.
4. Determination, if possible, of the influence of the removal of variable quantities of *P. homomalla* on the welfare of other reef fauna and flora.
5. Determination of the normal growth rates, size and reproductive capabilities of *P. homomalla* in Grand Cayman waters as compared to Florida and Jamaican waters.

These studies were begun in December 1970 with the continuing advice and recommendations of Drs. Bayer and Kinzie.

Methods and Results

To gain some insight in the quantity and distribution of *P. homomalla* in the Cayman Island waters, we chartered a fishing boat in North Sound and went to the location where Dr. Stone had made his collection in October 1970. A map of the area is shown in Figure 1. After showing the local charter boat captain the species we were interested in, he was very

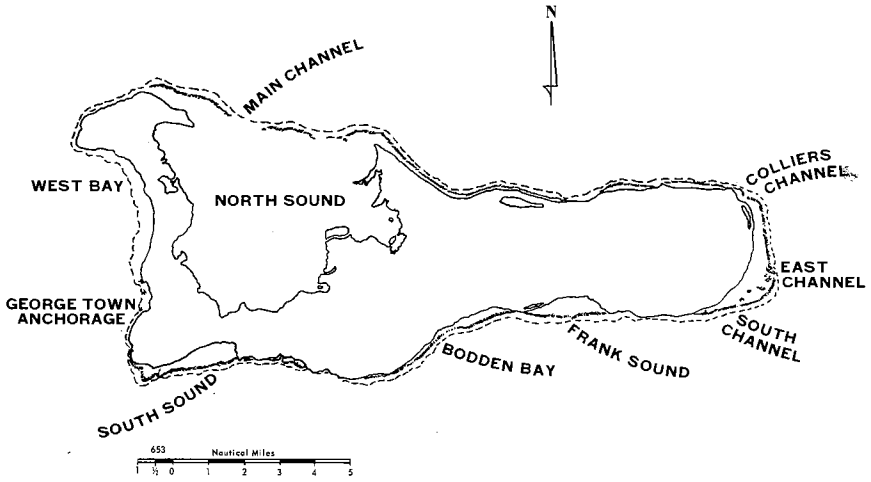


FIGURE 1. Grand Cayman Island, British Indies.

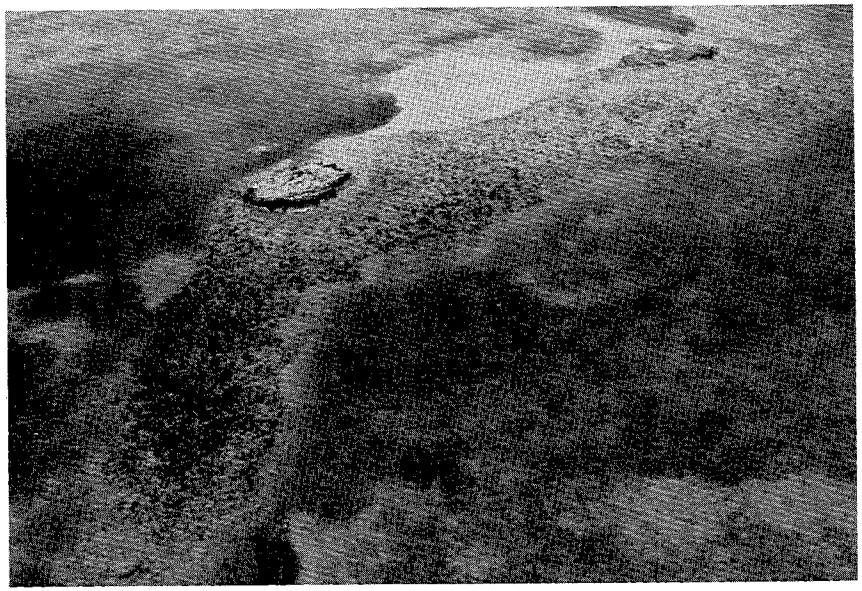


FIGURE 2. Low-altitude aerial photograph of a small area toward the west end of North Sound. View shows two small islands known as the Vidal Cays. Mottled dark region is gorgonian growth; uniform gray regions are turtle-grass flats in clear, shallow water.

helpful in taking us to locations where he knew it grew in abundance. Small analytical samples of *P. homomalla* were collected by divers from each of these locations and preserved in methanol for shipment to Kalamazoo. We soon gained the impression that there was an abundant supply of *P. homomalla* in the shallow water areas inside the barrier reef. Individual colonies seemed to average larger in size than those from the Florida coast and the proportion of *P. homomalla* to other gorgonian species appeared to be much higher. At this point, however, these were only qualitative impressions.

Aerial Photographs.—Noting that the major stands of *P. homomalla* occurred in extremely clear water over shallow, hard, white flats, we decided to try aerial surveys and photographs as an aid in locating *P. homomalla* and estimating its abundance. We chartered a light plane and starting with North Sound, which we had already studied from the surface and underwater, we surveyed the coastal waters of all three Cayman Islands. This method proved to be extremely effective in eliminating areas which did not contain *P. homomalla* and it also enabled us to tentatively identify areas containing gorgonians. Followup examination of the likely areas by diving was of course, necessary for positive identification. Using this technique we found and sampled large areas containing gorgonians with a high proportion of *P. homomalla* not only in North Sound, but also in South Sound,

FIGURE 3. Low-altitude aerial photograph of experimental harvest area west of the Vidal Cays in North Sound. Arrows point to marker buoys spaced 100 feet apart. At the time this photo was taken in June 1971, over a ton of *P. homomalla* had been harvested from the region adjacent to the left-arrow. Note diminished intensity of black spots in this region.

Frank Sound and inside the reef at the east end of the Island. In addition, there were substantial amounts of *P. homomalla* growing in the scenic and more difficult-to-harvest regions just inside and just outside the barrier reefs. The laboratory results from the small analytical samples taken from these areas were interesting: samples taken from the waters around Grand Cayman generally contained PGA_2 and its derivatives with the 15S or mammalian configuration rather than the 15R, although, as has been reported previously [4], these samples also contained the 5-*trans* PGA_2 and its esters.

With this information in hand, we decided for practical reasons to select North Sound* as our study area and proceeded with the investigation. Figures 2 and 3 illustrate the usefulness of aerial photographs for location of stands of *P. homomalla* and estimation of quantity in a particular area. Figure 2 is a low-level aerial photograph showing how gorgonian patches can be distinguished from turtle grass stands in these waters. The region shown here, adjacent to two small islands known as the Vidal Cays, was set aside as a control area where none of the colonies was harvested but two groups were tagged, measured and photographed in an effort to determine the normal growth rate under conditions typical of the region.

*The environments and organic communities of North Sound, Grand Cayman Island, have been described by Roberts (1971).

Aerial photos of this type were used to estimate the quantity of *P. homomalla* available in North Sound. By including a measured reference distance in the aerial photos and weighing the *P. homomalla* harvested from a measured small area within the larger area photographed, it was possible to calculate the approximate tonnage available in a given patch. On the advice of the marine biologists it was assumed that for the initial experiments it would be unlikely that any irreparable damage would be done to the eco-system if the experimental harvest was limited to less than 10 per cent of the *P. homomalla* available.

Figure 3 is a low level aerial photo of the main harvest area just west of the Vidal Cays off the Head of Barkers. The marker buoys visible in this picture are spaced 100 feet apart. Near the center of this photo there is an area where the density of dark spots is perceptibly diminished. From this area, over 1 ton of *P. homomalla* had been harvested. This is the region where the regrowth studies are underway. Similar experiments are being conducted at the east end of North Sound off Rum Point.

Weighed Harvests from Measured Areas.—to obtain baseline information on the amount of *P. homomalla* per unit area, to evaluate regeneration growth, and at the same time collect material for our laboratory experiments, the following procedures were used. In December 1970, square areas 25 feet on a side (625 sq. ft.) were measured off on the ocean floor and all *P. homomalla* colonies within the marked area were harvested, by drastic pruning in one case and by complete removal in another. These control experiments were carried out in the part of North Sound between Head of Barkers and the barrier reef–the region shown in the aerial photo, Figure 3. The specimens were collected by divers, allowed to drain for a few minutes, ground, packed in plastic bags and weighed. The 625-sq. ft. area from which every colony of *P. homomalla* was harvested was considered 100 per cent. A similar-sized area adjacent to this, harvested by drastic pruning but leaving the bare "stumps," yielded 80 per cent. We would like to emphasize that these are the only cases where such drastic harvest methods were used and to point out that the regrowth data on these areas will be reported later. Other 625-sq. ft. areas close by were harvested by the technique recommended by Dr. Kenzie, i.e., careful pruning of up to 75 per cent of major branches from larger (more than 12 inches in height) colonies and leaving all colonies less than 12 inches in height intact. In addition to being the preferred harvest method from the point of view of conservation, this method also provides a richer source of prostaglandins because the desired compounds are concentrated in the living soft tissue. In this part of North Sound the Kinzie method of harvesting provided about 62 per cent of the base amount obtained by total harvest. Similar studies were carried out in the Rum Point region using the conservative pruning technique and preliminary results indicate that the yield from this region would be roughly half that determined for the other test area. It is apparent from these studies that the density of *P. homomalla* growth varies widely

from one part of North Sound to another, and while these waters clearly contain many tons of *P. homomalla,* more data are required to determine the exact amount.

Estimation of Portion of Gorgonians represented by P. homomalla.—As indicated earlier, casual underwater inspection of the Grand Cayman waters gave us the impression that *P. homomalla* occurred in higher proportion to other gorgonian species than was usually observed in other regions of the South Atlantic and the Caribbean Sea. In an attempt to quantitate this observation, we stretched a 200-foot yellow and blue nylon line on the ocean floor in several representative locations in gorgonian stands in North Sound. Observers snorkelled the length of the line and counted: (1) *P. homomalla* colonies more than 12 inches in height, (2) *P. homomalla* colonies less than 12 inches in height and (3) all other gorgonian species regardless of size. In all cases only those specimens crossed by the line were counted. Results of several of these "line counts" are summarized in Table 1, confirming the impression that *P. homomalla* is the dominant gorgonian species in these waters.

TABLE 1

RATIO OF *P. homomalla* TO OTHER GORGONIAN SPECIES IN NORTH SOUND

Location	*P. homomalla* <12 inches	*P. homomalla* >12 inches	Other Gorgonians	*P. homomalla* %
Rum Point	5	16	16	57
Rum Point	9	20	20	59
Head of Barkers	9	31	41	50

Experimental Harvest Technique.—With the exception of the two 625-sq. ft. areas mentioned previously, all harvesting was carried out using the conservative pruning method recommended by Dr. Kinzie. Only large colonies were harvested, i.e., those which were obviously in excess of one foot in height. Many of the colonies in these waters are 3 feet or more in height. This is in marked contrast to specimens in Florida waters where a 17-inch specimen was reported as large. Up to 75 per cent of the main branches of large colonies were pruned using a hawkbilled pruning shears as illustrated in Figure 4. Care was taken to sever the branch near the base with a single, clean cut perpendicular to the length of the branch. This exposed the minimum area of axis and reduced the possibility of overgrowth by millepore. It is interesting to note that pruning of a colony was always accompanied by the release of brown "cloud" which appears to be laden with prostaglandins and perhaps other pharmacologically active substances.

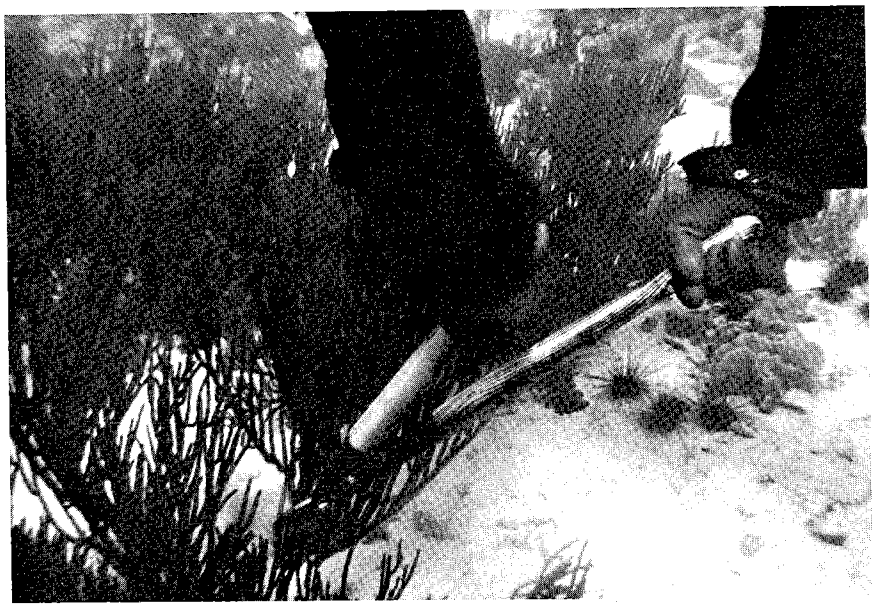


FIGURE 4. Illustration of pruning technique.

The cut branches were carried by hand or in a diver's bag to the boat. In the early harvesting experiments, the collected *P. homomalla* was ground to a fine pulp, packed in plastic bags and frozen quickly with dry ice. The plastic bags were packed into insulated shipping cartons and these were stored in the Mariculture freezer until they were shipped by air freight to Kalamazoo.

Regrowth Studies.—At the outset, the most important unknown among the factors involved in this study was whether or not *P. homomalla* colonies harvester by the methods described would survive and regenerate. Consequently, a major objective was to acquire reliable and quantative measurement to answer these important questions. A fundamental requirement for accomplishing this objective was a reliable method for identifying individual colonies and finding them again after various time intervals. The following method was devised and used in the test areas just west of Vidal Cay in North Sound.

It was considered unwise to use any kind of tagging system that involved attachment of a foreign substance to the colony because it would be difficult to determine what influence it might exert. To minimize the problem of finding the tag it was decided to use a float which would keep the marker a foot or so above the ocean floor. The tagging equipment used is illustrated in Figure 5.

The colony identification number was stamped and printed on a plastic tag which was attached to a nylon line securely anchored to a mountain-

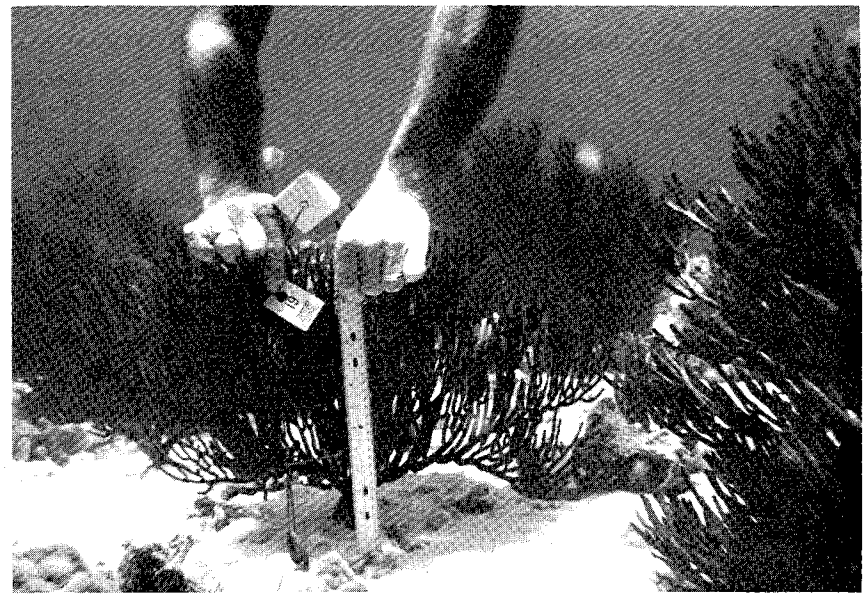


FIGURE 5. Tagging equipment used for identification and relocation of individual *P. homomalla* colonies.

climber's steel piton driven into the hard rock bottom. The other end of the nylon line was attached to a cube of low density polyurethane which served as the float to keep the tag suspended for maximum visibility. The number tags were color-coded to indicate the study area involved.

Using this tagging method, 10 colonies were identified in each regrowth study area. Each study group was located by surface bearings. Fearing that tags might be lost during severe weather or from vandalism, an additional precaution was taken to assist in locating the individual colonies. Near the center of each study group, a two-foot length of 2-inch pipe was driven into the ocean floor. The compass direction and the distance of each test colony from the pipe was recorded providing a map of the type illustrated in Figure 6. Thus far this method has proved quite satisfactory although in spite of these precautions we have lost track of a few of our tagged colonies.

Devising a method for accurate recording of new growth proved to be a technical problem almost as difficult as the determination of the total quantity available. We started by taking a close-up photo of the tagged colony with a plastic ruler behind it. Our hope was that we could use the photograph as a permanent record and read the measurements of growth change from it. Unfortunately, this did not prove to be very satisfactory for precise measurement, so we substituted a grid with white background with black lines forming 1-inch (2.5 cm) squares for the ruler. This was an improvement in some respects but the large white background contributed

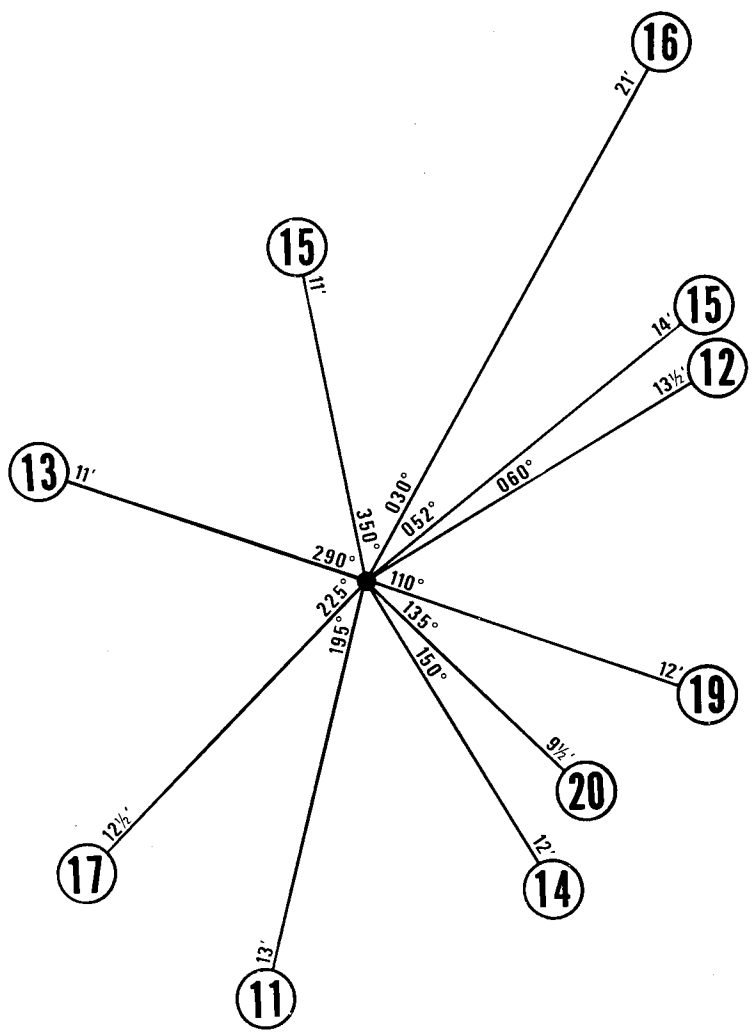


FIGURE 6. Example of map used for relocation of individual in a study area.

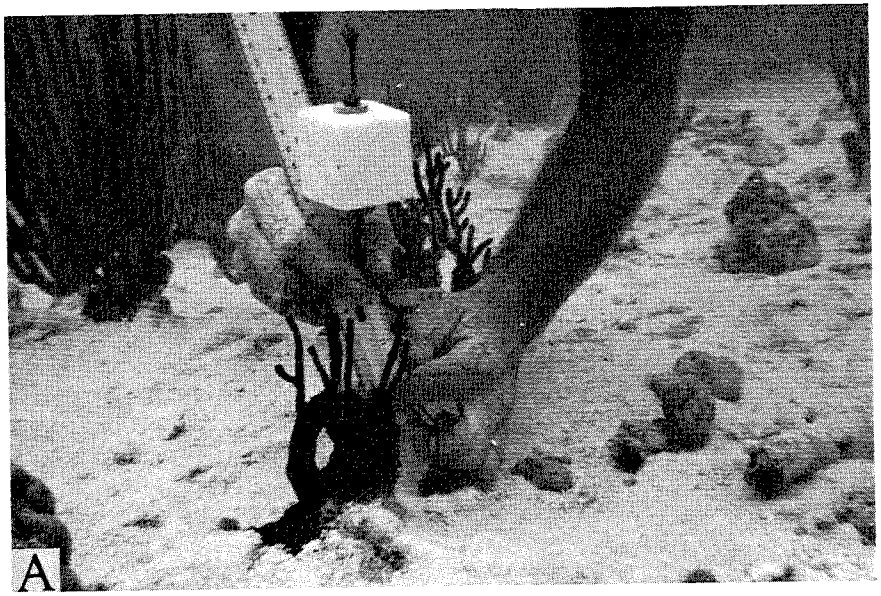
to problems of exposure and depth perception with the photography. This difficulty has been largely overcome by using a grid which has a blue-green background and white lines. We encountered other simple practical problems which we should have anticipated but did not. For example, we were not as careful as we should have been in selecting test colonies without other specimens or obstructions so close as to complicate the photography. And we did not always make follow-up photos from exactly the same position and distance.

Still another difficulty was related to the amount of pruning the colony had been subjected to. Considering the colonies in the test area which had been drastically pruned, it was easy to detect new growth. On the other hand, colonies pruned conservatively by the standard harvest method of Kinzie have so many branches and shoots on them that it is difficult to assess new growth, especially from the kind of photographs we were able to get. However, it was possible even in the latter case, on close visual inspection, to recognize unmistakably new growth from the cut branches. Although it was a tedious job, these were measured and recorded. This is what we have done with two of the study groups to get some quantitative assessment of the regrowth rate. This, of course, is not an accurate indication of the total replacement of bio-mass because all of the branches new and old are growing, but it does provide quantitative measurements of linear growth of new shoots.

The photographs in Figure 7 were selected to illustrate these findings with colony No. 149 in the A-1 test group, the only test group which was subjected to drastic pruning. In December 1970, this colony was pruned free of all small shoots and major branches were cut to within 3 inches of the holdfast. By February 1971, new tissue had covered the exposed axis, but no new shoots were in evidence. When observed in June 1971, it was clear that several small shoots had emerged from the base (Photograph A). Continued growth and branching of the shoots were noted six months later (Photograph B) in December 1971. About a month later it was observed that some soft tissue had been accidentally removed from two of the new shoots (Photograph C). The last opportunity to observe this colony was in May 1972. Photograph D shows that new soft tissue had overgrown the exposed axis and further growth and branching had taken place.

Table 2 summarizes the regrowth data for the tagged colonies in the A-1 test group (those drastically pruned in December 1970). At the beginning of this experiment there were ten tagged colonies in this test group. At the last visit we failed to locate two of them. Of the remaining eight, only one has failed to produce new growth. The number and size of the new growth varied considerably. For the chart notations, branched growth was counted only as one shoot and only the length of the longest branch was recorded.

Figure 8 shows the record photos for Colony No. 4 in the B-1 test group. These colonies were harvested in March 1971 using the more conservative pruning technique. Photograph A shows Colony 4 as it appeared in June 1971 and it illustrates the problem of assessing new growth from



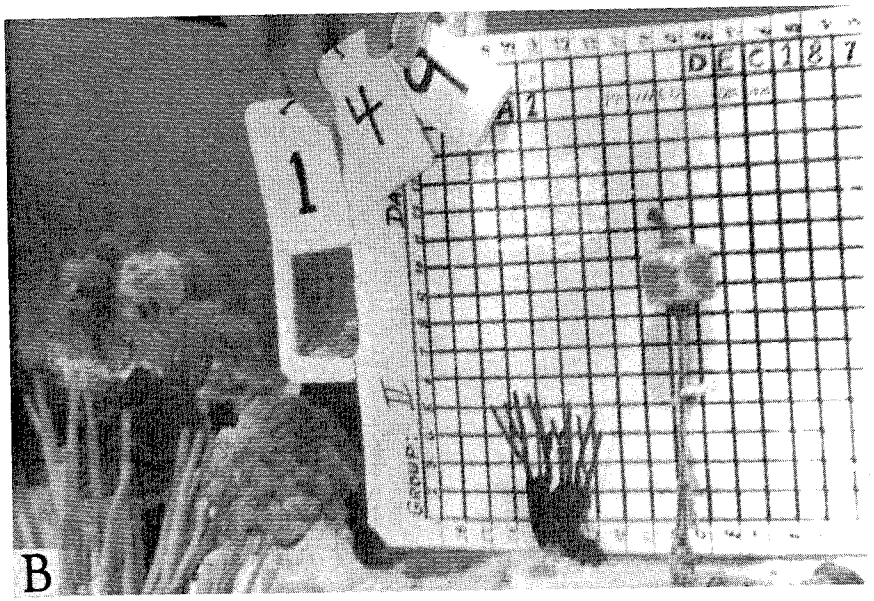
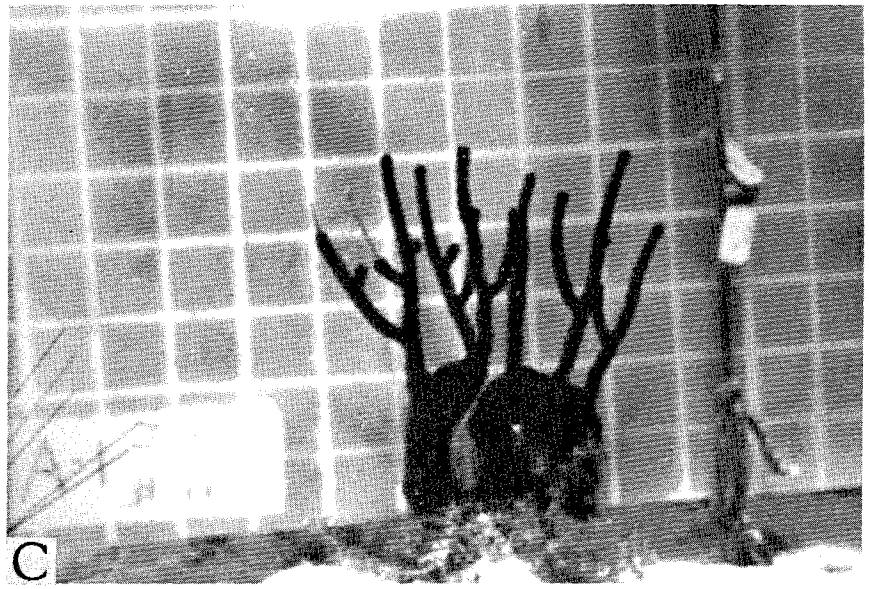


FIGURE 7, A, B. Colony no. 149 of test group A-1 after drastic pruning: A, 6 months after pruning; B, 12 months after pruning.



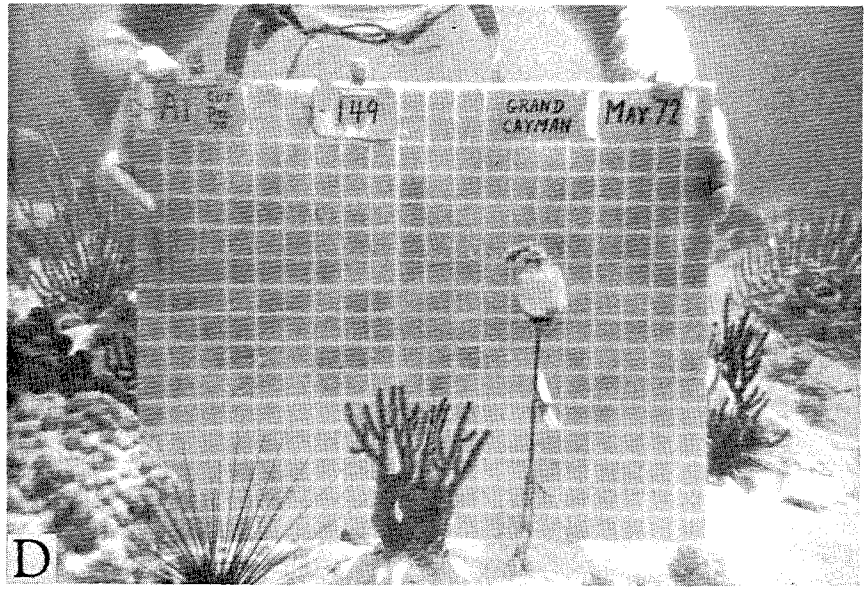


FIGURE 7, C, D. Colony no. 149 of test group A-1 after drastic pruning: C, 13 months after pruning, showing damaged shoots; D, 23 months after pruning, showing healing of damaged shoots.

photographs of colonies which had been pruned by the more conservative method. It is difficult to distinguish new shoots from shoots which had not been pruned. However, this can be detected and measured by close visual inspection. For example, the branch immediately in front of the ruler in photograph A shows two new shoots growing from the base of the harvested branch. Photograph B shows the same colony in December 1971; photograph C, January 1972, and photograph D, May 1972. Qualitatively it can be seen that this colony has survived and is growing, but actual measurement of new shoots was required for quantitative assessment.

Table 3 summarizes the regrowth data for the B-1 test group. Again one of the colonies failed to show new growth, but is still surviving. The other nine colonies appear to be increasing their biomass at a variable but fairly

TABLE 2

REGROWTH OF TAGGED COLONIES
1 YEAR AFTER HARVESTING

(A-1 TEST GROUP)

Colony Number	Number of new branches	Length of new branches (cm)	Average
32	9	4,4,3.5,3,1.5,4.6,2.5,4	3.6
149	7	7,9,7,6,9,7,8	7.6
209	4	8,9,8,7	8.0
251	0	—	—
260	8	7,3,4,9,4,7,4,14	6.5
279	2	2.5,3	2.8
300	6	3,6,3,4,1,1	3.0
423	4	1,1,1,4	1.8

respectable rate. The observation period clearly is not long enough yet to to indicate accurately the length of time required for regrowth of the biomass removed by harvesting. We can say, however, that it is definitely more than one year. These findings are very preliminary, but they are encouraging in that they suggest that the vast majority of *P. homomalla* colonies in these waters, when harvested by the methods described, not only survive but have the capability of generating new growth.

Control Studies.—Inasmuch as there are no reports in the literature on the normal growth rate of *P. homomalla* in the waters of this region, it seemed worthwhile to initiate some experiments designed to determine this rate. As previously mentioned, an area just west of the Vidal Cays was reserved for these studies. Two groups of 5 small colonies each, designated as test groups C-1 and C-2, were mapped and tagged using the same procedure

as described for the harvested colonies. Figure 9 shows a representative member of this group (Colony No. 7 in C-2) as it appeared in June 1971, and almost a year later, in May 1972. It is difficult to determine from these pictures alone, but on-the-spot observations and measurements suggest that the branches have increased in length by only 1 to 2 cm at most, although there does appear to be a detactable increase in total biomass. These observations, which are only preliminary at best, still suggest the possibility that regrowth of pruned large colonies may be capable of regenerating a greater total of new biomass than that represented by normal growth of undisturbed small colonies. This is but one of many interesting questions which we are trying to answer during the course of this project.

TABLE 3

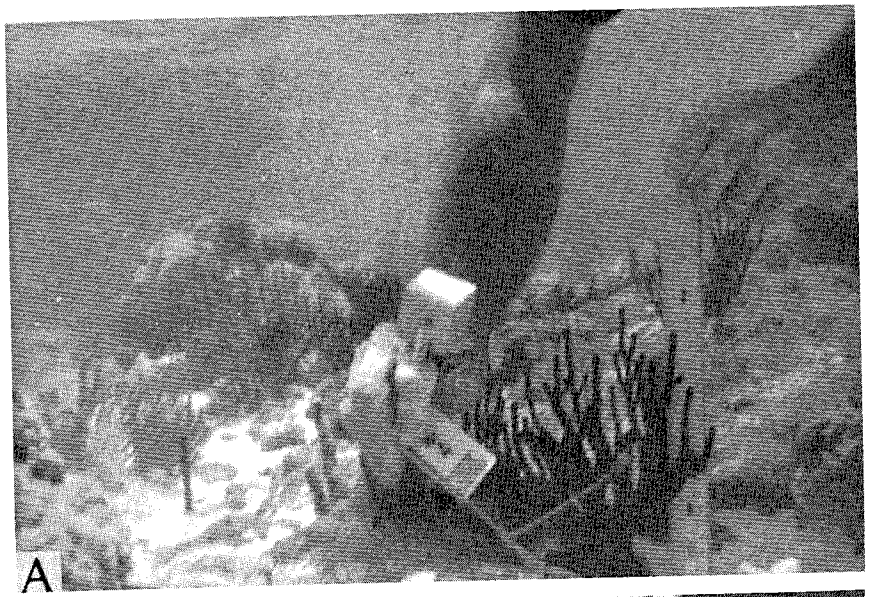
REGROWTH OF TAGGED COLONIES
10 MONTHS AFTER HARVESTING

(B-1 TEST GROUP)

Colony Number	Number of new branches	Length of new branches (cm)	Average
1	0	—	—
2	2	2,4	3.0
3	17	3,2,7,3,3,4,7,8,6,8,1,1, 2,7,5,3,5	5.0
4	9	8,3,12,7,4.5,8,10,7,9	6.6
5	11	3,6,6,5,4,7,6,8,4,5,6	5.5
6	2	3,2	2.5
7	15	4,4,8,2,4,3,5,1,1,3,2, 5,2,2,3	3.2
8	10	1,2,3,6,5,2,1,3,1,1	2.5
9	8	3,3,4,7,5,5,1,1	3.6
10	3	5,5,5	5.0

SUMMARY AND CONCLUSIONS

Stimulated by the discovery of Weinheimer and Spraggins and assisted by many outstanding marine biologists and through the cooperation of the Cayman Islands Government and Mariculture, Ltd., we have had the unique opportunity to investigate the feasibility of harvesting *P. homomalla,* a marine resource rich in intermediates which can be used for the preparation of clinically useful prostaglandins. In our judgment these studies to date indicate that *P. homomalla* can be harvested by methods which permit regrowth and do not inflict any discernible damage to the ecosystem involved.



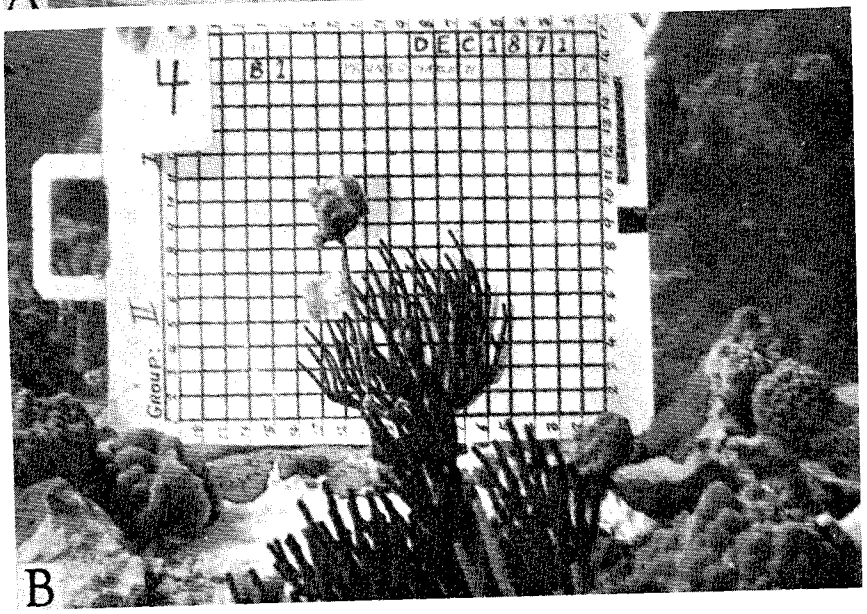
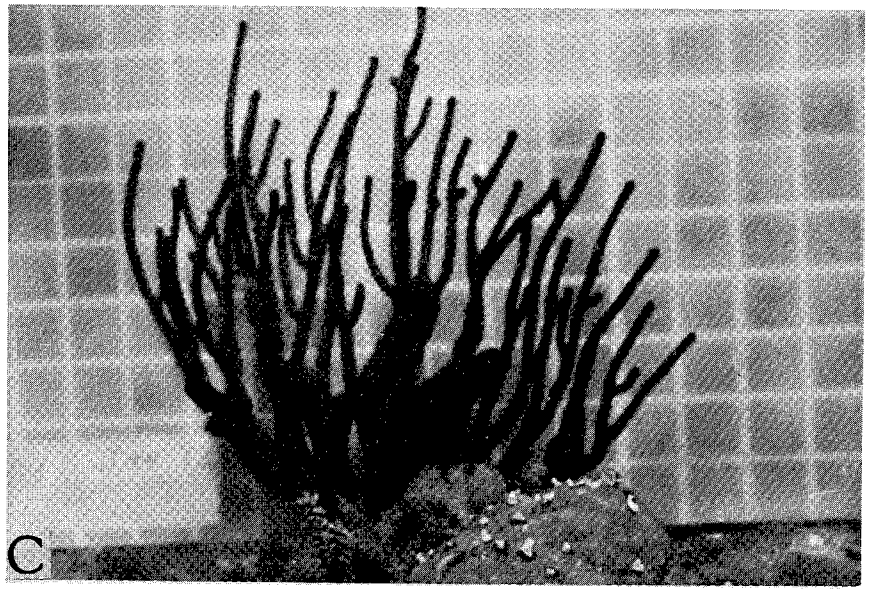


FIGURE 8, A, B. Colony no. 4 of test group B-1 post harvest: A, 3 months after pruning; B, 9 months after pruning.



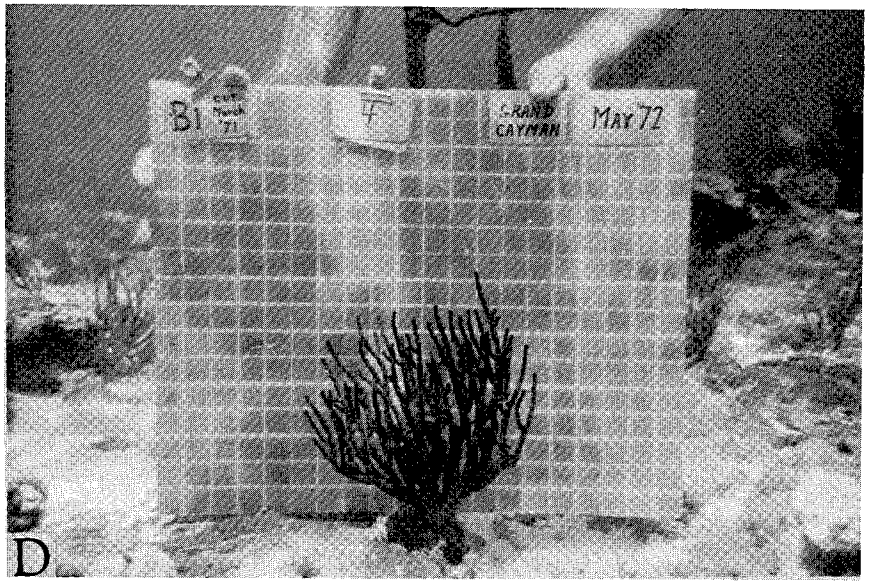


FIGURE 8, C, D. Colony no. 4 of test group B-1 post harvest: C, 10 months after pruning, close-up; D, 14 months after pruning.

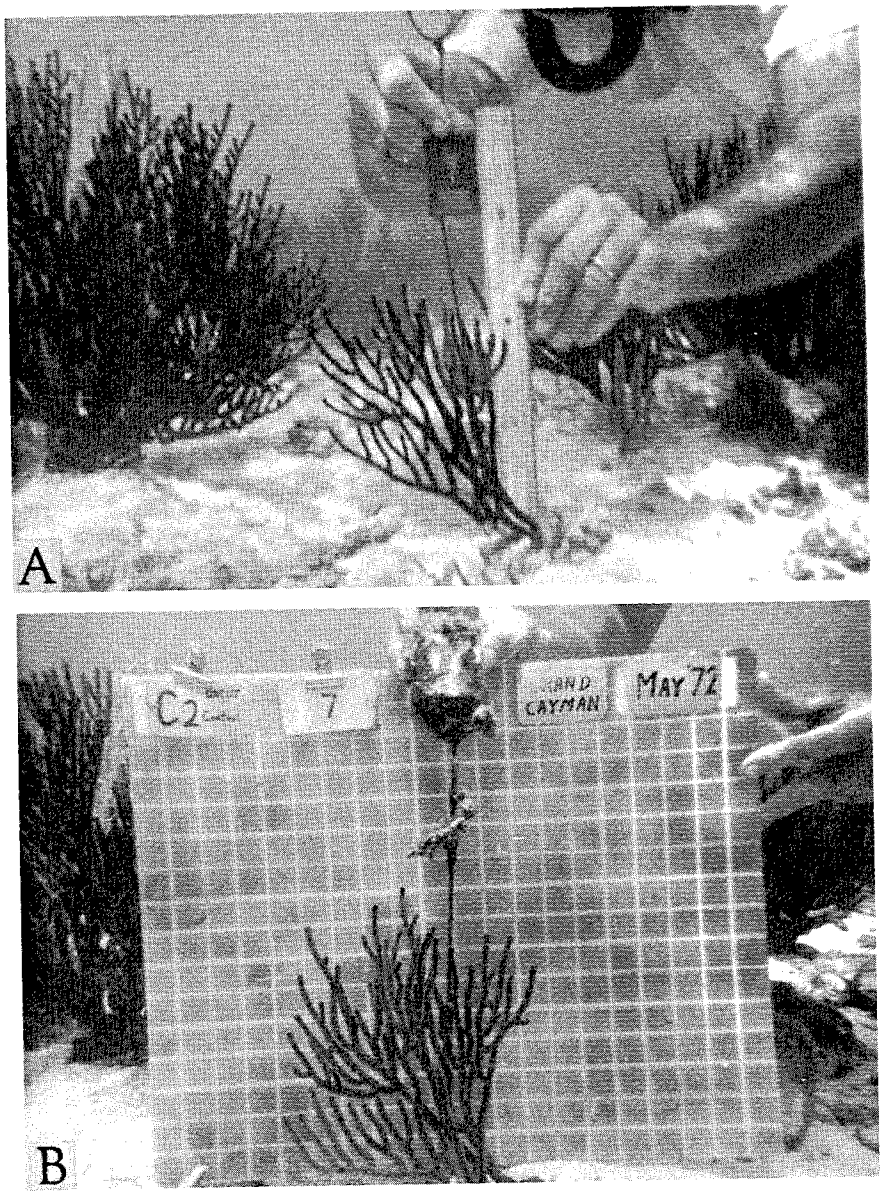


FIGURE 9, A, B. Colony no. 7 of control group C-2: A, photograph taken in June 1971; B, photograph taken in May 1972.

REFERENCES

1. WEINHEIMER, A. J. AND R. L. SPRAGGINS, 1969. Two new prostaglandins isolated from the gorgonian *Plexaura homomalla* (Esper). In: *Food-Drugs from the Sea Proceedings,* H. W. Youngken, Jr., Ed., pp. 311-314. Marine Technology Society.
2. WEINHEIMER, A. J. AND R. L. SPRAGGINS, 1969. The occurrence of two new prostaglandin derivatives (15-epi-PGA_2 and its acetate, methyl ester) in the gorgonian *Plexaura homomalla. Tetrahedron Lett.,* 5183-5188.
3. BAYER, FREDERICK, M., 1961. *The shallow-water Octocorallia of the West Indian Region.* Martinus Nihjoff, The Hague.
4. BUNDY, G. L., E. G. DANIELS, F. H. LINCOLN AND J. E. PIKE, 1972. Isolation of a new naturally occurring prostaglandin, 5-*trans*-PGA_2. Synthesis of 5-*trans*-PGE_2 and 5-*trans*-PGF_2. *J. Amer. Chem. Soc. 94:* 2124.
5. ROBERTS, H. H., 1971. Environments and organic communities of North Sound, Grand Cayman Island, B.W.I. *Carib. J. Sci. 11:* 67-79.

THE SEXUAL CYCLE IN *PLEXAURA HOMOMALLA**

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ABSTRACT

Colonies of the prostaglandin-containing gorgonian *Plexaura homomalla* (Esper) were tagged in the Florida Keys. Samples were taken at monthly intervals during a two-year period in an effort to determine the nature of the sexual cycle. Paraffin sections of apical polyps have shown that colonies are diecious. During eight to nine months of the year only female or sexually indeterminate colonies are found. Male colonies are discernible only during the summer, particularly in June and July. At this time all specimens are mature and appear to be sexually active.

INTRODUCTION

Despite the prominence of the Octocorallia in many marine habitats, studies on their sexual cycle have been few in number. Six orders compose the group and of these, nothing is known of any of the Telestacea or Coenothecalia. Our knowledge of the life history of the Stolonifera is limited to a brief description of a species of *Clavularia* [5]. The sexual cycle of the soft corals (order Alcyonacea) has been described in the tropical family Xeniidae thanks to the work of the same author [4, 6], while reproduction and development in the European alcyonacean *Alcyonium digitatum* (L.) has been studied by Matthews [12]. Reproductive biology of octocorals is probably best known among the pennatulaceans (sea pens). Wilson's study of *Renilla* [15] and Berg's study of *Funiculina* [2] are two of the more noteworthy papers in this group. The sexual cycle in the order Gorgonacea is known from the Mediterranean species *Corallium rubrum* (L.) [14] and *Eunicella stricta* (Bertolini) [13]. Grigg made similar studies on the life cycles of two California species of *Muricea* [7]. Only three gorgonian species have been studies in the Atlantic-Caribbean area. Wilson noted the development of eggs and planulae in *Leptogorgia virgulata* (Lamarck)

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[15] as did Cary in *Plexaura flexuosa* Lamouroux [3]. More recently, Kinzie studied the larval ecology and life history of *Pseudopterogorgia bipinnata* (Verrill) in Jamaica [8].

The sexual cycle, larval morphology and development of most all octocorals studied to date demonstrate remarkable similarities. Although it is tempting to generalize on this basis, it should be noted that Kinzie's study is the only detailed account available for any Caribbean species [8]. Considering the diversity of gorgonians in this area [1, 8] it would seem desirable to study others as well. Recent interest in the prostaglandin-containing gorgonian *Plexaura homomalla* (Esper) has provided impetus for such an investigation. This species is one of the more distinctive and easily recognized reef-dwelling forms. It is known from Bermuda, southern Florida and the Caribbean islands [1], but appears to be restricted to well-developed reef situations. *P. homomalla* seems to thrive in shallow water, but becomes increasingly rare with relatively slight changes in depth. In Forida waters it is most uncommon in depths below 10 m.

MATERIALS AND METHODS

A total of 75 mature colonies of *P. homomalla* were tagged in a depth of 2.4 m in the vicinity of Bache Shoal in the northern Florida Keys. Fifty colonies were tagged in 1971 and sampled for one year. The tags were lost or destroyed during 1972 and this led to tagging 25 new colonies in 1973. Every six to eight weeks, samples were taken at random from the branch tips of each colony. These samples were immediately fixed and decalcified in Bouin's fluid for 4-21 days after collecting. Paraffin impregnation was accomplished with an Autotechnicon using standard histological techniques. Longitudinal serial sections were cut at a thickness of five microns and were stained with hematoxylin and eosin.

RESULTS AND DISCUSSION

The data shown in Table 1 clearly demonstrate the sharp periodicity of the sexual cycle in *P. homomalla*. Through most of the year, specimens are either sexually unrecognizable or female. In the former case, no sexual products could be discerned in sections. Specimens identified as female bore eosinophilic, nucleated eggs with diameters ranging from 0.05-0.35 mm. Mature females were easily recognizable with eggs nearly filling the coelenteric cavity. The maximal number of eggs per polyp is not known, but Kinzie reports that polyps from a winter-breeding *Pseudopterogorgia* could each produce 6-10 planula larvae [8]. However, neither planulation nor cleavage of eggs was ever noted during the present study.

Samples taken in early June were the first in which male gonads were found. The spermaries were approximately 0.14 mm in diameter and stained deeply with hematoxylin. They were packed with developing sperm and were readily distinguishable from the larger, yolk-laden eggs. Male and female gonads were never found in the same colony. However, a few

occasions were noted when a given colony appeared to change sex in successive months especially in the collections made in 1971. Careful sampling in 1973 revealed only one such "transsexual" colony. We are inclined to attribute this to collecting error. Similarly, the colonial and intrapolypar hermaphroditism reported in precious red coral, *Corallium rubrum,* by Lacaze-Duthiers [11] was not confirmed by Vighi in an examination of 123 colonies [14]. The hermaphroditism in *Primnoa resedaeformis* (Gunnerus) reported by Kükenthal has not been re-examined critically but there is no reason to assume this observation is incorrect [10]. Our ignorance of the basic biology of such deep water gorgonians approaches totality. Gohar has demonstrated hermaphroditism in several species of alcyonacean octocorals from the Red Sea [4]. Thus, we are not at present in a position to make general statements about octocoral life cycles.

TABLE 1

MONTHLY RECORD OF SEXUAL DEVELOPMENT IN *Plexaura homomalla* AT BACHE SHOAL, FLORIDA

Date	Total No. Specimens Sampled	Percent Sexually Indeterminate	Total Males: Females
September 1972	20	50.0	0:10
November 1970, 1972	68	55.9	0:30
January 1971, 1973	41	58.5	0:17
March 1971, 1973	47	53.2	0:22
April 1971, 1973	43	48.8	0:22
June 1971, 1973	65	0	30:35
July 1973	18	5.5	10:8
August 1971, 1972, 1973	66	39.4	8:32

As shown in Table 1, April collections were devoid of males. By early June, however, the male popuation became recognizable, indicating rapid development during the month of May. The decline noted in the number of discernible males in August is evidence that June and July are the months of greatest sexual activity in *P. homomalla.* No males were found outside of the May-August period.

The fate of the asexual colonies was predictable to the extent that 70 per cent of such cases became recognizable males by June. The remainder developed eggs. Since only large colonies were tagged, all specimens were mature and none remained asexual all year. The critical size and age for the onset of sexuality is not known.

The length of time a colony remains sexually active appears to be unpredictable. We have several instances of female colonies remaining identifiable throughout the year. In other cases, colonies were asexual until the May-June period, became sexually recognizable and then reverted to the asexual state before July.

Cary [3] found that *P. flexuosa,* another reef-dwelling species common in Florida, has a similar sexual cycle: "In June 1910 almost every colony of *P. flexuosa* was carrying mature eggs . . . In July 1911 some colonies of *P. flexuosa* contained ripe eggs, but the greater number were without gonads. Late in August 1912 not a single specimen was found in which gonads were recognizable . . ." Female gonads are apparently not present outside of the breeding season in this species. The significance of this difference remains to be determined.

REFERENCES

1. BAYER, F. M., 1961. The shallow-water Octocorallia of the West Indian region. Martinus Nijhoff, The Hague, 373 pp.
2. BERG, S. E., 1941. Die Entwicklung and Koloniebildung bei *Funiculina quadrangularis* (Pallas). *Zool. Bidr. 20:* 1-100.
3. CARY L. R., 1914. Observations upon the growth-rate and ecology of gorgonians. *Pub. Carnegie Inst. Wash. 182:* 79-90.
4. GOHAR, H. A. F., 1940. Studies on the Xeniidae of the Red Sea. *Publ. Mar. Biol. Sta. Ghardaqa 2:* 25-120.
5. GOHAR, H. A. F., 1948. A description and some biological studies of a new alcyonarian species *Clavularia hamra* Gohar. *Pub. Mar. Biol. Sta. Ghardaqa 6:* 3-34.
6. GOHAR, H. A. F. AND H. M. ROUSHDY, 1961. On the embryology of the Xeniidae (Alcyonaria) (with notes on the extrusion of the larvae). *Pub. Mar. Biol. Sta. Ghardaqa 11:* 45-72.
7. GRIGG, R. W., 1970. *Ecology and population dynamics of the gorgonians, Muricea californica and Muricea fruticosa, Coelenterata: Anthozoa.* Ph.D. dissertation, University of California, San Diego, 260 pp.
8. KINZIE, R. A., 1970. *The ecology of the gorgonians of Discovery Bay, Jamaica.* Ph.D. dissertation, Yale University, New Haven, Conn., 107 pp.
9. KINZIE, R. A., 1973. The zonation of West Indian gorgonians. *Bull. Mar. Sci. 23*(1): 93-155.
10. KÜKENTHAL, W., 1924. Gorgonaria. *Das Tierreich 47,* 132 pp.
11. LACAZE-DUTHIERS, H. 1864. *Histoire naturelle du corail.* J. B. Baillière, Paris.
12. MATTHEWS, A., 1916. The development of *Alcyonium digitatum* with some notes on the early colony formation. *Quart. J. Micr. Sci. 62:* 43-94.
13. THEODOR, J., 1967. Contribution à l'étude des gorgones (7): Ecologie et comportement de la planula. *Vie et Milieu* 18(2A): 291-301.
14. VIGHI, M., 1970. Ricerche sul ciclo riproduttivo del corallo rosso [*Corallium rubrum* (L.)] del Promontorio di Portofino. *Atti Accad. Lincei [Roma],* Ser. 8, *10*(1): 1-26.
15. WILSON, E. B., 1883. The development of *Renilla. Phil. Trans. Roy. Soc. London* Pt. 3: 723-815.

STUDIES ON THE ANATOMY AND HISTOLOGY OF *PLEXAURA HOMOMALLA* IN FLORIDA*

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ABSTRACT

Aspects of the anatomy and histology of *Plexaura homomalla* as shown by a study of serial paraffin sections of Bouin-fixed material from Florida are described and illustrated. The distribution of zooxanthellae in the entoderm of zooids and coenenchymal canal system is discussed.

INTRODUCTION

The discovery of prostaglandins in *Plexaura homomalla* (Esper), a common West Indian gorgonian coral [21], drew unprecedented attention to this group of animals and raised many fundamental questions about their biology. The role of prostaglandins in the physiology of *P. homomalla* and their relationship to the reproductive cycle are unknown, as is the extent of involvement of the symbiotic zooxanthellae in the production and concentration of prostaglandins by the coral.

In an effort to shed some light on some of these subjects, a program of periodic sampling of tagged colonies was begun in Florida at the end of November 1970 and is still continuing. The main purpose of this program was to determine the sex ratio in the natural population and the breeding period of *P. homomalla* in the Florida Keys. As there is no external clue to the sex of colonies of *P. homomalla,* paraffin sections must be made from every sample for which identification of sex is desired. Early in the investigation, it was found that male colonies cannot be recognized during winter months even from histological sections because their gonads are inactive and therefore undetectable. Therefore, a study of the general histology and anatomy of this species was undertaken to seek means of recognition. The results of this study originally formed a part of the paper on sex ratio and reproductive cycle by Walter Goldberg and Ramon D. Hamilton presented at this symposium, but were considered inappropriate for oral presentation because of their highly descriptive nature. They are presented here as a separate paper because the histology and anatomy are so sharply distinct from the subject of sex ratio and reproductive cycle.

This investigation is by no means exhaustive, but must be considered as a preliminary exploration of a complex subject. Examination of hundreds of sections has revealed numerous interesting features worthy of study but which have necessariy been neglected from lack of time. Much is yet to be learned by ordinary light microscopy of the histology of *Plexaura homomalla.* When it can be studied with the electron microscope, even greater advances in our knowledge of this important coelenterate surely will be forthcoming.

*Contribution No. 1714 from the Rosenstiel School of Marine and Atmospheric Sciences, University of Miami.

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HISTORICAL BACKGROUND

Plexaura homomalla was first described and illustrated, as *Gorgonia homomalla,* by E. J. C. Esper [8] in 1792, as has been mentioned already [3]. Esper's colored figure so clearly represents the characteristic appearance of dried specimens of this common and widespread gorgonian that it leaves no doubt as to what the author had in hand. This is not to say that no taxonomic problems revolve around *Plexaura homomalla.* It is well known, for example, that specimens from inshore localities in the Florida Keys and elsewhere are smaller and more slender than "typical" colonies of *homomalla* but, apart from a sometimes weaker crown, their skeletal characters do not differ significantly. This form was treated by me [2] under the name *P. homomalla* forma *kükenthali* Moser. Moreover, some colonies of typical *homomalla* form are softer and more flexible than others and have weaker anthocodial armature and fewer coenenchymal spicules (Fig. 6). Also, the species collected from a depth of 55 meters in the Tongue of the Ocean, called *Plexaura nina* by Bayer & Deichmann [4], shares several characters in common with *P. homomalla,* including the production of prostaglandins, but differs in growth form and in details of spiculation. The validity of this nominal species and its relationship to *P. homomalla* remain to be clarified.

Although 19 species were included in the genus *Plexaura* by Moser in his outline revision [17], followed by Kükenthal [16], some of these belong to other genera and some were synonymized [2], leaving only *P. homomalla, P. nina,* and *P. flexuosa.* The last shares many features with various species placed in the genus *Eunicea,* and it does not concentrate prostaglandins. It now seems highly probable that *P. flexuosa* Lamouroux must be removed from *Plexaura* and reassigned elsewhere in the family.

The most complete and, in the present context, the most pertinent, investigation of the anatomy and histology of any gorgonacean coral is that

of *Pseudoplexaura crassa* Wright & Studer (properly known as *Pseudoplexaura porosa* [Houttuyn]) by Chester [7]. In an unpublished thesis [1], I described the microanatomy of four species of plexaurids and one gorgoniid but, as publication has never ensued, only passing reference will be made to that work. The present investigation of *Plexaura homomalla* agrees with the major points brought out by Chester [7], but differs in some details and in some interpretations.

Materials and Methods

Samples for histological study were collected in June 1972 from untagged colonies at Bache Shoal off Elliott Key, Florida. These were fixed in Bouin's fluid and were not further treated for decalcification. They were processed according to standard procedures for the paraffin method by Mrs. Fay Mucha in the histology laboratory of the Rosenstiel School of Marine and Atmospheric Science. Both longitudinal and transverse sections of the branches were cut at a thickness of 8μ and stained with haematoxylin and eosin or Gomori's Trichrome Stain. The latter was modified by doubling the amount of light green in order to achieve adequate staining of the mesogloea. In addition, some series were stained with aldehyde-fuchsin and some with haematoxylin-safranin-light green.

Explanation of Lettering

anth. w.—anthocodial wall
ax. cort.—cortex of axis
ax. epi.—axis epithelium
ax. loc.—loculi of axial cortex
ax. med.—medulla of axis
cl. ax. ep.—cell of axis epithelium
cl. ect. phar.—ectodermal cell of pharyngeal lining
cl. ent.—entodermal cell
cl. eos.—corpuscle-like eosinophilic cell
cl. epi.—epidermal cell
cl. fth.—frothy cell
cl. gdr. phar.—gastrodermal cell of pharynx
cl. gl.—gland cell
cl. gr.—cell with coarse red-staining granules
cl. int.—interstitial cell
cl. mgl.—mesogloeal cell
cl. str.—cell-strands
cl. str. lin.—cell lining the coenenchymal cell-strands
cnbl. a.—cnidoblast of acidophilic nematocyst
cnbl. n.—nucleus of cnidoblast
cr. mcl.—circular muscle
dbl. z.—double zooid
desm. pl.—"desmocyte" plaque
ect.—ectoderm
ent.—entoderm
epi.—epidermis
epi. o. d.—epidermis of oral disc
fibr. contr.—contractile fibers?
fil. asulc.—asulcal septal filament
gdr.—gastrodermis
gdr. phar.—gastrodermis of pharynx
gvc.—gastric cavity
ic. sp.—intercellular space
int. tub.—intercellular tubular structure
lum. sol. sub.—lumen of subepidermal solenium
lum. st. can.—lumen of stem canal
mcl.—muscle fibers
mcl. retr.—retractor muscle
mgl.—mesogloea
mth.—mouth
nem. a.—acidophilic nematocyst
nem. b.—basophilic nematocyst
ov. cyt.—cytoplasm of egg
phar.—pharynx
r. gr.—red-staining cytoplasmic granules
sclbl.—scleroblast
sept. asulc.—asulcal septum
sept. sulc.—sulcal septum
siph.—siphonoglyph
sol.—solenium

sol. coen.—solenia in coenenchyme
sol. sub.—subepidermal solenia
sol. w.—solenial wall
sp. d.—developing sperm
sp. lac.—lacuna remaining after solution of spicule
sp. z.—tailed sperm
spic. anth.—anthocodial spicule
spic. coen.—coenenchymal spicule
spm.—spermary
st. can.—longitudinal stem canal
zx.—zooxanthellae

Anatomy and Histology

General Features.—As the gross structure and anatomy (Figs. 1, 4) of *Plexaura homomalla* conform closely with those of other holaxonian gorgonaceans [7, 16], they will not be described in detail here. *P. homomalla* forms arborescent colonies of substantial size (as much as 1.5 m in height) composed of a supporting axis of scleroprotein (Fig. 1) over which is spread a layer of mesogloea containing spicules ("coenenchyme"). The polyps, or zooids, are embedded in this coenenchyme and are connected with one another by way of small canals ("solenia") lined with entoderm, which in turn open into a series of large, longitudinal stem canals which closely surround the axis in the smallest branchlets as well as in the large branches and main stem. The surface of both coenenchyme and zooids is covered with ectoderm, and the axis is invested throughout with an ectodermal axis epithelium which is responsible for its formation [15]. The lining of the polyps and canal system consists of entoderm, except for the two asulcal septal filaments, which are ectodermal. *Pexaura homomalla* has gastrodermal solenia of two distinctly different kinds and is unusual among gorgonaceans in the great extent of their development, with consequent reduction of mesogloea.

A peculiarity of *P. homomalla* soon noticed by anyone working with it in the field or laboratory is its tendency to begin disintegrating much sooner after collection than do other species. It also is very difficult to maintain under aquarium conditions, where colonies commonly die in 24-48 hours, even in a circulating seawater system. This property can be explained by the scanty mesogloea, which is reduced to a thin layer immediately surrounding the spicules, and thin mesolamellar sheets between the solenia and cell-strands. The spicules are not held in place by mesogloea, as they are in most other species, but by cellular tissue of a rather diffuse nature.

Vegetative Reproduction.—Vegetative reproduction in gorgonaceans normally takes place in the coenenchyme, where new zooids arise from the peripheral solenia. I know of no reported departure from this method in the Gorgonaca, but Studer [18, 19] described an instance of apparent longitudinal fission ("fissiparité") in the alcyonacean *Schizophytum echinatum*. In this case, one colony had an unusually large zooid with 16 tentacles, and another had a large zooid with two complete oral disks, each with mouth and 8 tentacles. Studer apparently did not section either of these "dividing" zooids [18] and did not figure the arrangement of septa.

Sections made from part of a colony collected at Bache Shoal near Miami revealed zooids with 16 septa and two pharynges (Fig. 15). Closer examination of the remainder of the sample, in which the zooids are not

completely retracted, showed that zooids with supernumerary tentacles could be recognized externally (Fig. 3). Additional serial sections prepared from this sample showed the double zooids to be similar in structure to those found in the first series. Each has two pharynges with eight complete septa enclosed in a common gastrovascular cavity (Fig. 5). Septal linkages

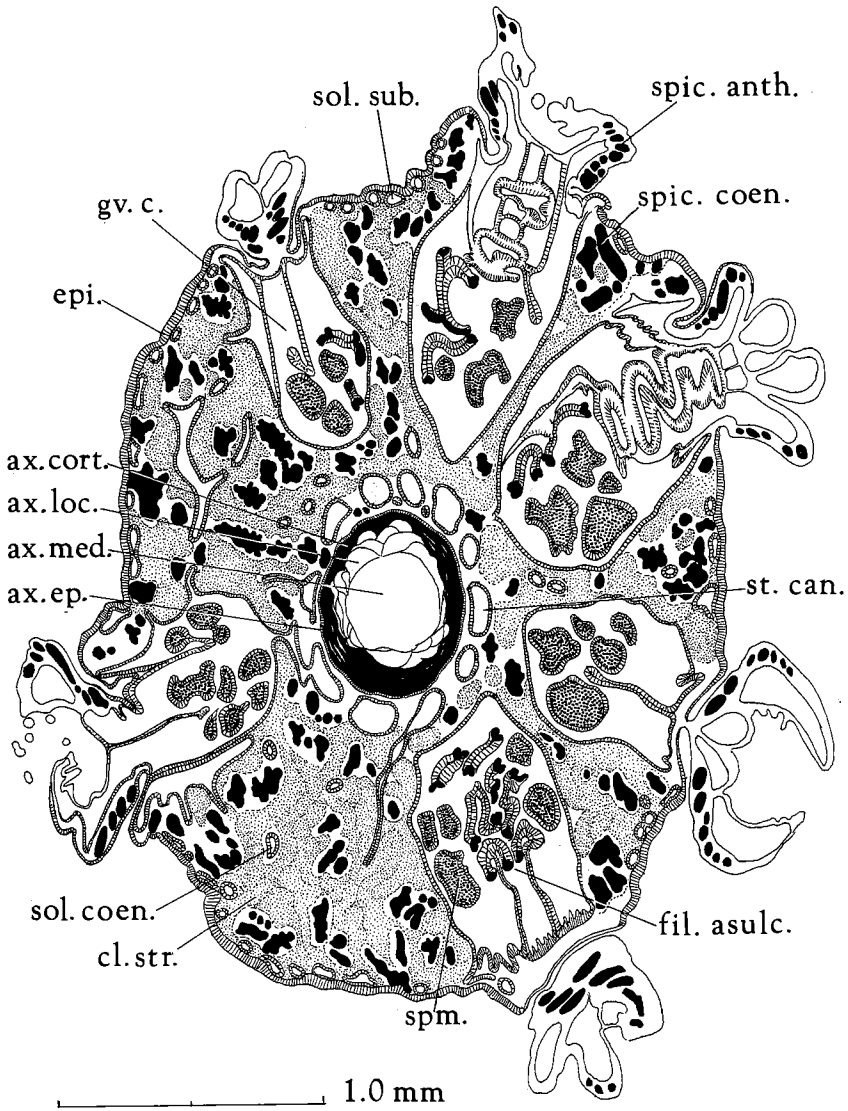
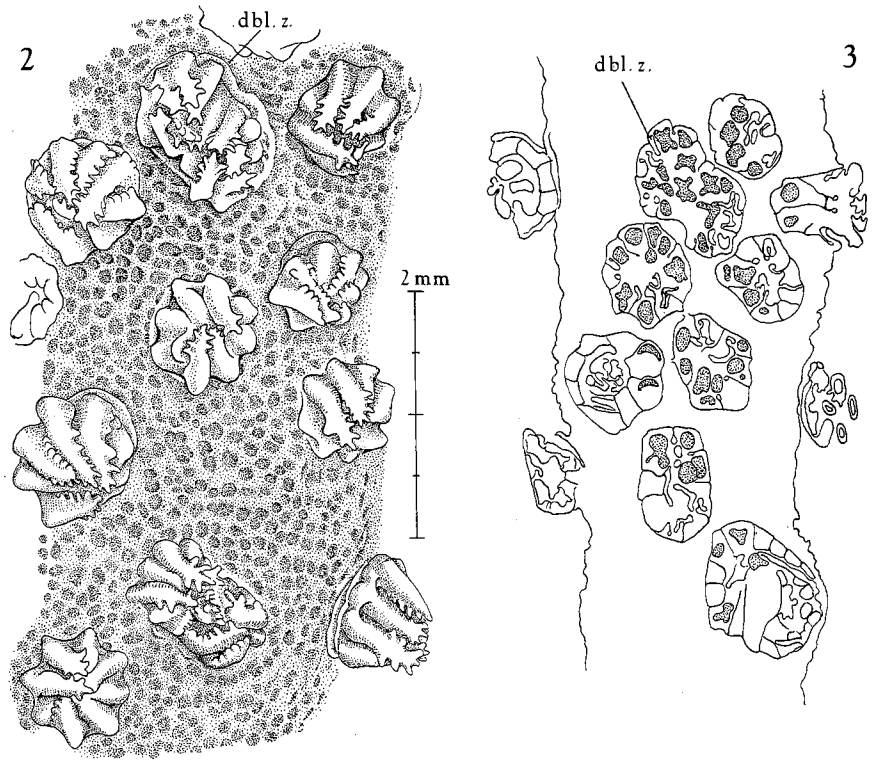


FIGURE 1. Semidiagrammatic cross section of terminal branchlet of *Plexaura homomalla.*

between the two pharynges do not occur in any of the double zooid observed. Consequently, all of the septa extend from pharynx to body wall, with the result that the double zooids share a common interseptal chamber that appears to have only one large tentacle. Thus, the double zooids have 15 tentacles instead of 16.

There seems to be no consistency in which of the interseptal chambers is shared. Double zooids with common ventral, lateral, and ventrolateral chambers were found, and in one case the shared chambers of the two halves did not correspond. In this instance, the sulcal chamber of one half opens into the ventrolateral chamber of the other half, and there is a well developed siphonoglyph in both pharynges. In other double zooids, one or both pharynges may lack a siphonoglyph.

This zooidal twinning can hardly be regarded as a form of intratentacular budding as there is no obvious mechanism whereby the shared interseptal chamber of the double zooids could produce two tentacles so that the two halves could separate into normal, identical daughters, nor is



FIGURES 2-3. Terminal branchlet of *Plexaura homomalla:* 2, surface showing two double zooids among normal individuals (decalcified).—3, Tangential section of the same branch; the plane of the section does not coincide exactly with the viewpoint in Fig. 2.

there any evidence that they ever separate. However, the high incidence of twinning in this colony suggests that it is not merely fortuitous but may have a genetic basis. Therefore, it can be interpreted as the kind of anomaly that may at one time have led to the one process of intratentacular budding in anthozoans.

Ectoderm.—In general, the ectoderm of *Plexaura homomalla* agrees with that of *Pseudoplexaura crassa* as described by Chester [7, p. 747]. It is divided rather indistinctly into an outer epithelial and an inner subepi-

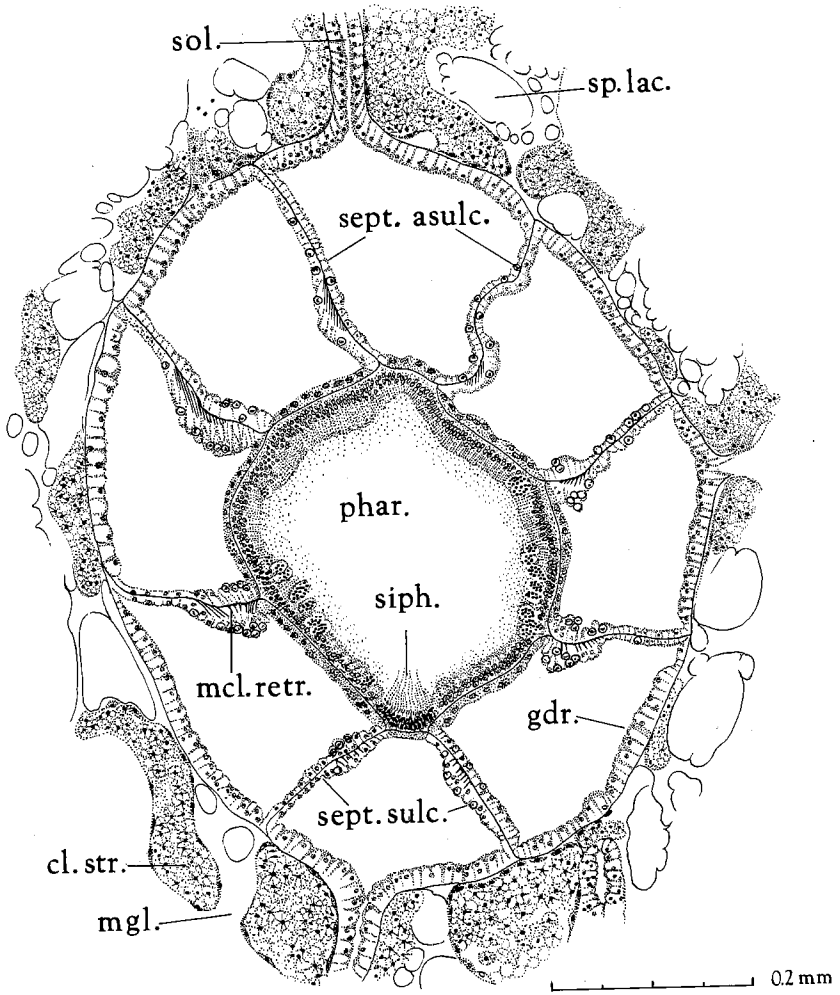


FIGURE 4. Cross section of a normal zooid of *P. homomalla* just below the surface of the coenenchyme.

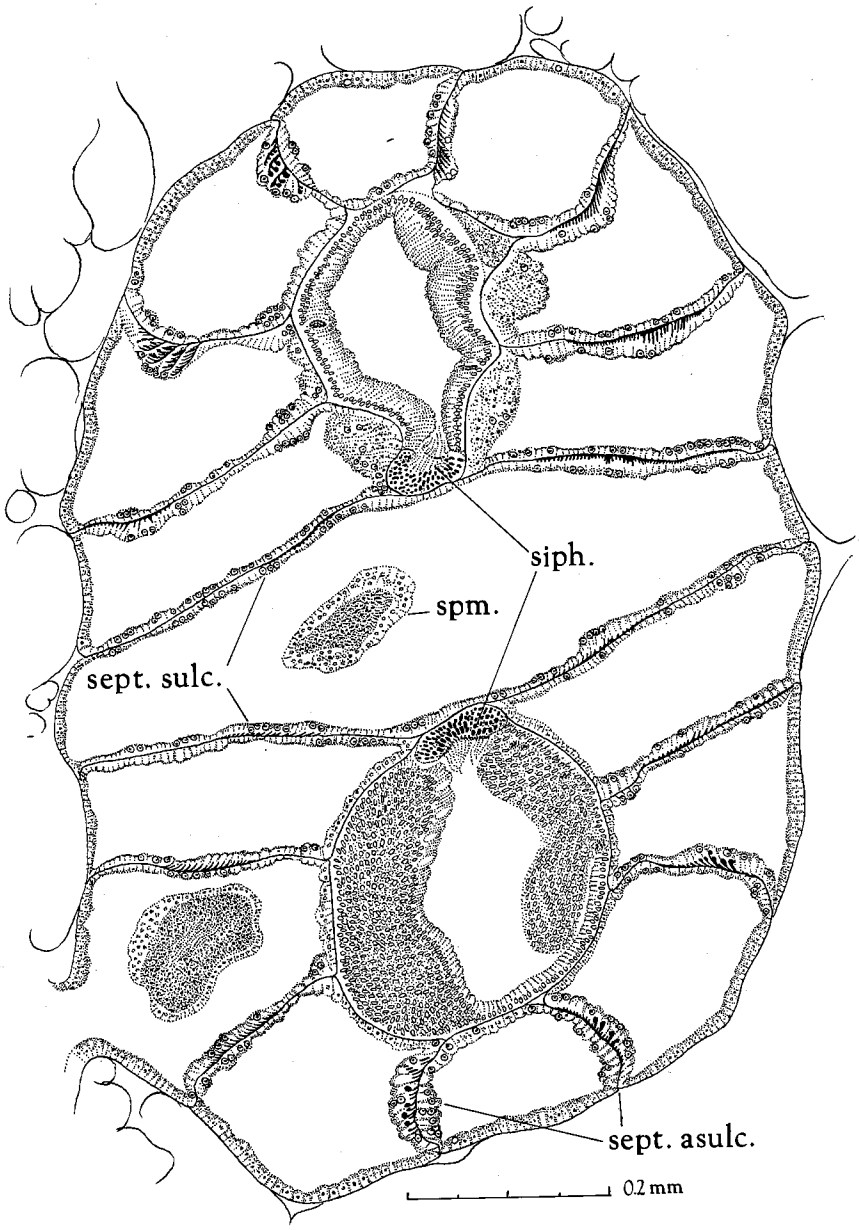
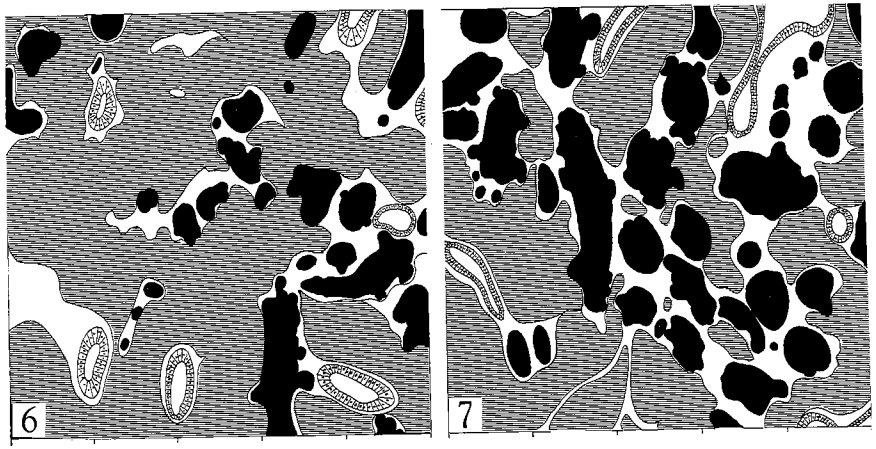


FIGURE 5. Cross section of a double zooid of *P. homomalla* just below the surface of the coenenchyme.

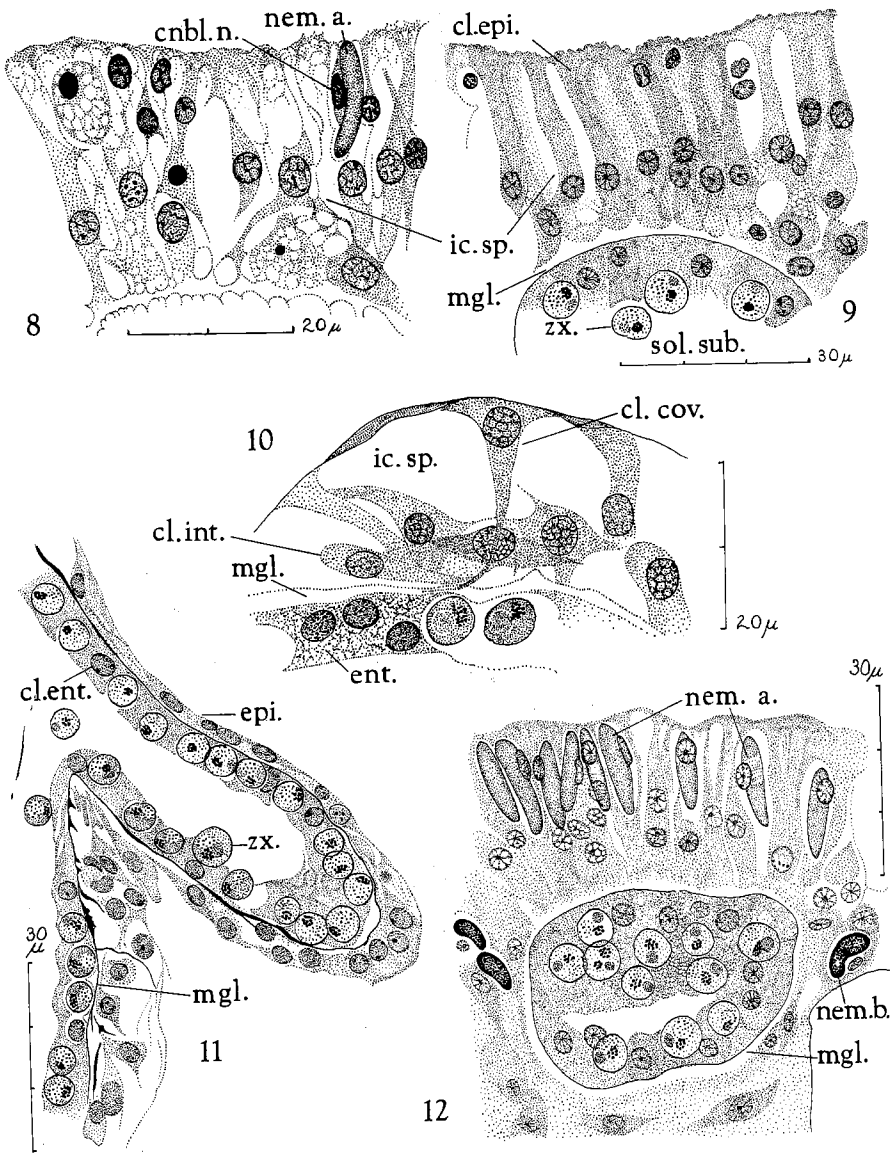


FIGURES 6-7. Diagrammatic sections through the coenenchyme of hard and soft variants of *P. homomalla:* 6, soft form; 7, hard form. Spicules black, mesogloea white; frothy-cell cords hatched; entoderm of solenia dotted, surrounding white lumen.

thelial layer. The epithelial layer consists chiefly of peltate, mushroom-shaped, or tall-columnar cover cells, whose expanded outer ends form the epidermal surface and whose tapered or columnar stems pass through the subepithelial layer to meet the underlying mesogloea.

The epidermis of the anthocodia is low, cuboidal, in some places so low as to approach the squamous epithelial type to be seen in many other kinds of organisms (Fig. 10). The epithelium is generally lower and flatter on the oral surface of the tentacles, on the sides of the pinnules, on the oral disc (Fig. 15), and in areas overlying spicules; it tends to be thicker (cuboidal or low columnar, sometimes several layers in thickness) on the aboral surface of the tentacles, on the tips of the pinnules, and in areas between spicules. However, examples contradicting each of these generalities are easily found on any slide, and even on the same section.

The appearance of the coenenchymal epidermis (Fig. 50) is highly characteristic. The elongated cover cells taper downward from their expanded outer ends toward the mesogloea, against which they terminate either flat or in one or more narrow points. Their cytoplasm is finely granular and contains many small vacuoles; their nuclei lie at a level deeper than midheight, are large, and stain lightly because of their fine, open chromatin reticulum. Commonly the cytoplasm contains an irregularly spiral or twisted fibril, as was noticed also in *Pseudoplexaura* by Chester [7; p. 750, pl. 3, fig. 24], which stains deep red in Gomori's Trichrome stain. The pyramidal or columnar bodies of the cells are separated by commodious intercellular spaces. Among the cover cells, several other cell types can be distinguished: cells with large, lightly stained nuclei like those of cover cells, with tapering cytoplasmic (pseudopodial?) processes (Fig. 8);



FIGURES 8-12. Histology of *Plexaura homomalla:* 8, epidermis; 9 epidermis of coenenchyme and part of subepidermal solenium; 10, longitudinal section of pinnule showing ectoderm, entoderm with zooxanthellae, and contractile fibers; 11, epidermis of coenenchyme showing cluster of nematocysts, and subepidermal solenium; 12, section through base of tentacle, showing epidermis with tall cover cells and voluminous intercellular spaces, very thin mesogloea, and entoderm with zooxanthellae.

oval cells with large, pale nuclei, chiefly in the subepithelial layer next to the mesogloea (Fig. 11); cells generally fusiform in shape, with smaller, more darkly stained nuclei (Fig. 8); broadly oval cells with copiously vacuolated cytoplasm and very dense, deeply stained nuclei smaller than those of cover cells (Fig. 8); nematocytes and their nematocysts (Figs. 8, 11, 50, 51); and oval "blood-corpuscle-like" cells taking both eosin and chromotrope very deeply (Figs. 50, 53).

Of these, the first type probably is not distinct from the tall, peltate cover cells and may represent one of the forms of interstitial cells described in *Pseudoplexaura* by Chester [7; p. 748]; the second type may be Chester's globular interstitial cells; the third type seems to be identical with the "sense cells" described by Chester [7; p. 748, pl. 2, figs. 8, 13], but their sensory nature is not established in the present material; the fourth type appears the same as the cells filling the mesogloeal strands throughout the coenenchyme, and was not reported in *Pseudoplexaura;* the nematocysts and their

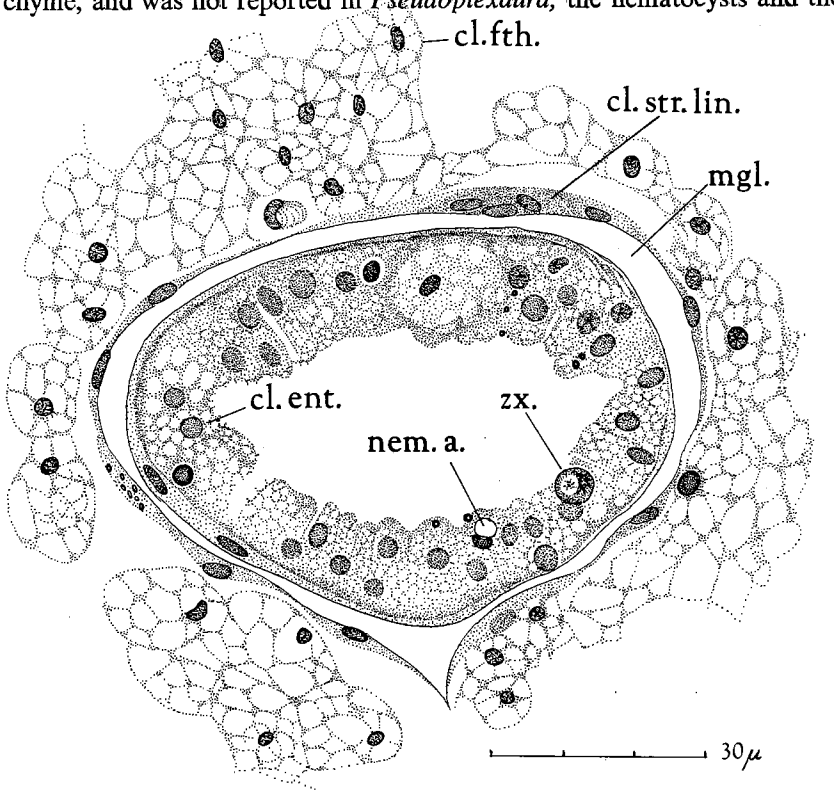


FIGURE 13. Histology of *Plexaura homomalla:* cross section of coenenchymal solenium surrounded by thin layer of mesogloea, embedded in cords of frothy cells; frothy cells separated from mesogloea by very flat entoderm.

cells occur in clusters in the epidermis, chiefly that of the coenenchyme, but originate in the mesogloeal cell strands; and the sixth type, of unknown function, also originates in the mesogloeal cell strands and apparently moves through them to scattered positions in the epidermis. This last type is scarce in some colonies, abundant in others.

Here and there, the surface of the epidermis shows (in Gomori preparations) peculiar lobed projections, very delicate and stained pale grey, which seem to be of cuticular nature (Fig. 30). Study of many examples shows intimate relationship with the adjacent epidermal cells between which they seem to pass, but no unequivocal evidence of continuity with them has been seen so far. However, pink-staining epidermal cells (as is characteristic of Gomori preparations) with similarly shaped processes were found. It seems possible that the surface of these epidermal projections becomes cuticularized and their cytoplasmic contents retreat, leaving hollow remnants. The epidermal cells in this region show red-stained fibers identical in appearance with contractile fibers elsewhere.

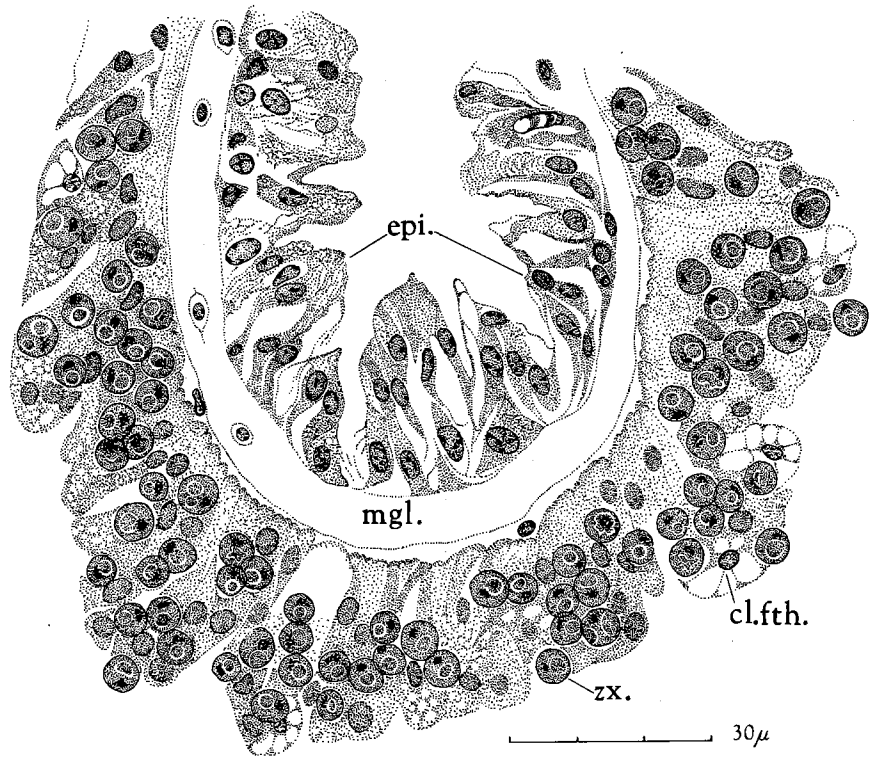
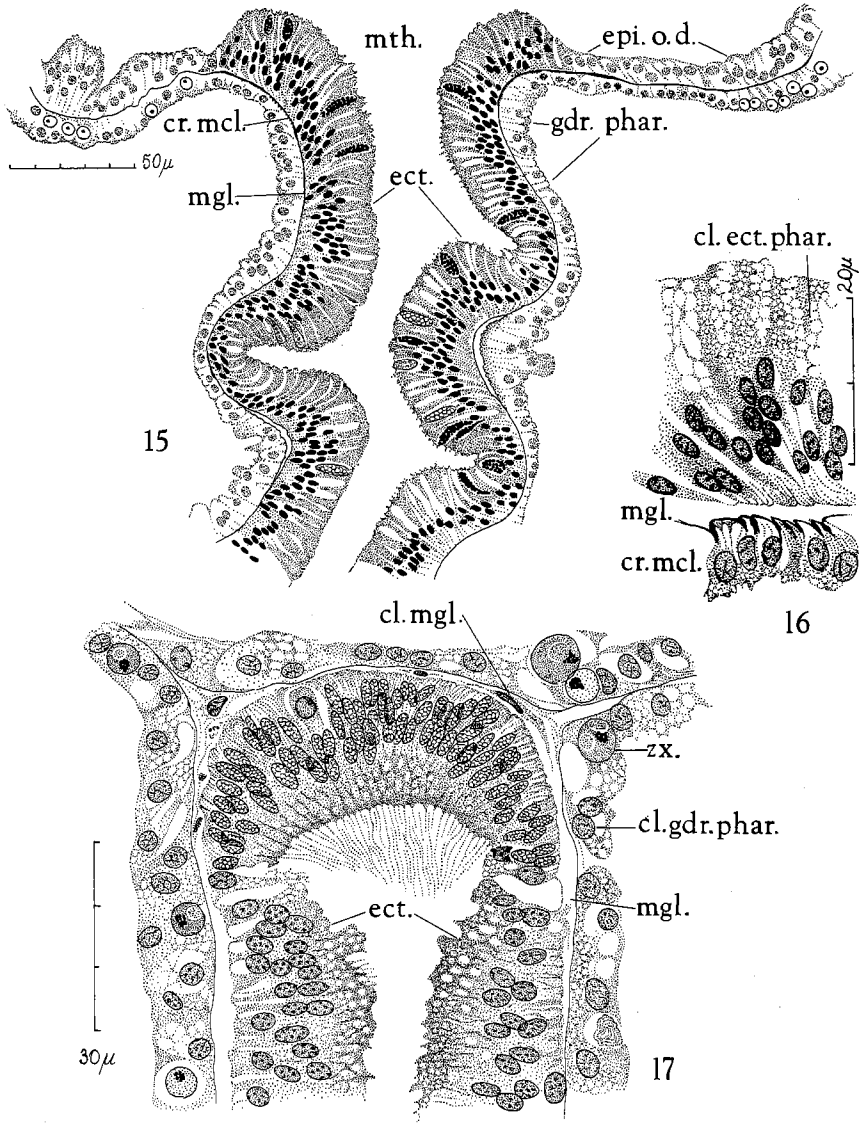
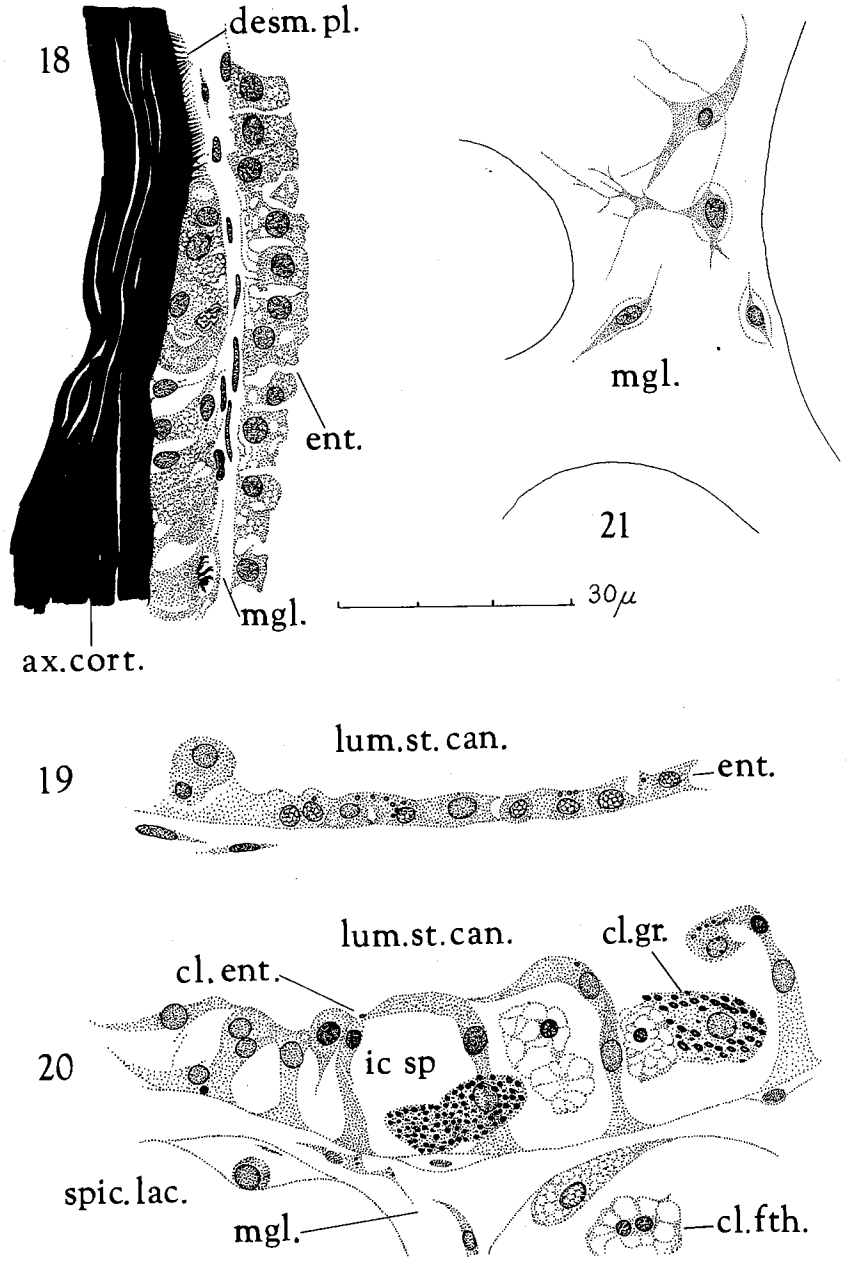


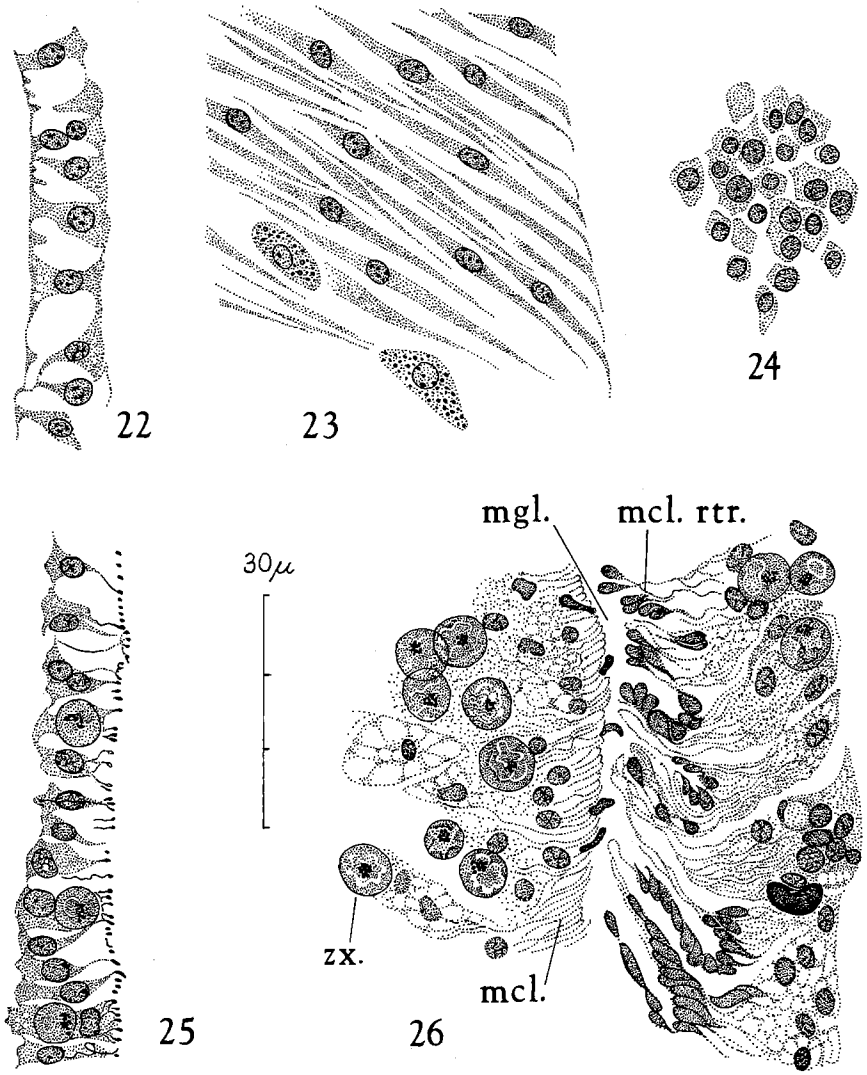
FIGURE 14. Histology of *Plexaura homomalla:* section through infolded neck zone of zooid showing epidermis thrown into folds.



FIGURES 15-17. Histology of *Plexaura homomalla:* 15, longitudinal section through mouth and upper part of pharynx; 16, section through pharyngeal wall just inside mouth to show circular muscle fibers in entoderm; 17, cross section of part of pharynx to show siphonoglyph.



FIGURES 18-21. Histology of *Plexaura homomalla:* 18, cross section of branch showing part of axial cortex with axis epithelium and desmocytes, separated from the entoderm of a longitudinal canal by a thin layer of mesogloea containing elongated cells; 19, entoderm of longitudinal stem canal; 20, entoderm of longitudinal canal, containing frothy cells and coarsely granular cells; 21, mesogloeal cells.



FIGURES 22-26. Histology of *Plexaura homomalla:* 22, transverse section of longitudinal stem-canal wall (entoderm); 23, deep tangential section through same, showing extremely elongated cells; 24, tangential section through surface layer of same; 25, entoderm of zooid wall showing circular muscle fibers; 26, section of septum showing longitudinal retractor muscles on one face and longitudinal muscle fibers on opposite face.

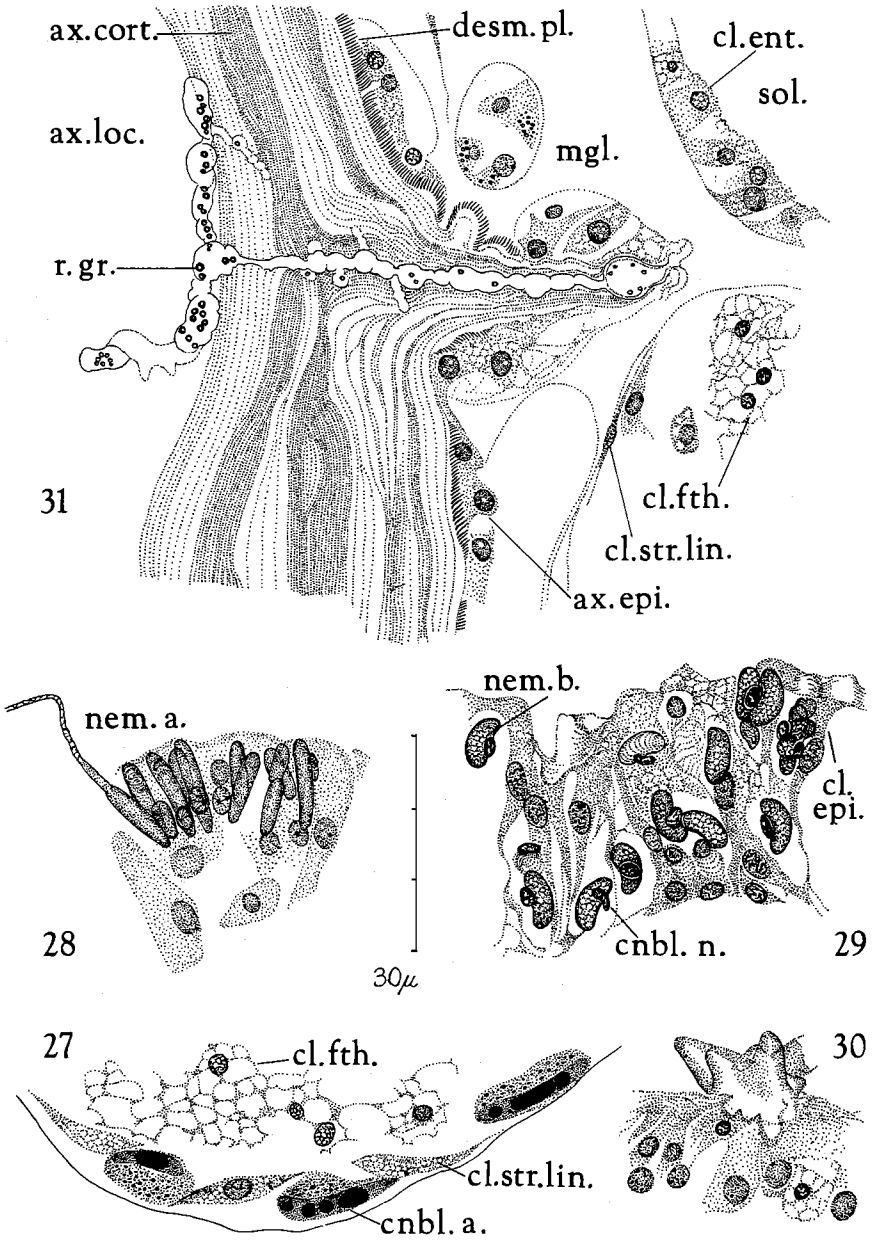


FIGURE 27-31. Histology of *Plexaura homomalla:* 27, entodermal wall of frothy-cell cord, showing cnidoblasts containing developing nematocysts; 28, battery of nematocysts, with one discharged capsule; 29, reniform nematocysts in anthocodial epidermis; 30, lobulated projections of anthocodial epidermis; 31, tubules penetrating axial cortex.

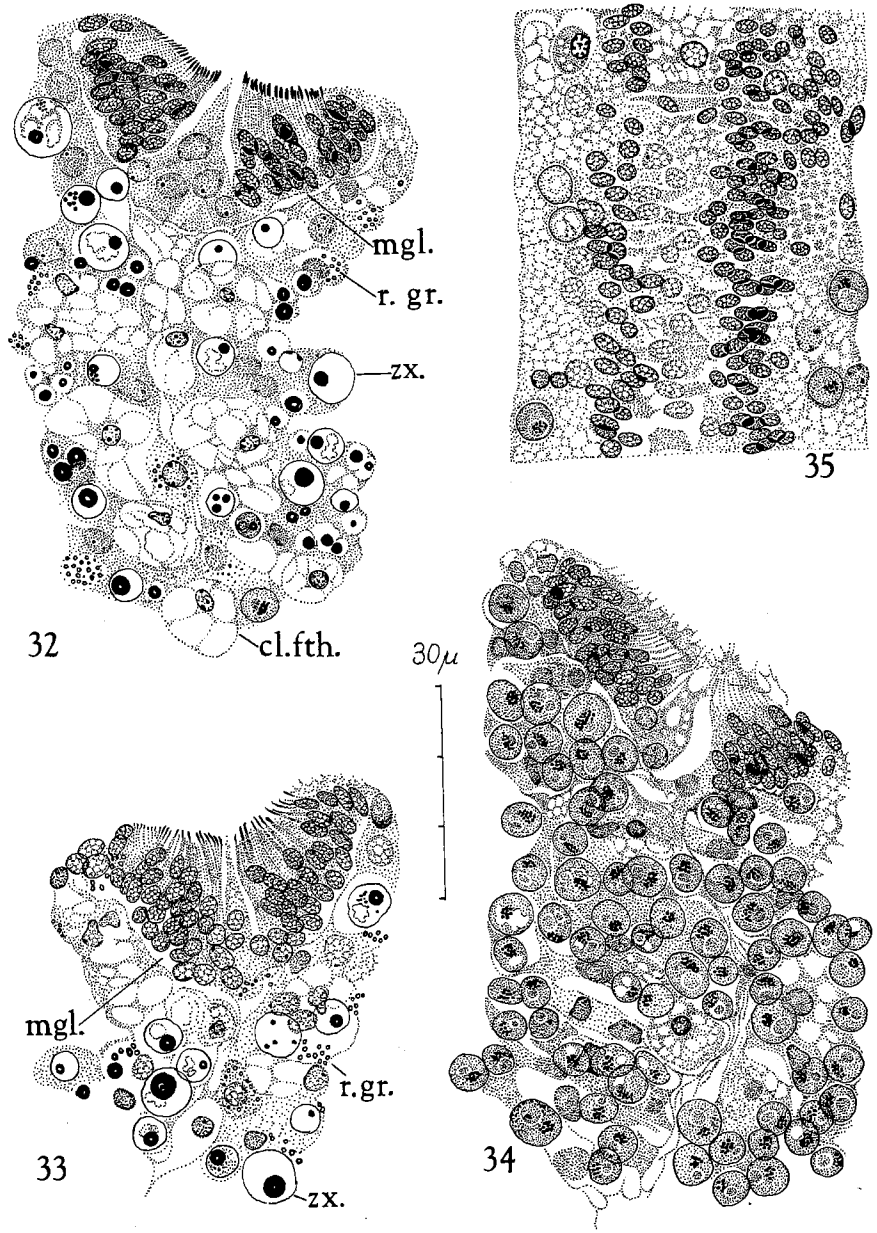
Nematocysts are present in the epidermis, abundantly in that covering the coenenchyme, usually less numerous in that of the anthocodiae, although they do not originate in the epidermis. Chester [7; pp. 747-748] reported two kinds of nematocysts, one type 10-14 μ in length unexploded, the other, less common, 5-8 μ. In most colonies of *P. homomalla,* I have been able to find only one kind of nematocyst in the epidermis, where they are aggregated together in batteries (Figs. 8, 11, 50, 54). They are mostly 15 μ long, occasionally to 17 μ, and 4.5$\times$ as long as broad. In haematoxylin and eosin and in Gomori's Trichrome, they stain bright pink, in haematoxylin-safranin-light green, dark green. Chester [7; p. 748] observed nematocytes with formative stages of nematocysts in the epidermis as well as in the mesogloeal cell strands [7; p. 753], but nematocyst formation in *P. homomalla* does not seem to occur in the epidermis. In this species, nematocytes containing nematocysts in various stages of development are abundant in the thin walls of the coenenchymal cell strands (Fig. 27), especially those near the gastric cavities of zooids.

Some preparations of *homomalla* also have nematocysts of a different shape (Fig. 52), up to 9 μ long, which stain purple in haematoxylin and eosin and blue-grey in Gomori. These are located in the mesogloeal cell-strands but generally not in the epidermis. However, one specimen sectioned was found to contain large numbers of this type of nematocyst, not only in the cell-strands of the mesogloea but also in the epidermis of the aboral surface of the basal parts of the tentacles (Fig. 29), where they vastly outnumbered the larger, eosinophilic nematocysts. They were present in great abundance also in the walls of the mesogloeal cell-strands and among the frothy cells filling the strands.

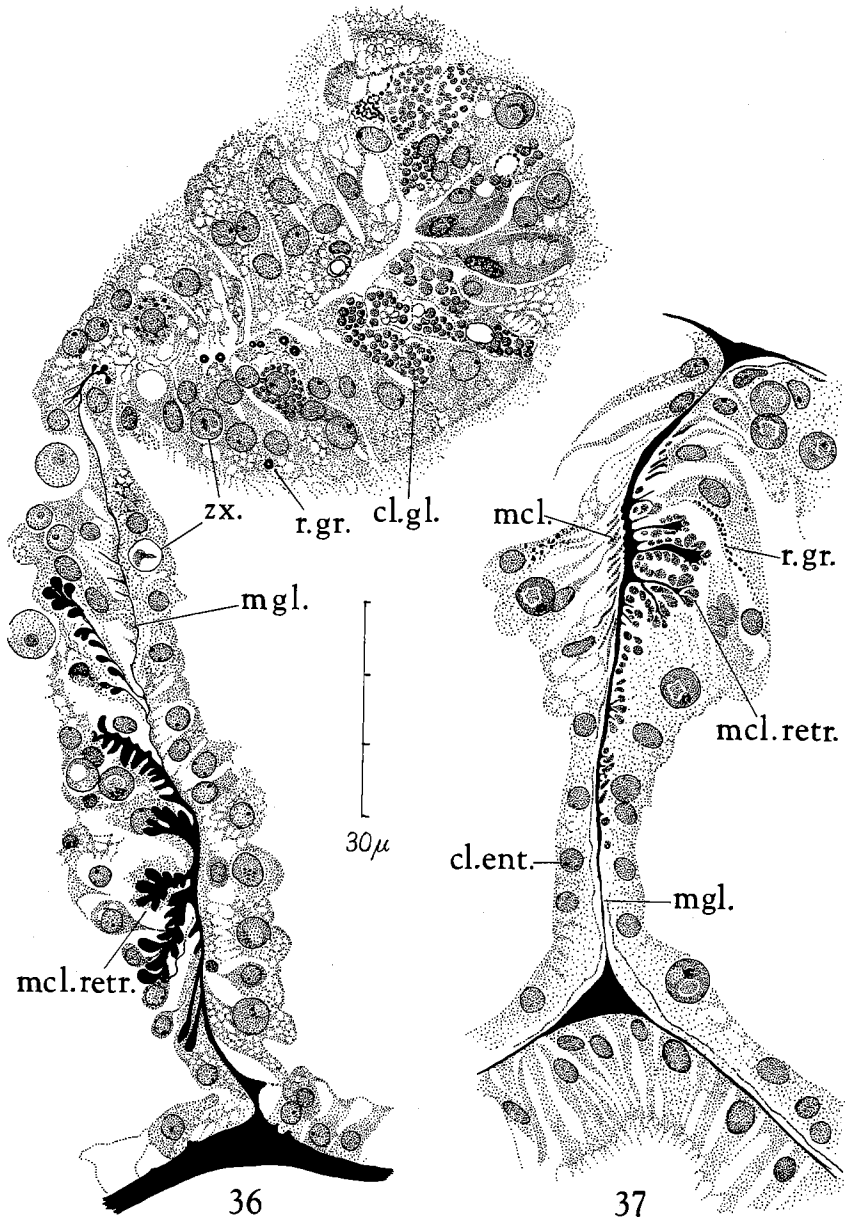
Although long, spindle-shaped cells like those described by Chester [7; p. 748] were found in association with the nematocyst batteries and elsewhere in the epidermis (Figs. 8, 11, 50), none showed anything that could be interpreted as a sensory bristle, and their sensory function cannot be demonstrated by any of the preparations we have studied.

The subepithelial layer of ectoderm contains interstitial cells with branching processes, as well as more rounded forms (Fig. 12), as reported by Chester, but we have not found any cells that could be interpreted as ganglion cells, nor any nematocytes with developing nematocysts. Also in the subepithelial layer are a few rounded cells with abundantly vacuolated cytoplasm and small, dark nucleus (Fig. 8). These seem to be identical with the frothy cells of the mesogloeal cell-cords, and probably originate from the latter where they pass through the epidermis.

Axis.—The principal structural support for the gorgonian colony is the proteinous internal axis [1, 2, 7, 9, 14, 15]. The structure and chemistry of the gorgonian axis have been investigated recently by Goldberg [9]. In *Plexaura homomalla,* the axis is composed of a cross-chambered central chord or axial medulla, which is elongated apically by the addition of successive thin, cap-like chambers (Fig. 1). Concentric layers of tough pro-



FIGURES 32-35. Histology of *Plexaura homomalla:* 32-34, asulcal septal filaments with zooxanthellae in various states of disintegration; 35, tangential section of asulcal filament.



FIGURES 36-37. Histology of *Plexaura homomalla:* 36, lateral septum and filament; 37, septum in pharyngeal region showing location of contractile elements.

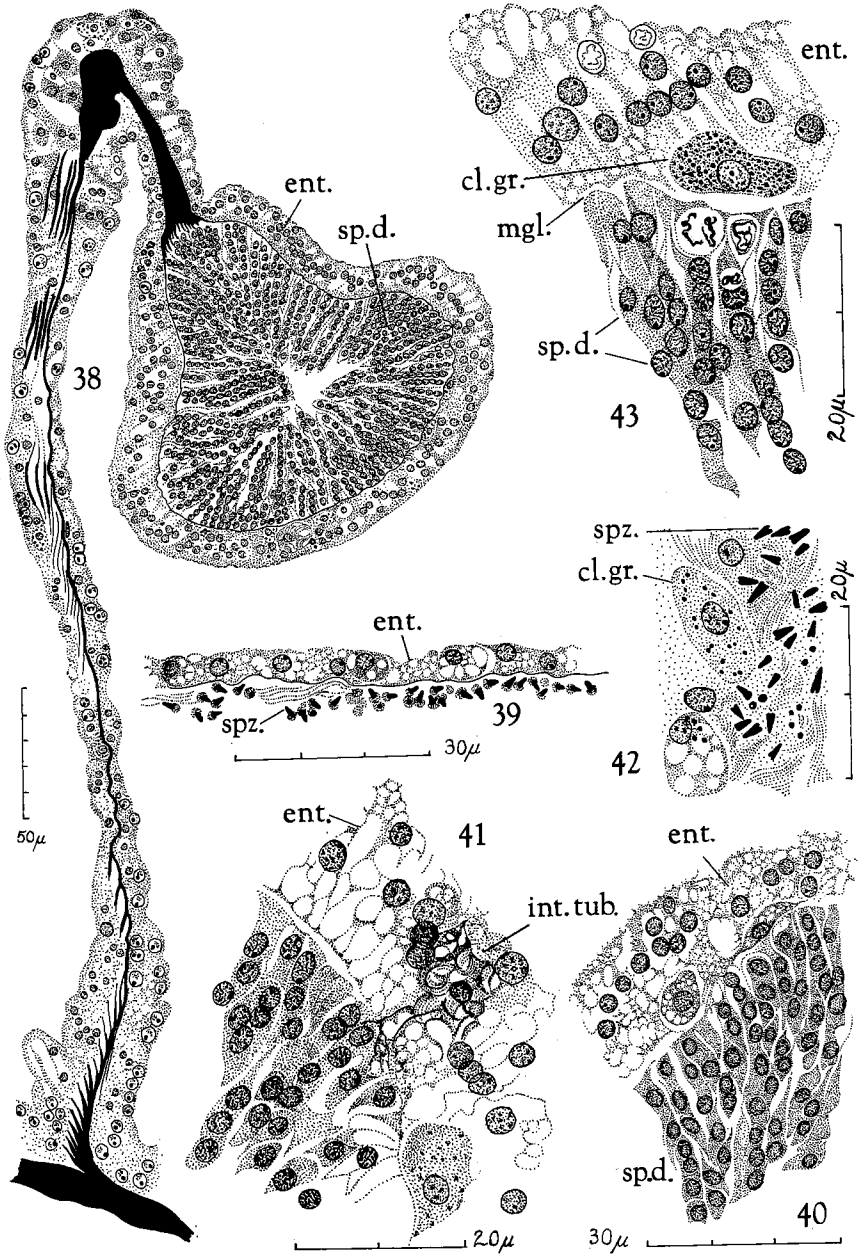
teinous material are added proximally to form the axial cortex, providing thickness and strength to support the colony as it grows. At first these layers are blister-like and enclose spaces ("loculi"), but later they become dense and compact, forming the familiar woody material of the axis.

The dense axial cortex would appear to seal off the chambers of the axial loculi and medulla from the living part of the colony, but sections show that it is penetrated by fine canals (Fig. 31). It seems likely that these canals serve to maintain physiological contact between the coenenchyme and the interior of the axis. The extent of physiological activity and morphological change that take place in the interior of the axis throughout the life of the colony is unknown.

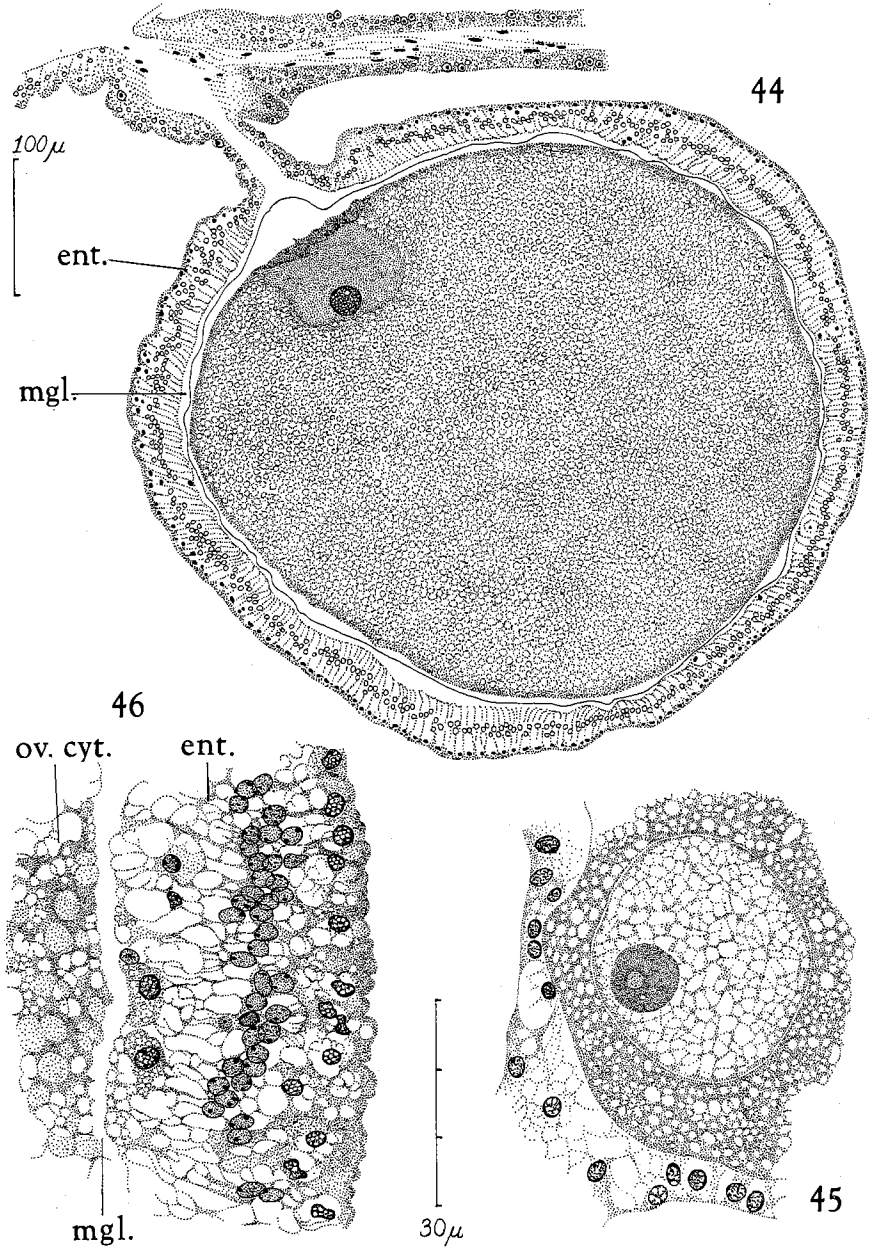
Axis Epithelium.—Although the ectodermal nature of the axis epithelium was disputed for a considerable time, the question was definitely answered by Kinoshita's investigations of *Anthoplexaura dimorpha* [13]. Chester [7] described the axis epithelium of *Pseudoplexaura crassa* in somewhat greater detail and reported both secretory cells and holding cells, the latter called "desmocytes" in accordance with Bourne's terminology [6] of similar structures in *Heliopora.*

The axis epithelium of *Plexaura homomalla* conforms closely with that of *Pseudoplexaura crassa* as described by Chester. In both longitudinal and transverse sections of *P. homomalla* stained with either Gomori's Trichrome or Mallory's Triple Stain, the axial material is delimited from the mesogloea by a narrow, interrupted zone stained red. Under high power, the red zone is seen to be striated normal to the surface of the axis (Fig. 48). These red striated zones are the "desmocytes" of Chester, and although that author maintained that he found nuclei in them, I have not been able to confirm his observation. Between the desmocytes, the interface between mesogloea and axis is bounded by epithelial cells, generally rather tall and separated by conspicuous intercellular spaces (Fig. 48) (possibly artifactual). Wherever the desmocyte has been cut across its widest part, its striations gradually disappear in the mesogloea and the red-stained striated zone merges into the blue- or green-stained mesogloea without any distinct boundary.

Longitudinal sections of branch that are tangential to the axis clearly show that the striated zones have an irregularly oval outline, and in such views the striations are radially arranged. In the most favorable sections, it can be seen that the epithelial cells surround the desmocytes (Fig. 47), which are continuous with the mesogloea. Therefore, the axis epithelium below the growing tip is an epithelium perforated by holes through which the desmocytes merge with the mesogloea. It thus appears that the desmocytes are not cells, but the products of the epithelial cells, and are areas where axis formation is actually taking place. I described the desmocytes of several species in 1954 [1] and speculated as to their depositional function, but did not then mention the perforate nature of the axis epithelium.



FIGURES 38-43. Histology of *Plexaura homomalla:* 38, lateral septum with spermary; 39, entoderm surrounding ripe spermary; 40, entoderm surrounding unripe spermary; 41, entoderm of spermary showing canaliculate structure; 42, nurse cells at center of spermary; 43, peripheral part of spermary showing karyokinetic figures.



FIGURES 44-46. Histology of *Plexaura homomalla:* 44, egg attached to septum; 45, germinal vesicle of egg at higher magnification; 46, entoderm surrounding mature egg.

W. Goldberg [9], using other techniques including electron microscopy, has confirmed that desmocytes are not cells but cell products and are areas of axis formation. Evidence obtainable by such means suggests that gorgonin is mesogloea converted by the epithelial cells [9]. As the desmocytes are least numerous on the growing tips of the axis, where the epithelial cells are very tall and lie in direct contact with the uppermost of the partitions forming the axial medulla, it seems probable that they are concerned mostly with the secondary thickening of the axis. Further, evidence afforded by many series of sections suggests that the production of desmocyte plaques is intermittent, so the secondary thickening of the axis is composed of many layers of overlapping plaques.

Because of the importance of desmocytes in axis formation and of the method of axis formation to the phylogeny of the Gorgonacea as a whole, specimens of several genera in both suborders were examined for the presence of desmocytes. They were found in all the genera of Holaxonia so far investigated, but not in any Scleraxonia.

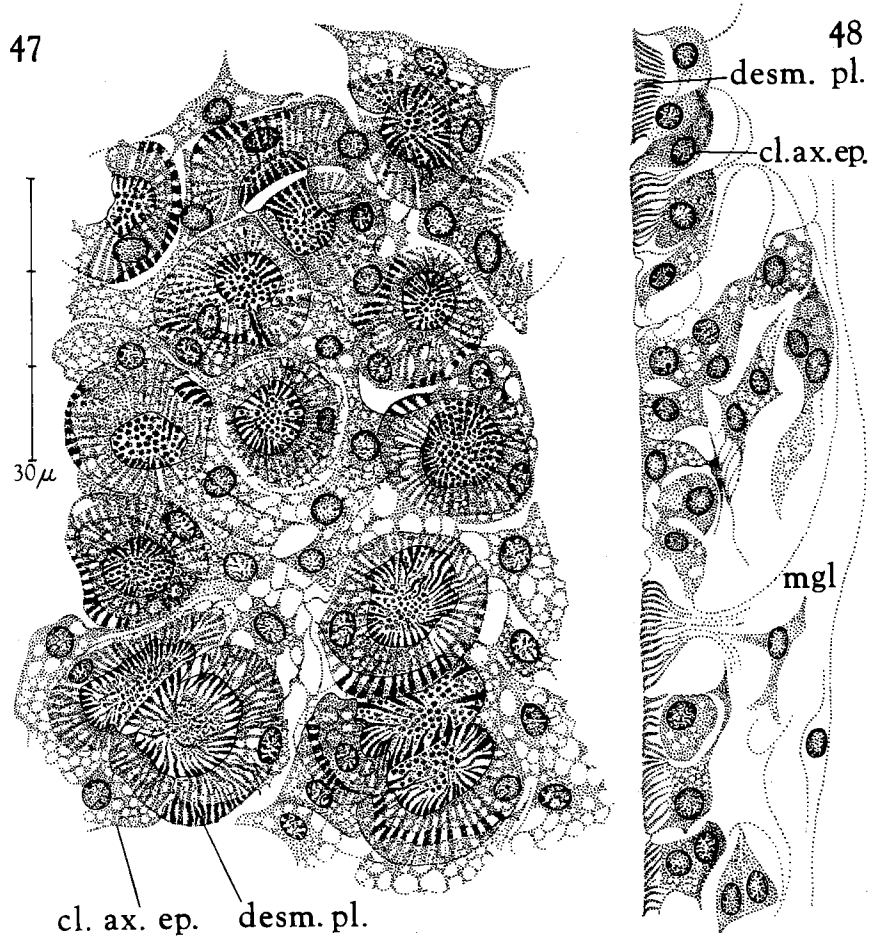
Coenenchyme.—The coenenchyme is composed of mesogloea, spicules, cell-strands, solenia, and entodermal canals, the whole covered by epidermis. The most conspicuous cellular component is a network of wide cell-strands without apparent lumen, composed of oval cells with small, very dense nucleus and conspicuously vacuolated cytoplasm (i.e., "frothy cells"). Among the cell-strands are typical entodermal solenia with lumen, as well as the lacunae originally occupied by spicules.

The mesogloea separating the cell-strands, solenia, and spicule-lacunae is thin in most places, commonly reduced to an inconspicuous mesolamella. Mesogloea surrounding the coenenchymal spicules is usually thicker than elsewhere, as it fills the spaces between the tubercles and projections of the spicules where solenia and cell-strands do not penetrate. Cells can be found throughout the mesogloea, even in the mesolamella of the anthocodial wall (Fig. 14) and pharynx (Fig. 17). They occupy a definite unstained space in the mesogloea and may be fusiform or amoeboid, with two or more extremely long processes extending through the mesogloea (Fig. 21).

Although the cell-strands lie very close to the solenia, longitudinal canals, gastric cavities of the zooids, and epidermis, they form a system that is for the most part sharply isolated. Lobes of frothy cells extend from the cell-strands outward between the peripheral solenia and push into the base of the epidermis (Fig. 50). Here and there, these lobes of frothy cells penetrate the epidermis and apparently discharge cells into the surrounding water (Fig. 51). In some samples, many of the lobes break through the epidermis, in others few do so. One of the specimens sectioned clearly demonstrates direct communication between the cell-strands and the gastric cavity of the zooids, which contain clumps of frothy cells and many zooxanthellae. Although direct communication between cell-strands and the longitudinal stem canals was not observed, the latter may contain clumps of frothy cells, so some route of communication must be assumed.

The cell-strands appear to be solid cords of frothy cells extending through the coenenchyme, but careful examination shows that they are surrounded by a thin epithelium composed of flattened, more or less elongated or fusiform cells (Figs. 50, 52). These cells probably are entodermal, and the frothy cells are actually the contents of a separate system of thin-walled solenia.

Entoderm.—The epithelium lining the zooids and the canals extending from them into the coenenchyme is entoderm ("gastrodermis" of many authors, following Hyman [12, pp. 263-264]). In the tentacles, pinnules



FIGURES 47-48. Histology of *Plexaura homomalla:* 47, tangential section of axis epithelium and desmocyte plaques; 48, cross section of axis epithelium and desmocyte plaques.

(Fig. 10) oral disk and generally in the anthocodia, it is low-cuboidal, with ill-defined cell boundaries—possibly syncytial. In areas of strong contraction, as at the anthocodial neck zone where the zooidal wall folds inward during retraction, the entoderm may be thrown into complex folds and appear stratified (Fig. 14). The entoderm lining both sides of the septa contains wide intercellular spaces and the cytoplasm is conspicuously vacuolated (Figs. 36, 37). Ovate or cuboidal cells with profusely vacuolated cytoplasm and small dark nuclei are not uncommon, clearly differentiated from the ordinary entodermal cells (Fig. 34). The entoderm of the septa is filled with zooxanthellae, which have a quite different appearance in the various specimens sectioned. The free edges of the two asulcal ("dorsal") septa form strongly flagellated septal filaments of the structure usually found in octocorals. These are histologically similar to the pharyngeal lining and are considered to be ectodermal. They have the form of a much convoluted, strongly flagellated cord with deep median groove, resulting in a bilobed appearance in cross section (Figs. 32-34). The two flagellated tracts, packed with darkly staining nuclei, are separated by a groove containing a ridge of rather tall, non-flagellated cells with larger, paler nuclei and vacuolated cytoplasm (Fig. 32). In *Plexaura homomalla,* these asulcal filaments are very well developed, occupying a substantial part of the gastrovascular cavity.

The six other septa (one ventral and two lateral pairs) have filaments without median groove, usually considered to be of entodermal origin [12]. These filaments are not convoluted, have abundant gland cells, and apparently lack flagellated cells entirely (Fig. 36). Zooxanthellae are present

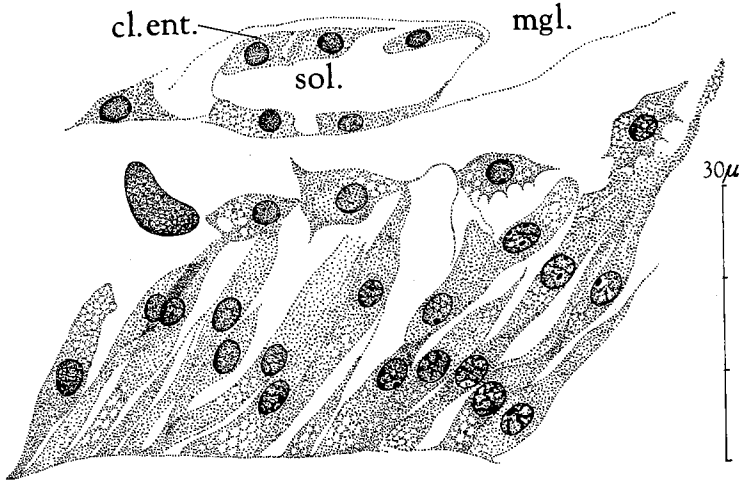


FIGURE 49. Histology of *Plexaura homomalla:* axis epithelium at branch tip where chambers of the axial medulla are formed.

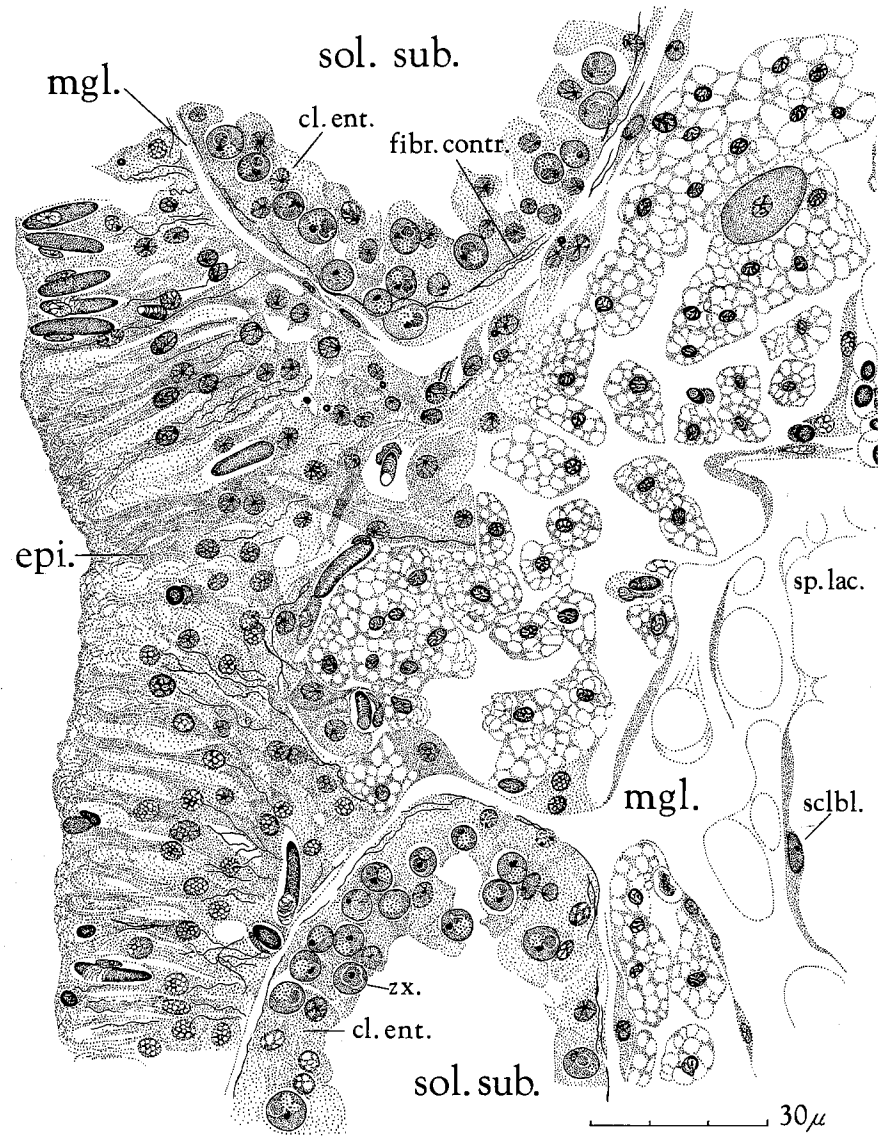


FIGURE 50. Histology of *Plexaura homomalla:* section through outermost layer of coenenchyme between two subepidermal solenia, showing approach of frothy-cell cord to surface.

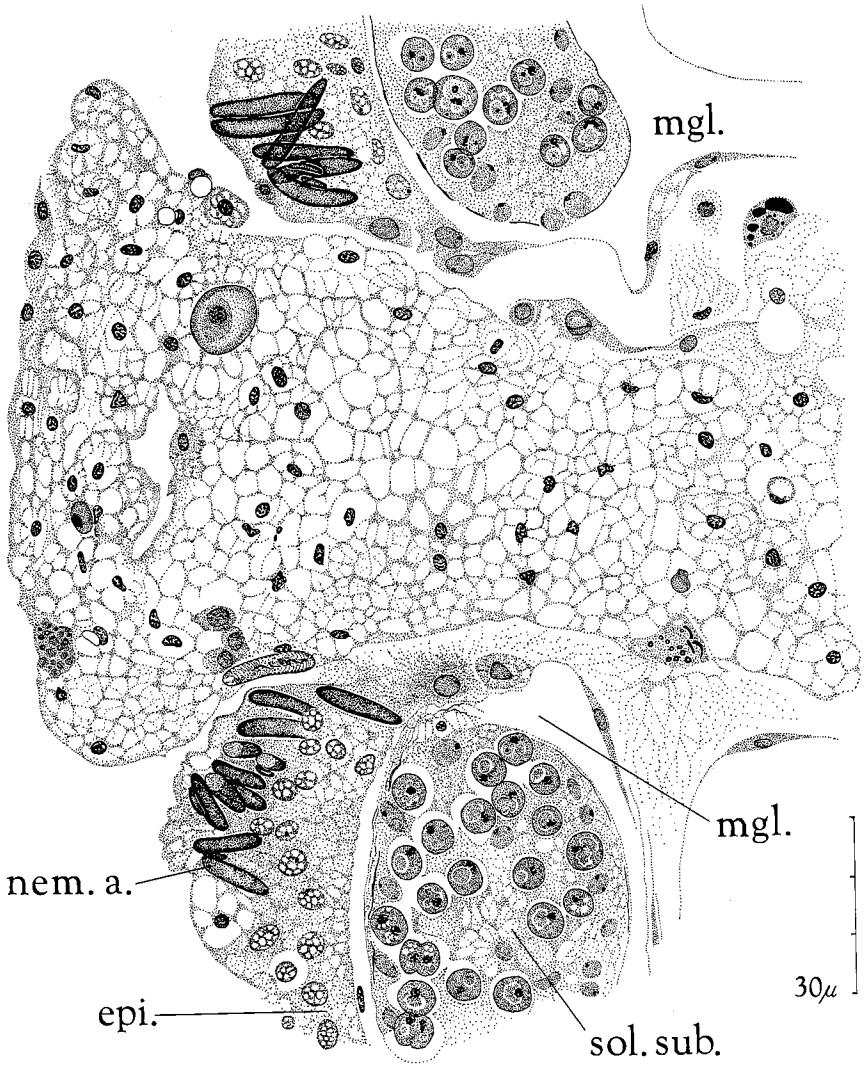


FIGURE 51. Histology of *Plexaura homomalla:* section through outermost layer of coenenchyme between two subepidermal solenia, showing perforation of epidermis by frothy-cell cord.

both in the filaments and in the entoderm of both septal faces, but not so abundantly as in the asulcal septa.

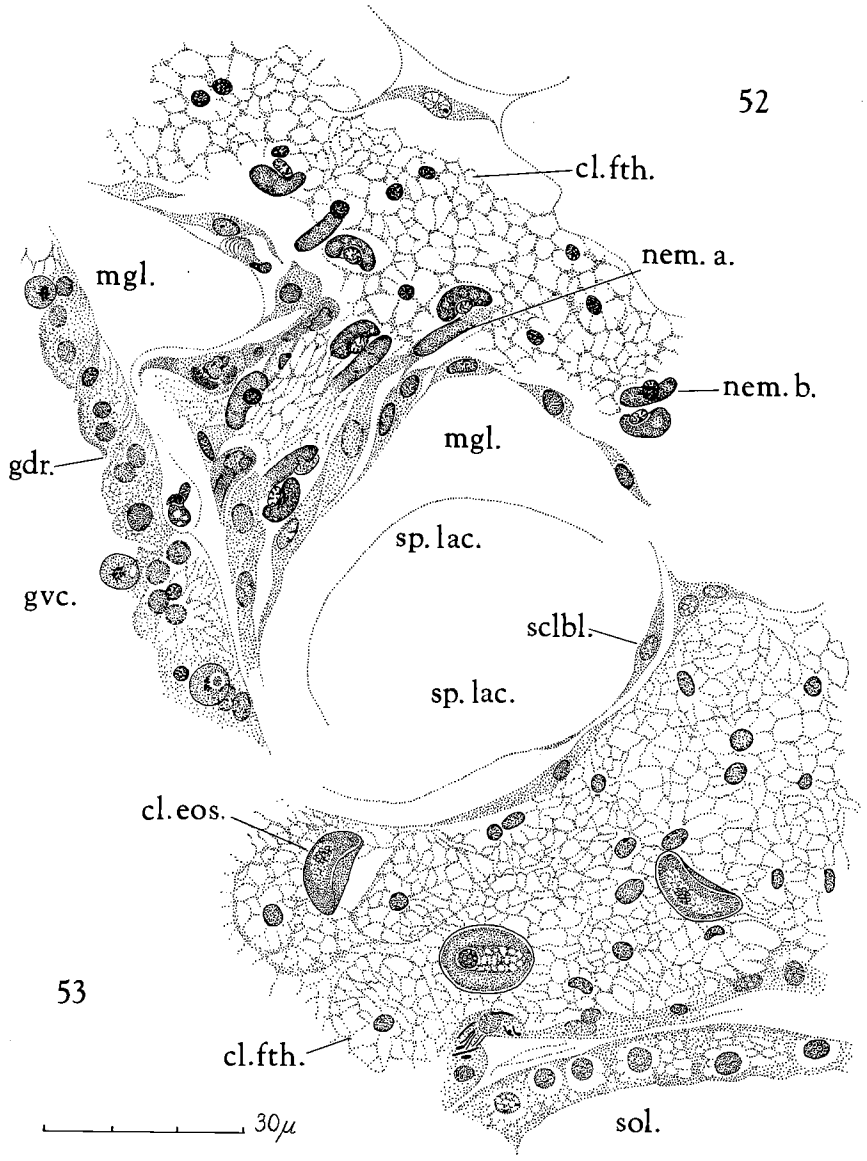
The pharynx is laterally compressed so that in cross section its two narrow ends lie at the so-called dorsal and ventral sides of the zooid. Its lining is commonly stated to be of ectodermal origin [12], but this has not been verified for *Plexaura homomalla* by embryological studies, so far as I am aware. Whatever its origin, the pharyngeal lining, except for that along the narrow edge ("ventral") toward which the septal retractor muscles face, consists of tall flagellated cells, abundantly interspersed with goblet cells containing large spherules (Fig. 15), in some cells stained bright red (Gomori's Trichrome), in others deep reddish brown. No nematocysts of the predominant type were found in the pharynx, but in one section a cell with irregularly coiled contents was seen. It stained neither pink like the common nematocysts nor purplish blue like the reniform nematocysts, and its interpretation is problematic. Also seen in the pharyngeal lining are the purplish-blue staining, fine tubular structures (apparently not cells) that were noted in the entoderm elsewhere.

The narrow ventral side of the pharynx is occupied by the siphonoglyph, a longitudinal tract of tall, crowded, flagellated cells with nuclei more elongated and more darkly stained than are those of the other pharyngeal cells (Fig. 17). Goblet cells are lacking, but there are a few cells with larger, paler nucleus.

Entoderm like that of the lining of the gastric cavity extends out into the coenenchyme as the lining of solenia. A network of these lies just beneath the epidermis (Figs. 1, 2), from which it is separated by a thin layer of mesogloea. This peripheral network connects directly with the gastric cavities of the zooids (Fig. 60) as well as with the more diffuse solenial network of the deeper coenenchyme. The solenia deep in the coenenchyme connect in turn with the large longitudinal canals immediately surrounding the axis.

The entodermal cells of the solenia, like those of the gastric cavities, are more or less columnar, moderately tall but not so tall as the epidermis, and, like them, commonly tapered from the epithelial surface to a rather narrow base; intercellular spaces are abundant. Red-staining fibers, apparently myonemes, may be present in the bases of those near the solenial openings into the gastric cavities, suggesting that the size of the openings can be controlled to some degree (Fig. 61). Similar fibers are present in the entodermal cells of the peripheral solenia, where they follow a circular course (Fig. 50), and in the lower part of the anthocodial wall (Fig. 25).

In the upper parts of the anthocodia, the septa, and in the walls of the peripheral canals beneath the epidermis, the entoderm is crowded with zooxanthellae, and the boundaries of individual entodermal cells cannot readily be distinguished. Zooxanthellae are fewer in the entoderm of the lower part of the gastric cavities and pharyngeal wall, and in the deeper solenia where they usually are absent altogether.



FIGURES 52-53. Histology of *Plexaura homomalla:* 52, close contact of frothy-cell cord with gastric cavity of zooid; flat entoderm surrounding frothy cells; two types of nematocysts in cell cords; 53, corpuscle-like cells in frothy cell cord.

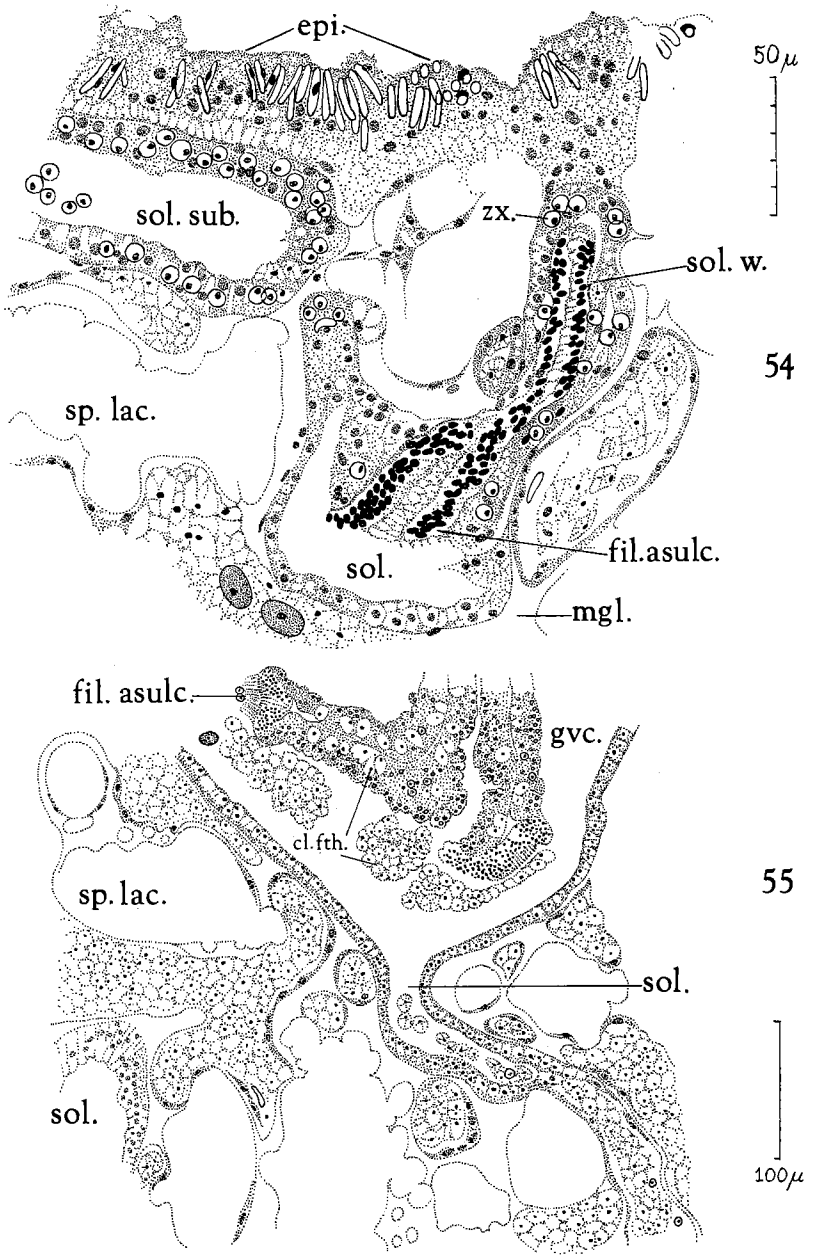
The lining of the longitudinal stem canals is not unlike the entoderm of the smaller solenia, but in some places is much lower (Fig. 19). It elsewhere may be very tall, with large intercellular spaces which may contain cells with coarse, red-staining cytoplasmic granules, and highly vacuolated frothy cells, both of which appear to have been phagocytized (Fig. 20). Masses of entodermal cells derived from the asulcal filaments, including flagellated cells, frothy cells, and zooxanthellae, are sometimes seen in the lumen of the longitudinal stem canals, and these may lie in close contact with the cells of the canal wall where it appears that phagocytosis may take place (Fig. 62).

In tangential sections, the entoderm of the longitudinal canal walls is found to be composed of two strata. That lying adjacent to the mesogloea consists of elongated cells deployed transversely or obliquely (Fig. 23), and may include scattered oval cells with coarsely granular cytoplasm. This is covered by a layer of cells rather squarish or irregularly polygonal in surface view (Fig. 24) but conical or pyramidal in section (Fig. 22), lining the lumen of the canals. In section, the canal walls are not noticeably stratified because the nuclei of the fusiform cells are widely scattered and only inconspicuous bits of cytoplasm are visible in the plane of section.

In addition to solenia and spicules with their thin investment of mesogloea, the coenenchyme contains abundant cell-strands composed of frothy cells, already mentioned. These cell cords are surrounded by a layer of mesogloea, usually very thin or lamellar, and inconspicuously separated from it by an extremely tenuous layer of flat epithelial cells whose most conspicuous feature is the nucleus (Figs. 50, 52). The origin of this epithelium or of the cells of the cell cords has not been determined. The epithelium apparently gives rise to nematocysts of both kinds observed in this species, and nematocytes containing formative stages of the cysts are abundant in the epithelial walls of the cell cords, especially in the cords lying adjacent to zooids. Fully developed nematocysts are commonly seen among the frothy cells, and they probably are conveyed to their functional positions through the cell-strands.

Muscle System.—Time has not permitted a thorough study of the muscle system of *Plexaura homomalla,* but in its general aspects it resembles that of *Alcyonium* [11] and *Pseudoplexaura porosa* [7]. The longitudinal retractor muscles form the most conspicuous element of this system. The muscle fibers are situated on simple or compound lamelliform folds of mesogloea (Fig. 36) and, as usual in octocorals, they lie on the sulcal faces of all septa (Fig. 4). In addition, there are smaller longitudinal muscles on the asulcal faces of the septa, best seen in the upper part of the zooids where the septa are attached to the pharynx (Figs. 26, 37).

Circular entodermal muscles are present in the anthocodial wall and in the pharynx. In longitudinal sections of the zooids, the muscle fibers lie at the base of the entoderm (Fig. 25) of the zooid wall, and on low meso-



FIGURES 54-55. Histology of *Plexaura homomalla:* 54, extension of asulcal septal filaments into solenia, where they closely approach the zooxanthellae; 55, frothy cells in entoderm of septa and in clumps in the gastric cavity of zooid; some frothy cells also can be seen in the solenium leading out of the gastric cavity.

gloeal ridges encircling the oral end of the pharynx (Figs. 15, 16), where they form a weak oral sphincter.

Cells and fibers of possibly contractile nature in other tissues have been mentioned elsewhere (Figs. 23, 50, 61).

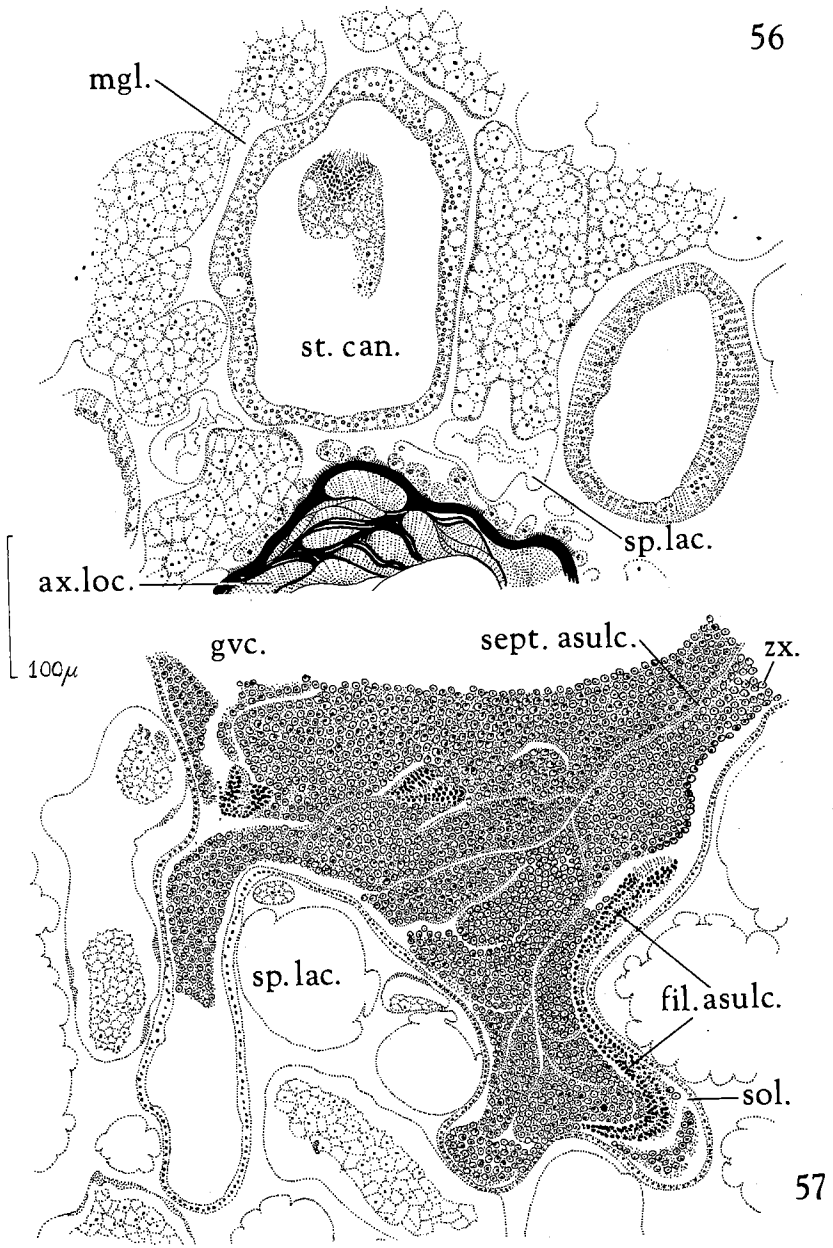
Gonads.—As in other octocorals, the gonads of *P. homomalla* develop near the edge of all septa but the asulcal pair. The sexes are separate and, as all the zooids in a given colony are vegetatively produced and genetically alike, colonies are entirely of one sex.

In female colonies, the six fertile septa bear several eggs, each with its own short mesogloeal stalk and surrounded by a thin mesolamella covered by gastrodermis (Fig. 44). The eggs (Figs. 44, 45) are similar to those described for other octocorals including *Alcyonium* [11], and were found in most of the female colonies sampled at all seasons, and in all during the summer months [10]. In none of the sections examined were dividing eggs or developing embryos observed, even at and just following the time of maximal sperm development and release.

The gastrodermis surrounding the ripe and nearly ripe ova is very thick, consisting of abundantly vacuolated cytoplasm, seemingly syncytial, containing nuclei stratified into three layers (Fig. 46). The peripheral layer consists of darkly stained nuclei commonly more or less distorted and sometimes lobulated; the inner layer, immediately surrounding the mesogloeal covering of the egg, consists of widely scattered cells with cytoplasm more darkly stained than that surrounding, and more or less distinctly differentiated from it. Midway between these two layers is a conspicuous, dense layer of ovoidal or spheroidal nuclei, less darkly stained than the others, thickly strewn through a froth of large vacuoles. Both inner and outer strata may be more or less inconspicuous, but the middle layer is distinct in all cases examined.

In male colonies, the germ cells occupy stalked spermaries, rounded when young but with maturity becoming indented by broad depressions and concavities. Immature spermaries (Fig. 38) are filled with spermatogenetic cells. These have darkly stained nuclei and converge in rows toward the center of the spermary (Figs. 38, 40), where there usually is a small hollow space. The developing male cells are small, and evidence of karyokinesis is scanty and difficult to observe. A few examples of probable karyokinetic activity, possibly at prophase stage, were found in a colony fixed on June 31, 1972. These invariably were peripheral in location (Fig. 43). Mature spermaries are packed with sperm with intensely stained, conical heads and well developed tails (Figs. 39, 42). The central space is usually conspicuous, and in many cases it contains several somatic cells. Some of these have conspicuous cytoplasmic granules stained bright red in Gomori's Trichrome, others a conspicuously vacuolated cytoplasm.

The gastrodermis surrounding the spermaries is not so clearly stratified as is that of the eggs. It consists of rather tall, columnar cells with con-



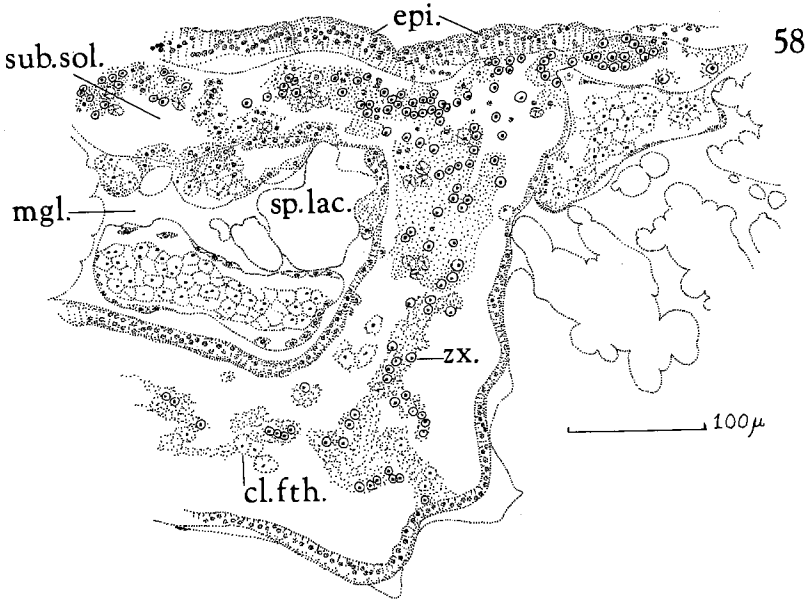
FIGURES 56-57. Histology of *Plexaura homomalla:* 56, asulcal septal filament in longitudinal stem canal; 57, asulcal filaments extending from gastric cavity into coenenchymal solenia.

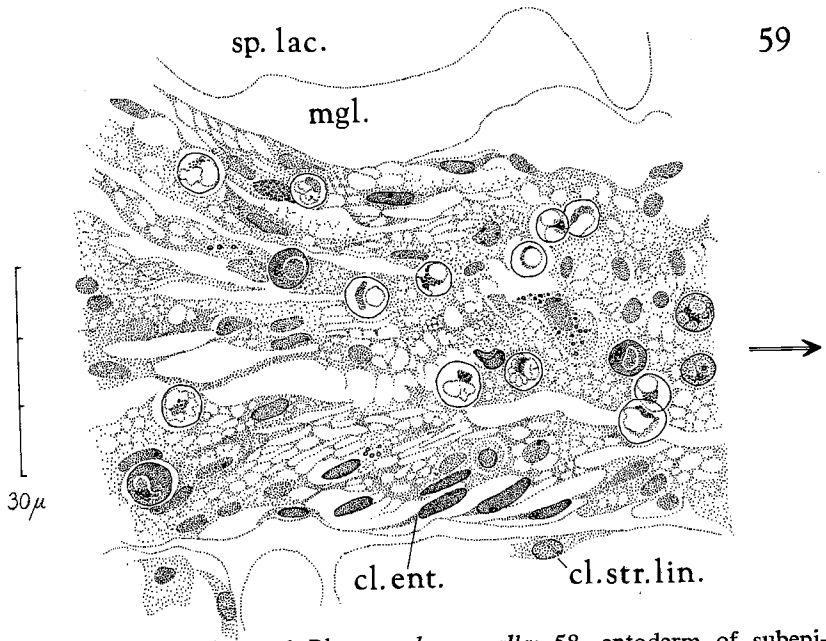
spicuously vacuolated cytoplasm and a few rounded or fusiform cells, some with vacuolated cytoplasm, some with bright red granules (Figs. 40, 43). The gastrodermis of fully developed spermaries (Fig. 39) is not so thick as that of immature ones (Fig. 38). Peculiar structures consisting of a convoluted mass of tubules whose walls stain dull blue in haematoxylin and eosin were also observed in the gastrodermis of the spermaries (Fig. 41). It was not determined whether they are intra- or extra-cellular nor what their function might be. Similar but less complex tubules were seen elsewhere in the gastrodermis lining the zooids, especially the filaments of the six fertile septa.

Males are recognizable only in the months of June, July and August. In June, the spermaries are clearly recognizable but contain no spermatozoa. In 1973, all males had sperm with tails by July 20, and by August 3 the spermaries of all but one colony had been completely resorbed. These observations indicate that males are sexually active for only a short time during the latter half of June and the first half of July. Present data do not permit a closer delimitation of the period of male activity, nor do they indicate whether temperature or the lunar cycle is involved in the controlling mechanism.

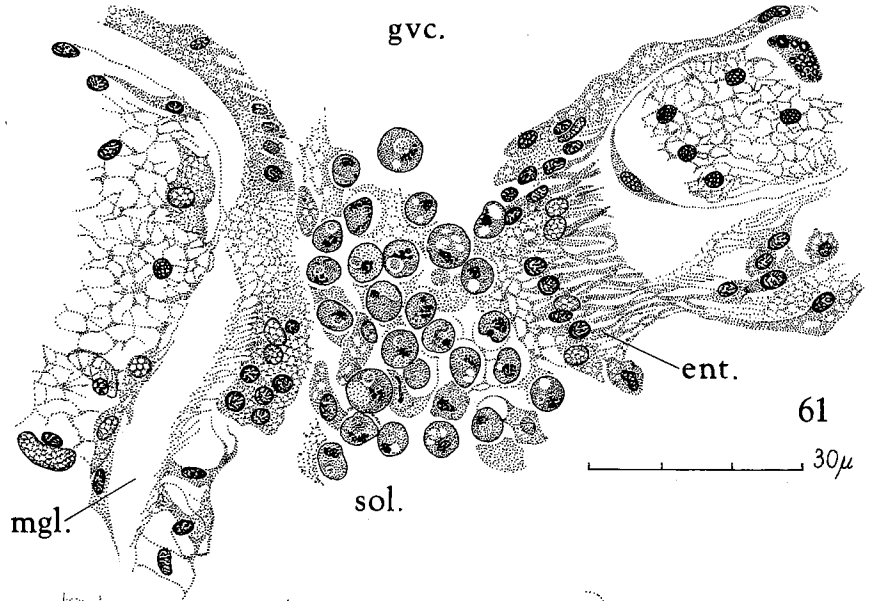
Zooxanthellae.—Examination of serial sections made from six specimens of *Plexaura homomalla* reveals differences in the appearance and distribution of zooxanthellae which suggest that the population of these algae undergo cyclic development and degeneration in their hosts. These observations have necessarily been of a superficial and uncontrolled nature, but they are sufficient to suggest that the zooxanthellae flourish, proliferate and then degenerate en masse. During this cycle, the algae change their location in the host, and it seems likely that at some point during this migration they are broken down by the coelenterate and the nutrients that they contain assimilated. It will be necessary to conduct repeated studies upon the same host colony, including routine histological examination as well as electron microscopy, in order to observe progressive change in the algal population and to record the morphological aspects in detail. Following such an investigation, appropriate experimental procedures might be designed to reveal something of the role played by the zooxenthellae in the metabolism of *Plexaura homomalla.* As has already been mentioned, this species has histological features unique among the known Gorgonacea. There is no reason to conclude that the relationship of the algae to their host is not similarly unique, and it also seems reasonable to conclude that this relationship may have a bearing upon the ability of the coelenterate to concentrate prostaglandins in large amounts.

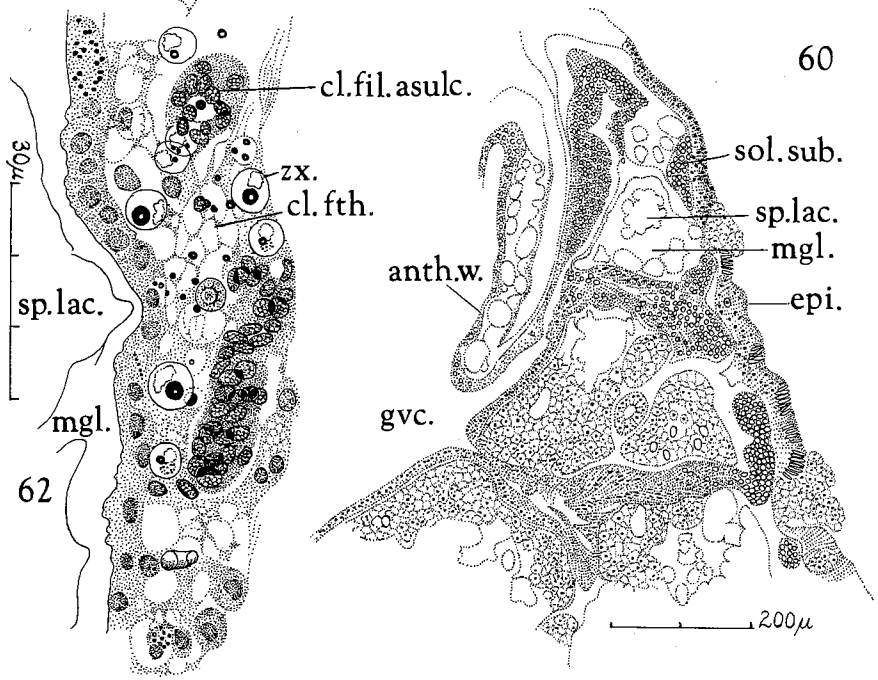
The present investigation suggests that the zooxanthellae are at one stage concentrated in the entoderm of the peripheral, subepidermal solenia (Figs. 9-11, 50) and anthocodiae (Fig. 10). They are fewer in the entoderm of the gastric cavity (Fig. 14), becoming less and less numerous deeper in the calices. They are present also in the entoderm of the septa





FIGURES 58-59. Histology of *Plexaura homomalla:* 58, entoderm of subepidermal solenia releasing zooxanthellae into lumen, and algal cells passing into deeper coenenchymal solenia; 59, zooxanthellae passing from subepidermal solenia into deeper canals.





FIGURES 60-62. Histology of *Plexaura homomalla:* 60, section of coenenchyme adjacent to gastric cavity, showing zooxanthellae free in subepidermal solenia and extending into solenia connecting with gastric cavity of zooid; 61, entrance of solenium into gastric cavity, showing apparent contractile elements whereby communication between solenium and zooid may be controlled; 62, entoderm of longitudinal stem canal in close contact with mass of tissue containing frothy cells, cells from asulcal filaments, and zooxanthellae in various states of disintegration.

(Fig. 34) but are uncommon in the adjacent wall of the pharynx, and generally absent from the deep coenenchymal solenia and longitudinal stem canals. These zooxanthellae have the aspect of the healthy algae as described in stony corals by Yonge and Nicholls [22].

In two specimens sectioned, the entoderm of the subepidermal solenia has in some places begun to discharge zooxanthellae into the lumen, and clumps of algal cells can be seen in the connecting solenia leading to the deeper coenenchymal network and to the gastric cavities of the zooids (Figs. 59, 60). In one specimen, large numbers of zooxanthellae lie in the gastric cavity, where they are accompanied by frothy cells like those of the coenenchymal cell-cords (Fig. 55). The entoderm of the asulcal septa below the level of the pharynx, where the filaments are thrown into complex convolutions, is packed with vast numbers of algae, and includes also many frothy cells, goblet cells with conspicuous red-stained (Gomori Trichrome) spherules, and oval cells with many coarse, dark red granules in the cytoplasm. In one specimen, the entodermal cells contain many red-stained globules, but in another they are few. The zooxanthellae appear to be undergoing progressive degeneration. In one sample, a few zooxanthellae stain bright red, although the nucleus, pyrenoid and assimilation product remain visible, while most stain very lightly (Gomori). In another, the contents of the algal cells are clear and unstained except for one or two large red globules (or several smaller ones). Toward the bottom of the gastric cavity, the septa are folded against the zooidal wall, leaving the center of the cavity open. The convoluted edges of the asulcal septa form conspicuous masses of zooxanthellae traversed by the darkly stained flagellated filaments. The mesogloea is here very thin in the septa, but it can be traced with phase contrast optics, and its position is usually indicated by the orientation of frothy cells along it. The gonads necessarily lie close to the wall of the gastric cavity as they are attached to the septa, and the ripe spermaries are here much flattened. Unripe male gonads and eggs of all sizes retain their shape and project noticeably into the center of the gastric cavity.

Folds of the asulcal filaments and adjacent parts of the septa extend outward into the solenial system of the coenenchyme, carrying many zooxanthellae with them (Fig. 57). Although shortage of time has precluded tracing these extensions carefully, it seems probable that they become detached from the septa and proceed through the solenia deep in the coenenchyme. Asulcal filaments definitely have been seen in the longitudinal stem canals (Fig. 56) and obviously have separated from the septa to which they initially were attached.

The presence of highly vacuolated frothy cells in the entoderm of the asulcal septa, the intermingling of frothy cells and zooxanthellae in the gastric cavities, the widespread penetration of cords of frothy cells throughout the coenenchyme, the presence of nematocysts in the cell-cords, and the undeniable evidence of the discharge of masses of frothy cells through the epidermis to the surrounding water all point to a function of these cords

as a transport mechanism, for nematocysts, for zooxanthellae, and possibly also for nutrients and metabolic waste products. Although much further research will be required to confirm the histological evidence, this system of coenenchymal cell strands in *Plexaura homomalla*—apparently unique among the gorgonians—may represent a primitive excretory system.

REFERENCES

1. Bayer, Frederick M., 1954. *The microanatomy of five holaxonian octocorals.* Unbuplished Master's Thesis, The George Washington University. Washington, D.C. 69 pp., 18 pls.
2. Bayer, Frederick M., 1961. *The shallow-water Octocorallia of the West-Indian region. A manual for marine biologists.* Martinus Nijhoff, The Hague, 373 pp., 101 figs., 27 pls.
3. Bayer, Frederick M., 1973. *Plexaura homomalla:* brief historical background. In: Prostaglandins from *Plexaura homomalla.* A Sympsosium . . . sponsored by The Upjohn Company. *Stud. trop. Oceanogr. Miami 12:* 1-8.
4. Bayer, Frederick M. and Elisabeth Deichmann, 1958. Two new plexaurid gorgonians from the Bahama Islands. *Bull. Mar. Sci. Gulf and Carib. 8*(3): 224-235, figs. 1-5.
5. Berg, Sten E., 1941. Die Entwicklung und Koloniebildung bei Funiculina quadrangularis (Pallas). *Zool. Bidr. Uppsala 20:* 1-100, pls. 1-7.
6. Bourne, Gilbert C., 1899. Studies on the structure and formation of the calcareous skeleton of the Anthozoa. *Quart. J. micro. Sci.* (new ser.) *41*(4): 499-547, pls. 40-43.
7. Chester, Wayland M., 1913. The structure of the gorgonian coral *Pesudoplexaura crassa* Wright and Studer. *Proc. Amer. Acad. Arts Sci. 48*(20): 737-773, pls. 1-4.
8. Esper, Eugenius Johann Christoph, 1788-1830. *Die Pflanzenthiere in Abbildungen nach der Natur mit Farben erleuchtet nebst Beschreibungen,* 3 vols., and *Fortsetzungen der Pflanzenthiere,* 2 vols., together 5 vols., Nürnberg, pp. xii + 320, 220, 285+, 230, 48, 428 pls.
9. Goldberg, Walter M., 1973. *The chemistry, structure, and growth of the gorgonian and antipatharian coral skeleton.* Ph.D. Dissertation, University of Miami.
10. Goldberg, Walter M. and Ramon D. Hamilton, 1973. The sexual cycle in *Plexaura homomalla.* In: Prostaglandins from *Plexaura homomalla.* A Symposium . . . sponsored by The Upjohn Company. *Stud. trop. Oceanogr. Miami 12:* 58-61.
11. Hickson, Sydney J., 1895. The anatomy of *Alcyonium digitatum. Quart. J. micr. Sci.* (new ser.) *37*(4): 343-388, pls. 36-39.
12. Hyman, Libbie H., 1940. *The invertebrates: Protozoa through Ctenophora.* McGraw-Hill Book Co., New York and London. xii + 726 pp.
13. Kinzie, Robert A. III, 1970. *The ecology of the gorgonians (Cnidaria, Octocorallia) of Discovery Bay, Jamaica.* Ph.D. Dissertation, Yale University.
14. Kinoshita, Kumao, 1910. Ueber die Postembryonale Entwicklung von *Anthoplexaura dimorpha* Kükenthal. *Journ. Coll. Sci., Imp. Univ. Tokyo 27*(14): 1-13, 1 pl.
15. Kükenthal, Willy, 1924. Gorgonaria. *Das Thierreich,* 47. xxviii + 478 pp., 209 figs.
16. Moser, Johannes, 1921. Ergebnisse einer Revision der Gattung *Plexaura* Lamouroux. *Zool. Anz. 53:* 110-118.

17. STUDER, TH., 1891. Cas de fissiparité chez un alcyonaire. *Bull. Soc. Zool. France* 16: 28-30.
18. STUDER, TH., 1901. Alcyonaires provenant des campagnes de l'Hirondelle (1886-1888). *Res. Camp. Sci. Monaco 20:* 1-64, pls. 1-11.
19. THEODOR, JACQUES, 1967. Contribution à l'étude des gorgones (7): Ecologie et comportement de la planula. *Vie et Milieu 18*(2A): 291-301.
20. VIGHI, M., 1970. Ricerche sul ciclo riproduttivo del corallo rosso [*Corallium rubrum* (L.)] del Promontorio di Portofino. *Atti. Accad. Lincei* (8) *10*(1): 1-26.
21. WEINHEIMER, ALFRED J. AND R. SPRAGGINS, 1969. The occurrence of two new prostaglandin derivatives (15-epi-PGA$_2$ and its acetate, methyl ester) in the gorgonian *Plexaura homomalla.* Chemistry of Coelenterates, XV. *Tetrahedron Lett. 59:* 5185-5188.
22. YONGE, C. M. AND A. G. NICHOLLS, 1931. Studies on the physiology of corals IV. The structure, distribution and physiology of the zooxanthellae. *Sci. Rep. Great Barrier Reef Exped. 1928-29, 1*(6): 135-176, pls. 1-2.

RECOLONIZATION AND REGROWTH OF A POPULATION OF THE GORGONIAN *PLEXAURA HOMOMALLA**

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ABSTRACT

An analysis is made of the size and structure of a population of the gorgonian *Plexaura homomalla* (Esper) occurring on a small patch reef near Miami, Florida. The maximum population density of the species was found to be 3.88 colonies/m^2 and the standing crop was calculated as 388 gm (dry weight)/m^2. Average-size colonies are 30 cm tall and weigh 70 gm (dry weight), and the largest colonies, which are 60-70 cm tall and weigh 800-1000 gm, are thought to be 25-30 years old. Estimates are also given for the rate of growth of individual colonies and for the rate of recruitment for populations on cleared and uncleared reef areas. The age of individual colonies, as estimated from their dry weight, appears to be closely correlated with the number of concentric rings on the basal part of the axis.

The results of the study indicate that *P. homomalla,* as well as most other species of gorgonians, can maintain a high degree of population stability.

INTRODUCTION

In 1958, the Biology Division at the Rosenstiel School of Marine and Atmospheric Sciences, University of Miami, began a series of ecological studies of nearby coral reefs [5]. As part of this program, and under the direction and supervision of Dr. F. M. Bayer, a study was undertaken to examine the distribution, abundance and diversity of shallow-water gorgonians [4]. It was not the original intent of this study to analyze the population dynamics of any particular species of gorgonian, and because of the method used, factors necessary for a comprehensive population study, such as recruitment and morality rates and age-specific growth rates, were not considered. However, because of the importance of population studies in regard to the careful and controlled exploitation of a marine resource such as *Plexaura homomalla* (Esper), and especially in light of the absence of available information on the subject, it is desirable to analyze any information pertaining to the population structure of the species which can be extracted from the above-mentioned coral reef studies. It must be emphasized,

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however, that more detailed studies would be needed to substantiate many of the points discussed in this paper.

Only one detailed study of the population dynamics of gorgonian corals has been made, and this was done by Grigg [2] for two species of eastern Pacific gorgonians, *Muricea californica* and *M. fruticosa*. Grigg found that *M. californica* had a population density ranging from 0.62 to 7.90 colonies/m^2, an annual rate of recruitment of 0.108 colonies/m_2, and an expected life span at settlement of 10.68 years. The mean density of *M. fruticosa* ranged from 0.10 to 2.35 colonies/m^2, recruitment was 0.041 colonies/m_2/year, and the expected life span at settlement for this species was found to be 3.63 years. The maximum age of *M. fruticosa* was estimated to be about 20 years, while that for *M. californica* was about 50 years. Under favorable conditions, populations of both species reached a steady state where mortality and recruitment rates were the same. Grigg considered population regulation to be controlled in most cases by space-limited recruitment, except where larval survival rates were extremely low. Distribution limits were apparently due to the effect of temperature on reproductive success and larval settling (Grigg, personal communication).

Population studies similar to those made by Grigg have not been attempted on *Plexaura homomalla,* and only a few general ecological studies have been carried out on the species. The only estimates of growth rates for the species were those made by Cary [1]. In studies in the area of the Dry Tortugas, Cary found that the average wet weight of *P. homomalla* was 1.0 lb and that four years were required to reach this "medium size," after which growth rates were considerably reduced. Cary was of the opinion that mortality from old age did not occur in gorgonians. He considered silting over and detachment of the colonies by waves and surge, and overgrowth by *Millepora* and bryozoans to be the major causes of gorgonian mortality.

Acknowledgments

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This paper has benefitted from discussions and correspondence with Dr. Richard W. Grigg, to whom I extend sincere thanks for his thoughtful comments and suggestions.

Materials and Methods

The data on which this study is based were obtained by the quantitative sampling of the gorgonian fauna at a small patch reef located approximately 17 miles south-southeast of Miami, Florida. The patch reef, known as Red Reef, is situated in Margot Fish Shoal about 1½ miles west of Long Reef, on the outer reef margin, and about 2 miles east of Elliott Key,

the nearest part of the island chain which encloses Biscayne Bay. The area was selected so as to be representative of a typical patch reef habitat and, as such, it supports a wide variety of corals, sponges, algae and gorgonians, as well as numerous species of other invertebrates and fishes. Descriptions and lists of the fauna and flora of such a patch reef habitat are given by Voss *et al.* [6]. A thorough hydrographic survey of Red Reef was made by Jones [3].

At Red Reef, a permanent survey site of 64 square meters was set up in 1962. Three separate collections of all the gorgonians occurring within the study area were made over the following five years; the first was made in August of 1962, the second on August 15, 1966, and the last on August 4, 1967. The gorgonians were air-dried, measured, weighed and identified. The identifications were made by Dr. F. M. Bayer. The collections were analyzed as to total number of species and colonies, population densities, biomass, and average height and weight of each species.

The sample taken in 1962 is representative of a natural population of *P. homomalla* from an undisturbed patch reef; the sample for 1967 is a natural population after one year of recolonization and regrowth on a cleared area of bottom, and the one for 1966 represents a four year old population.

Since the method of study did not include *in-situ* measurements of changes in the size of individual colonies from year to year, size-specific or age-specific growth rates could not be calculated directly. For similar reasons mortality rates were not determined. Growth rates and rates of recruitment were estimated from the examination of size frequency diagrams for each of the samples.

Relative Abundance of *Plexaura homomalla*

The relative abundance and average size of the colonies of *Plexaura homomalla* for each of the three samples are shown in Table 1. In 1962, 1736 colonies of gorgonians were collected at Red Reef. Twenty-eight species were represented in the collection. The total population density for all gorgonians was found to be 27.12 colonies/m^2, while that for *P. homomalla* was 3.88 colonies/m^2, or about 14 per cent of the total. This species was the second most abundant species at Red Reef and was surpassed in numbers only by the scleraxonian *Briareum asbestinum.* In terms of dry weight biomass, *P. homomalla* accounted for 338 gm/m^2, or about 24 per cent of the total gorgonian biomass (1399 gm/m^2).

In 1966, the population density of *P. homomalla* was 2.45 colonies/m_2 compared with a density of 17.12 colonies/m^2 for all gorgonians. In this sample the species made up 15 per cent (49 gm/m^2) of the total biomass which was approximately 326 gm/m^2.

Only 210 gorgonians (3.28 colonies/m^2) were found at Red Reef in 1967, and 17 percent of these were specimens of *P. homomalla.* The population density of the species was 0.56 colonies/m^2, and the species accounted for only 4 per cent (0.75 gm/m^2) of the total biomass of 18 gm/m^2.

Yr. of coll.	Entire gorgonian population: No. of spec.	Pop. density (col./m²)	Dry wt. biomass (gm)	Standing crop (gm/m²)	No. of spec.	Population of *Plexaura homomalla*: Pop. density (col./m²)	% of entire gorgon. pop.	Dry wt. biomass (gm)	Avg. ht. (cm)	Avg. wt. (gm)	Standing crop (gm/m²)	% of total gorgon. biomass
1962	1736	27.12	89505	1399	248	3.88	14.3	21630	30.2	70.2	337.97	24.2
1966	1096	17.12	20872	326	157	2.45	14.3	3117	21.1	19.8	48.70	14.9
1967	210	3.28	1086	18	36	0.56	17.1	48	9.3	1.5	0.75	4.4

TABLE 2

RELATIVE ABUNDANCE OF THE EIGHT COMMONEST HOLAXONIAN GORGONIANS AT RED REEF

Species	Percent of population 1962	1966	1967
Plexaura homomalla	14.3	14.3	17.1
Pseudopterogorgia bipinnata	13.0	17.3	9.4
Eunicea succinea	7.2	12.6	13.6
Plexaura flexuosa	7.9	5.9	4.6
Pseudoplexaura porosa	6.7	2.9	3.3
Muriceopsis flavida	3.2	2.6	2.7
Pseudopterogorgia americana	2.7	3.4	6.1
Gorgonia ventalina	2.7	2.6	3.6

TABLE 3

ESTIMATED AGE AND NUMBER OF GROWTH RINGS IN COLONIES OF *Plexaura homomalla*

No.	Ht. (cm)	Wt. (gm)	Est. age* (yr.)	Diam. of axis (mm)	No. of growth rings	Avg. width of ring (mm)
1	16	12	2.5	3	2-3	0.63
2	20	18	3.3	4	4-5	0.44
3	19	20	3.5	6	6-8	0.42
4	22	30	4.5	5	5-6	0.45
5	26	49	5.8	6	5-7	0.54
6	29	104	8.5	10	8-10	0.44
7	33	106	8.6	8	8-9	0.48
8	32	167	10.8	10	7-9	0.62
9	42	93†	8.2	10	14-15	0.34
10	46	304	14.5	13	12-14	0.50
11	43	308	14.6	18	15-16	0.58
12	65	465	18.0	15	16-18	0.44
13	59	487	18.4	19	22-24	0.41
14	60	732	22.4	20	25-29	0.37
15	88	736	22.4	23	30-31	0.38
16	66	205†	12.0	14	30-35	0.22

*Estimated ages based on dry weight as predicted in Figure 4.
†Incomplete specimens.

Although the relative biomass of *P. homomalla* dropped from 24 per cent in the 1962 collection to only 4 per cent of the total in the 1967 collection, the relative number of colonies of the species remained nearly constant at 14-17 per cent of the gorgonian population (Table 2). Furthermore, the relative abundance of the eight most common gorgonians (excluding scleraxonians) showed only small fluctuations in the three samples (Table 2). This would suggest that the gorgonian fauna at Red Reef had a relatively stable composition over the entire sampling period. It is postulated that this stability is indicative of a constant spawn output and a constant survival rate for the planulae of each species of gorgonian represented in the collections. In each species larval success would be dependent on the relative abundance of nearby adult populations which presumably were undisturbed over the entire sampling period. Maximum spawning activity generally takes place during the early summer, and thus the smallest colonies collected were probably about one year old. If the planulae settled after the collections were made. then the rate of recruitment may have been abnormally high due to the increase in available substrate resulting from the removal of the adult colonies. Since an average of 25 cm² of bottom were exposed by the removal of each colony, the total exposed area after the 1962 collection probably amounted to not more than 4.5 m².

DISCUSSION

The Red Reef collection of 1967 contained 36 colonies of *Plexaura homomalla*. The size-frequency distribution for this population is given in Figure 1. The average height of the colonies was 9 cm and the average dry weight 1.5 gm. The population density of the species was 0.56 colonies/m². This latter figure is a rough estimate of the annual rate of recruitment of the species on a cleared area of bottom, minus any mortality which may have occurred during the first year after settlement. A rate of recruitment of

FIGURE 1. Size-frequency diagram for the population of *Plexaura homomalla* at Red Reef in 1967.

0.56 colonies/m², coupled with low mortality rates in the following year-classes, would, in four years, produce a population of 2.24 colonies/m². The population of *P. homomalla* in 1966, after four years of regrowth, had a density of 2.45 colonies/m², only slightly greater than that calculated from the rate of recruitment for the 1966-67 population.

The average height of the colonies of *P. homomalla* collected in 1966 was 21 cm, and the average dry weight was 19.8 gm. To estimate the mean size of each of the four year-classes presumed to be represented in the population, the sample was divided at natural breaks into four size groups as shown in Figure 2. This was done under the assumption that variation in individual growth rates did not greatly alter the composition of each of the year-classes.

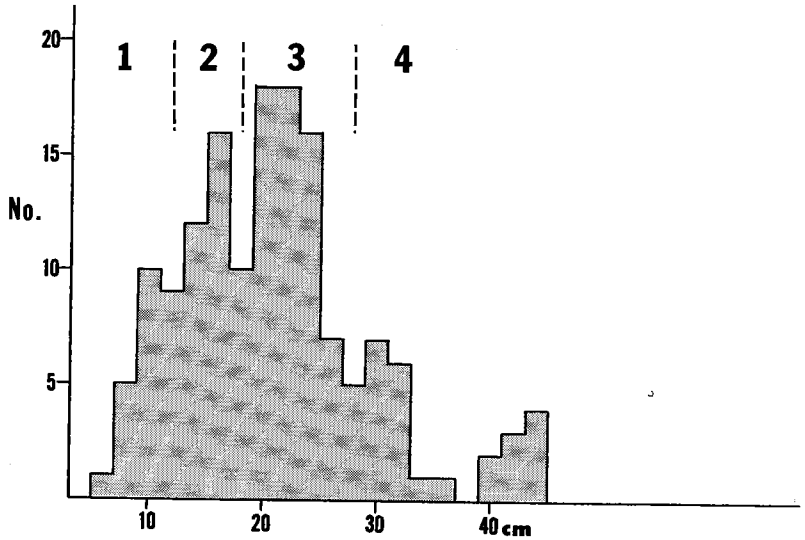

FIGURE 2. Size-frequency diagram for the population of *Plexaura homomalla* at Red Reef in 1966.

In the first year-class the average weight of the colonies is 3 gm; it is 8 gm in the second year-class, 15 gm in the third, and 25 gm in the fourth. From this data the annual increase in the rate of growth can be estimated to be 2-3 gm. Members of the fourth year-class, then, should show an average rate of growth of about 12 gm/year, and those of the fifth year-class a rate of 15 gm/year. If this rate of increase continued over the following years, as shown in the growth curve in Figure 3, then the largest colonies of *P. homomalla* collected at Red Reef in 1962, which were 60-70 cm tall and weighed 800-1000 gm, should be approximately 23-27 years old.

Although the age of the larger colonies was not determined directly, the accuracy of the growth curve given in Figure 3 was checked by comparing the age of the several colonies, as predicted by the curve, with the number of concentric layers of gorgonin in the basal part of the axis of the colonies. Grigg has shown that in *Muricea californica* and *M. fruticosa* the layers of gorgonin represent annual growth rings [2]. Such rings are often quite distinct in colonies of *P. homomalla* (Figure 4), and it is very likely that these are produced annually, especially in specimens from the waters off south Florida where there are seasonal changes in environmental conditions [3].

FIGURE 3. Theoretical growth curve for *Plexaura homomalla*.

Sixteen colonies of *P. homomalla* were selected randomly from several locations near the study area. The size, weight, estimated age, and number of rings of gorgonin present in the base of each colony is given in Table 3. In most of these specimens there is a close correlation between the number of growth rings and the estimated age based on the dry weight. Major discrepancies occur in four specimens (Table 3, No. 3, 9, 15 and 16), but in three of these, the height of each colony is much greater than the height of other specimens of similar weight. In colonies of similar height, the number of rings is about the same, therefore, it seems likely that these three colonies were abnormally low in weight.

The fact that the largest colonies examined possessed 25-35 growth rings would suggest that the growth curve given in Figure 4 gives a slightly lower age for the largest colonies, but a reasonable estimate for the age of small and average size colonies.

Presumably, the gorgonian fauna on Red Reef which was sampled in 1962 represented a natural population unaffected in previous years by collecting or massive mortality due to natural causes. On the basis of a

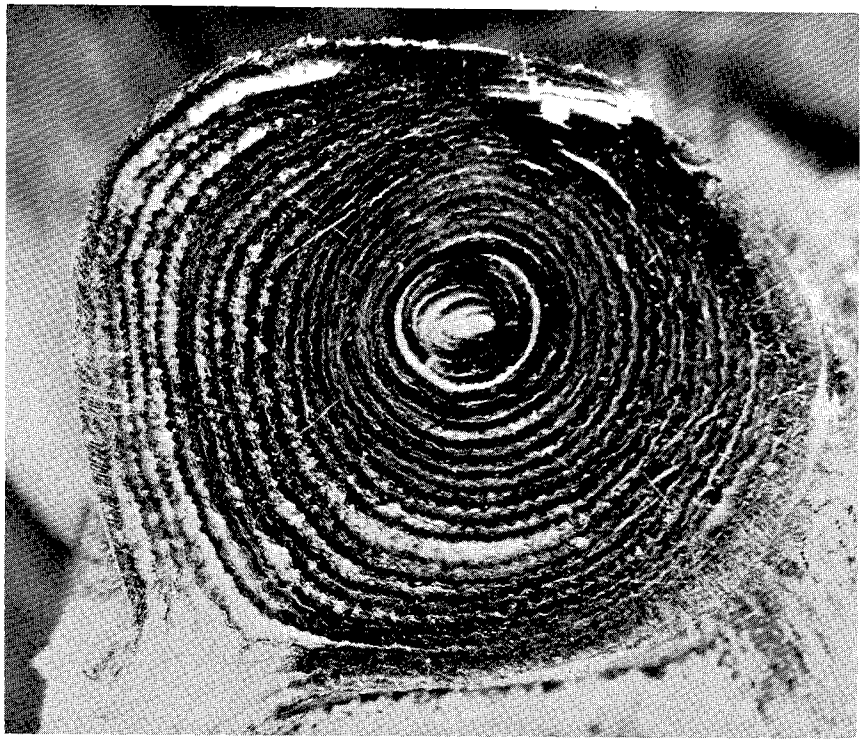

FIGURE 4. Cross section of the axis of a 60 cm tall colony of *Plexaura homomalla* (Table 3, No. 14) showing the annual growth rings. The maximum diameter is 1.93 cm.

population density of 38.8 colonies/m², and with a maximum rate of recruitment (minus mortality) of 0.56 colonies/m², as in the 1966-67 population, a minimum of seven years would be needed for *P. homomalla* to reach the same level of abundance after the reef had been cleared of all gorgonians. It is unlikely, however, that the recruitment rate would remain constant. In the 1966 sample, the size of the first year-class, and thus the recruitment rate for the 1965-66 season, was only 0.31 colonies/m². It is

also unlikely that the dry weight biomass of *P. homomalla* would return to its 1962 level after only seven years of regrowth, since in 1966, after four years of regrowth, the biomass of the species was only one-seventh of that in 1962.

The size-frequency data (Figure 5) for the 1962 sample is indicative of a relatively stable population. About 27 per cent of the colonies in the sample had an average weight greater than 65 gm and according to the growth curve presented in Figure 4, these colonies were probably more than seven years old. Furthermore, the rate of recruitment of the 1962 population as indicated by the size of the first year-class (which consisted of those

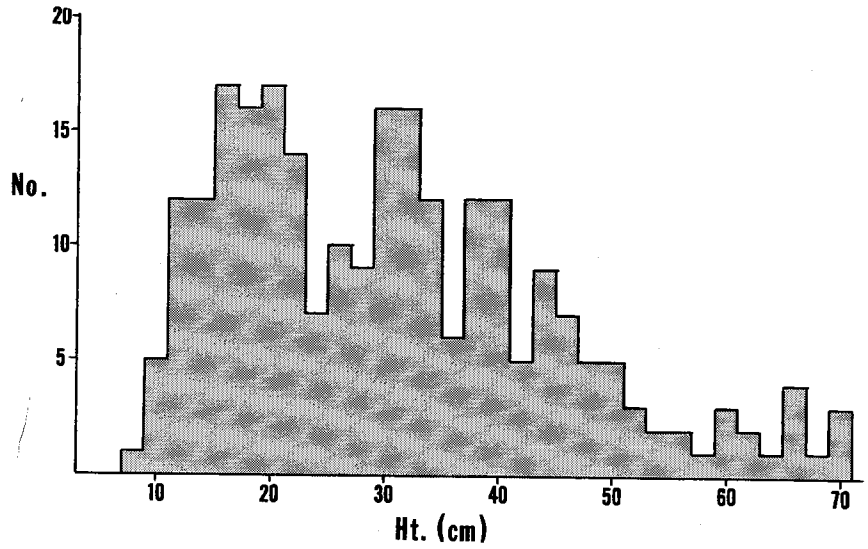

FIGURE 5. Size-frequency diagram for the population of *Plexaura homomalla* at Red Reef in 1962.

specimens weighing 3 gm or less and which were about 10 cm tall), was only 0.16-0.23 colonies/m².

If it is assumed that the 1962 population of *P. homomalla* was near a steady state where the rate of recruitment equalled the mortality rate and if 0.16-0.23 colonies/m² is a reasonable estimate for the annual rate of recruitment, then it can be calculated that the expected longevity of members of such a population would be 17-24 years. This figure seems to agree reasonably well with estimated growth rates for the first four year-classes, with the average age of the population, and with the predicted age of the largest colonies.

SUMMARY

The maximum population density of *Plexaura homomalla* at Red Reef was found to be 3.88 colonies/m^2, and the standing crop was 338 gm (dry weight)/m^2. The average height of the colonies was 30 cm and the average weight 70 gm. The largest colonies were 60-70 cm tall and weighed 800-1000 gm; these were estimated to be about 23-27 years old. Rates of growth, based on changes in total biomass, were estimated to increase 5 gm/colony/year in the first year-class to about 12 gm/colony/year in the fourth year-class. The maximum rate of recruitment on a cleared area of reef was found to be 0.56 colonies/m^2/year, while the rate of recruitment in a stable population was estimated to be 0.16-0.23 colonies/m^2.

It would appear that natural populations of *P. homomalla* can reach a high level of stability. Furthermore, the uniform composition of the fauna and the relative abundance of all species of gorgonians at Red Reef would suggest that such an assemblage of diverse species can also maintain an equally high degree of population stability.

REFERENCES

1. CARY, L. R., 1918. The Gorgonacea as a factor in the formation of coral reefs, *Carneg. Inst. Wash., Publ. No. 213, Papers from the Dry Tortugas Lab. 9:* 341-362, 6 pls.
2. GRIGG, R. W., 1970. Ecology and population of the gorgonians, *Muricea californica* and *Muricea fruticosa,* Coelenterata: Anthozoa, Doctoral dissertation, 260 pp., University of California, San Diego.
3. JONES, J. A., 1963. Ecological studies of the southeastern Florida patch reefs. Part I. Diurnal and seasonal changes in the environment, *Bull. Mar. Sci. Gulf & Carib. 13:* 282-307.
4. OPRESKO, D. M., 1973. Abundance and distribution of shallow-water gorgonians in the area of Miami, Florida, *Bull, Mar. Sci.* (in press).
5. VOSS, G. L. AND F. M. BAYER, 1968. Ecology and dynamics of coral reef populations, Florida. In: *Natl. Geogr. Soc. Res. Repts.,* 1968: 225-230.
6. VOSS, G. L., F. M. BAYER, C. R. ROBINS, M. GOMON AND E. T. LAROE, 1969. The marine ecology of the Biscayne National Monument. A Report to the National Park Service, Dept. of the Interior. (Unpublished report.)

OBSERVATIONS ON THE VEGETATIVE CULTURE OF *PLEXAURA HOMOMALLA* IN THE CAYMAN ISLANDS

ROBERT E. SCHROEDER

ABSTRACT

Experiments were conducted to determine the feasibility of commercial culture of *Plexaura homomalla* as a source of prostaglandins. Artificial substrates and methods of attachment were devised, and the viability and rate of growth of both large and small cuttings taken from both large and small donor colonies were compared.

INTRODUCTION

The experimental propagation of the alcyonarian *Plexaura homomalla* from cuttings was undertaken to investigate the feasibility of commercial culture, and to develop methods for implementing it. Both Kinzie and Goldberg (personal communications) had demonstrated that *P. homomalla* cuttings will survive on an artificial substrate. Among the initial questions to be answered in developing the method for commercial use were:

1. How rapidly will *P. homomalla* cuttings attach themselves to the substrate?
2. Will such cuttings grow into adult colonies?
3. What size cuttings attach and/or grow most rapidly?
4. What size parent colonies provide the best cuttings?
5. How are attachment and growth affected by such factors as wave action, depth, current, temperature, and bottom type?
6. Can a practical substrate (a "pot") be made cheaply enough for commercial purposes?

As yet, the work is no more than fairly begun. Even so, the results provide considerable information, suggest many new lines of thought, and pose a host of new questions.

MATERIALS AND METHODS

The first "pots" were made by filling aluminum piepans with concrete, and standing 8- to 15-cm pieces of ⅜-inch PVC waterpipe in the middle. Cuttings were attached by wedging them in with spring-clips of PVC welding rod.

Later pots were made by substituting PVC jaws of ¼-inch flat stock for the pipe. Two pieces of PVC stock, 3 cm by 10 to 15 cm, were held together by a rubber band, and inserted in the wet concrete. When the concrete hardened, the jaws were held firmly in place. Cuttings were inserted

by springing the jaws apart, then allowing them to close on the cut end of the cutting's axis.

Experimental groups of 25 to 35 cuttings were set out in two locations, one of 1 to 1.5 m depth, and the other of 3 m depth. Consecutively numbered pots were laid out in straight lines, then planted with cuttings from a nearby stand of *P. homomalla.*

Parent colonies were selected to represent extreme groups of "large" and "small" colonies by eye. From each, cuttings were taken to represent experimental groups of "large" and of "small" cuttings. Thus, experimental groups fell into four classes (Figures 1 and 2):

1. Large cuttings from large colonies.
2. Large cuttings from small colonies.
3. Small cuttings from large colonies.
4. Small cuttings from small colonies.

When in place, each cutting was photographed against a grid background, the grid lines being 25 mm apart. Groups were rephotographed periodically for measurement.

The two experimental locations were adjacent to dense natural stands of *P. homomalla,* but so situated that wild colonies were not interspersed among the cuttings. The bottom in these areas varied from bare to sand-and-rubble with sparse turtle grass. Altogether, 368 cuttings were set out, comprising twelve experimental groups.

Results

The first pots, using PVC pipe and spring-clips, were not successful. Within a few days, most of the cuttings were dislodged. Of those that remained in the pots, only about 10 per cent succeeded in attaching themselves. After seven months there was almost no sign of growth, apart from three cases where extensive attachments were formed.

At first, pots that lost cuttings were replanted, using a variety of techniques designed to improve the chances of attachment. None were particularly successful. With the development of the jawed pot the problem was solved, and these efforts were abandoned.

In these later groups there was extensive attachment to the PVC jaws of the pots. New branches, and apical growth up to 20 mm in 89 days were observed. In the final three experimental groups here reported, 94 per cent of the cuttings remained in place after three months.

Both in degree of attachment and in growth rate, the most successful groups comprised large cuttings from large parent colonies. The attachments consisted largely of soft tissue: very little—if indeed any—axis material was evident on gross examination. These data all were from groups in approximately 3 m of water. The shallow groups were notably unsuccessful, and were not followed up.

It is noteworthy that the cuttings exhibiting the most obvious apical growth did not show signs of extensive attachment to the pots. The amount of tissue spread over the PVC jaws ranged from moderate to virtually none.

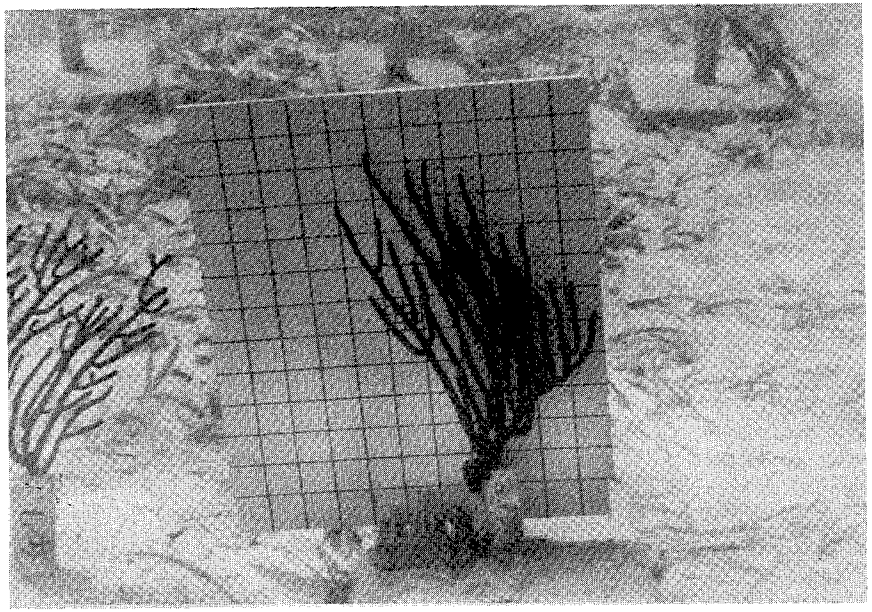

FIGURE 1. Typical large cutting 89 days after placement; cutting from large parent colony. Note attachment to jaws of pot.

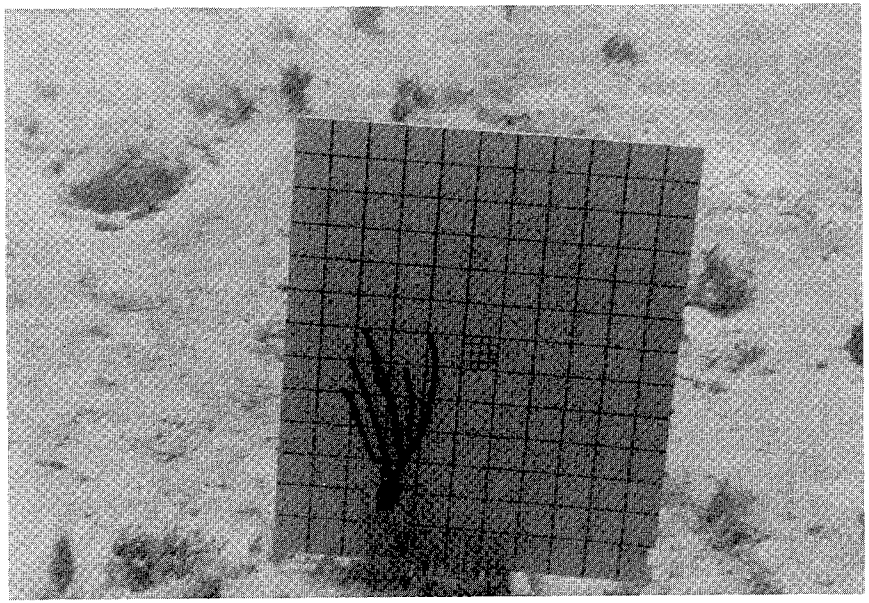

FIGURE 2. Typical small cutting 120 days after placement; cutting from large parent colony. Note attachment.

Groups comprising small cuttings from large colonies attached themselves almost as well as large cuttings, but there was little evident growth. Cuttings from small parent colonies, large or small, were the least successful in attaching themselves, and exhibited no apparent growth. When last examined, most of them remained in the jaws of the pots, but in many cases the bare axis of the cutting was exposed for four or five mm above the point of contact. All cuttings from small parent colonies had been in place at least a month longer than those from large parent colonies.

Discussion

From these data, very little more may be concluded than that *Plexaura homomalla* cuttings from large parent colonies will, when appropriately mounted, begin to attach themselves, and may exhibit some tip growth. There is the suggestion that the thicker the axis, the better the chance of attachment: cuttings from large colonies do have noticeably thicker axes. than cuttings of the same size from small colonies. It remains to be seen whether the cuttings will become typical adults.

It is plausible that continuing tissue damage at the point of contact with the substrate stimulates the cuttings to attach. Until the growth of an adequate holdfast, flexing would bruise soft tissues at this point. Too much motion, as in the first pots, does enough damage to prevent attachment altogether.

One would suspect that alcyonarians possess a mechanism that inhibits apical growth while tissue damage is occuring at the point of attachment. Something of the kind must prevent wild colonies from overstressing their holdfasts by too rapid growth. Such a mechanism would help account for the superior performance of cuttings from large colonies. These possess a substantially thicker axis than do similar cuttings from small colonies. The stiffer axis would be less subject to flexing at the point of attachment, and thus would permit less tissue damage than the thin axis of the cuttings from small colonies.

This hypothesis accords well with the observation that the greatest tip growth occurred in those large cuttings from large parent colonies that exhibited only modest growth of attaching tissues. Perhaps the grip of the pot's jaws on the thick axis of these cuttings provided adequate support from the first, so that tip growth might begin as soon as the initial damage was healed.

There is nothing in these data that indicates the vegetative farming of *P. homomalla* is infeasible. A rational continuation would place experimental groups in diverse habitats, and investigate quantitatively the questions of optimum cutting size and shape.

Numerous questions about the feasibility of large-scale *Plexaura* culture could be answered only by practicing it. A material increase in the density and extent of the natural stands around Grand Cayman Island certainly will bring corresponding changes in the associated bottom flora and fauna. One way or another, this is certain to affect the operation. Possibly it also will affect the recreational use of the island's waters.

THE EFFECT OF WAVE ACTION ON THE POPULATION DYNAMICS OF *GORGONIA VENTALINA* LINNAEUS

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Abstract

Recruitment, growth and mortality in a population of *Gorgonia ventalina* were recorded during a 230 day period of heavy surf conditions on the open coast of Panamá. The population in an adjacent area was experimentally reduced by 83 per cent to determine the effects of intraspecific competition on rates of growth and recruitment. The mean growth increments of individuals within this period (38.4 cm^2 surface area or 2.6 cm height) did not differ significantly between these areas of dense and sparse populations. The size distribution of specimens washed up on shore was found to be an inaccurate estimate of the relative mortality of different size classes due to wave shock. The force of wave action placed a size limitation to the growth of *G. ventalina*, but the greatest rate of mortality was in individuals smaller than the mean size of the population. The relative effects of wave shock, competition and grazing and the relative productivity of individual *G. ventalina* and populations in calm areas and surf zones are discussed.

Introduction

Cary [1], in the Dry Tortugas, Florida, observed that during periods of strong wave action large numbers of gorgonaceans were torn off the substratum to which they were attached and thrown up on the beach. It was quite apparent that the probability of being ripped away increases with the age of the gorgonacean as its surface area increases. This poses the question of what advantage there is for a sea fan in growing large. At Punta Galeta, Panamá, on the opposite side of the Caribbean, the same process occurs. Great numbers of *Gorgonia ventalina* are torn up by the surf and deposited on the intertidal reef flat or in subtidal pits or channels. The specimens torn loose apparently tend to be larger than those still attached.

Three aspects of the relationship between wave action and population dynamics of *Gorgonia* should be clarified before further studies are undertaken. First, the large numbers of *Gorgonia* thrown ashore suggest that wave action is the predominant cause of mortality. However, since the area of the living *Gorgonia* population which is contributing specimens and the amount of time involved in accumulating the specimens is unknown, a direct determination of the rate of mortality in the shallow population is

necessary. Second, since there appears to be a greater probability in being pulled up and killed as size increases, there must be some significant advantages in growing dangerously large. In order to determine the magnitude of these advantages, the exact relationship between the tendency to be ripped up and surface area should be measured. Third, the removal of individual *Gorgonia* by wave shock may be correlated with an increase in growth rate of remaining individuals or an increase in rate of recruitment in the area. This report presents the results of attempts to resolve these questions.

Acknowledgments

This study was a minor part of a project supported by the Federal Water Quality Administration Contract No. 14-12-874. James P. Stames was very helpful with the field work and in the construction of equipment. John Birkeland helped with the planimeter work. I wish to express my gratitude to Frederick M. Bayer for the identification of the *Gorgonia* sp. in this population. The few specimens I submitted were of the greatest combined variation I could find, so I am confident that the entire population consists only of *G. ventalina* Linnaeus.

Methods

The *Gorgonia ventalina* population in this study was located on a flat reef shelf extending from the intertidal to a depth of 2.3 m where there was an abrupt edge to a vertical wall which dropped to the sand bottom at 10 m. In the region of the quadrats of mapped *G. ventalina,* at a depth of 2.2 m, the horizontal distance from the intertidal to the vertical wall was 30 m. During much of the dry season, December to April, the entire shallow shelf was exposed to a rough surf (waves up to 1.5 m in height).

Twenty permanent 4 m^2 quadrats were marked by railroad spikes hammered into the reef with a sledge hammer (Fig. 1). In order to avoid frequent disorientation, a different color of forester's polyethylene plastic flagging was tied on each set of three spikes parallel with the direction of surf. The different colors of boundary lines roughly perpendicular to shore allow easy orientation while working. The polyethylene flagging has to be replaced after two months or cleaned of encrusting algae which makes it difficult to see.

In order to test the effects of the thinning of populations on larval settlement and rates of growth of remaining individuals, 102 colonies of *Gorgonia ventalina* were removed from a 20 m^2 quadrat containing 123 individuals. This quadrat was located right at the edge of the dropoff so that the few remaining *G. ventalina* were on the offshore edge of the population (Fig. 1). Most of the mapped quadrats were a few meters within the population so that much of the water reaching them had been in recent contact with other *G. ventalina*.

The height of each *G. ventalina* was measured and recorded on the map of each quadrat on plastic slates. Records of disappearance of mapped specimens and recruitment of new specimens were based on these maps. Surface area measurements were made on selected *G. ventalina* which were constructed in only one plane with no lateral branchlets. It would have been very difficult and time-consuming to work with the surface areas of those in more than one plane so the assumption was made that total growth rates are the same whether the growth is carried out in a branch in one plane or in branches in several planes.

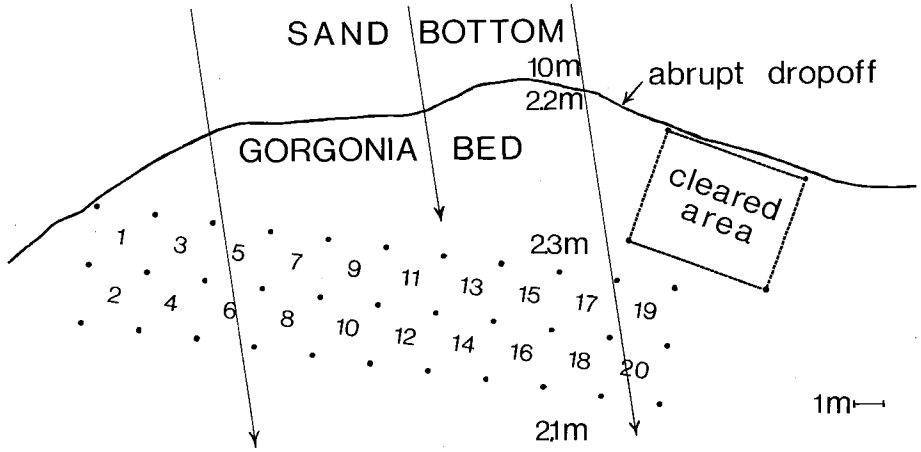

Figure 1. Relative positions of the clear area (a 20 m^2 area with 83% of the *G. ventalina* population removed) and the twenty 4 m^2 quadrats with the *G. ventalina* population measured and mapped. The population of *G. ventalina* essentially ends at the dropoff. Depths are indicated in meters. Arrows indicate the direction of the surf.

The surface areas were measured with a Gelman planimeter from precise drawings of two dimensional *G. ventalina*. These drawings were obtained in the field by two methods: tracing against a plastic slate or photographing against a background grid. Those which had their outlines traced were small enough to be completely outlined on a plastic slate 26 by 21 cm in area. Larger individuals were photographed against a plastic sheet 1 m tall by 60 cm wide marked with a 10 cm grid. For rigidity, the plastic sheet was nailed to a plywood board. The wood, of course, is awkward to handle in the water because it floats, so channels were cut into the board and filled with molten lead. When the lead hardened, the wood was covered with fiberglass to hold in the lead and to prevent the wood from warping. Two pairs of handles were nailed to the back to allow holding the sheet easily in each of two directions.

The traced outlines of small *G. ventalina* were life-sized. The photographs of other *G. ventalina* taken against a 10-cm grid were projected onto paper mimeographed with 2.5-cm grids. The planimeter measurements were multiplied by sixteen. The photographs were taken with the Nikonos 28-mm lens which is modified to correct for distortion caused by the refraction of light from water through the lens. It was attempted to hold the camera as perpendicular as possible to the plane of the background grid sheet and *G. ventalina* so that the squares of the photograph would match with the squares on the paper.

The camera could never be held perfectly perpendicular but the deviations were corrected by aiming the projector from the angle of correction on the opposite side of the perpendicular. It was sometimes necessary to line up the projector in several intervals for different regions of the outline. Planimeter measurements were taken only on outlines traced when the squares lined up perfectly.

TABLE 1

OUTCOME OF A POPULATION OF *Gorgonia ventalina* AFTER 230 DAYS OF GENERALLY ROUGH SURF CONDITIONS (23-IX-1971 TO 10-V-1972)*

	Control Area (80 m²)			Cleared Area (20m²)
	Number of individuals	Per cent of initial 393	Number of individuals with recorded surface area	Number of individuals
Total remaining alive	374	95		20
grown	267†	68†	41	14
unchanged	71†	18	11	6
torn	36†	9	5	0
Total that died	19	5		1
ripped up	15	4		1
dead, but still attached	4	1		0
Total recruitment	13			14
regeneration	1			6
reproduction	12			8

*By 23-IX-1971, 393 individuals were measured and mapped in control area and 21 in cleared area. Originally, 123 Gorgonia were present in cleared area but 83 per cent were experimentally removed.

†Prorated from ratio of measured individuals (third column).

RESULTS AND OBSERVATIONS

The outcome of the mapped population of 393 *Gorgonia ventalina* during 230 days of generally rough surf conditions is given in Table 1. Only 5 per cent died. Of those that died, 20 per cent remained attached. Considering the large number of specimens tossed on the beach by the surf, it was somewhat surprising that the population at a depth of 2 meters contributed only 4 per cent of its individuals during the season of heavy surf. However, many of the specimens found on the beach were very eroded and worn, and were probably lying on the beach for more than a year.

The height distribution of the 393 mapped individuals was compared with the height distributions of 15 that were removed from the population by surf and with 103 thrown up on the reef flat (Fig. 2). The mean heights of all three groups differ significantly ($P<0.001$ by t-test). As with bivalve shells [2, 3], the size distribution of sea fans washed up on the beach does not represent an accurate size sample of those that died. Since the size distribution of individuals torn off the substratum is not representative of those found, the number of specimens found on the beach is in even greater contrast than a direct comparison to the low percentage removed from the population.

Only 16 of the 103 found washed up on the shore were broken off at the holdfast. The rest were still attached to the holdfast which contained a chunk of limestone from the substratum. In other words, the substratum broke loose, not the *G. ventalina*.

The *Gorgonia* completely removed probably resulted from the force of surf action. Four individuals died but were still attached. One was largely overgrown by *Millepora* when last seen alive and was completely overgrown 230 days later. The smallest of the four was being eaten by *Cyphoma gibbosum* and was heavily browsed when last seen alive. The other two dead ones were overgrown by algae and hydroids, but no idea was obtained about what actually caused the deaths.

Populations in shallow surf zones appear to be comprised of relatively small *G. ventalina* in abundance, while in deeper sheltered calm water areas the populations appear to consist of larger individuals in lower abundance. To obtain a quantitative estimate of these differences, the abundances and heights of the *G. ventalina* at 6 to 9 meters depth in a sheltered area in the San Blas Islands was surveyed (Table 2).

The surface areas of 57 *Gorgonia* which remained alive were recorded at the beginning and end of the 230 day period. Growth was measured in 41 of the individuals. Five individuals had pieces torn off, presumably by wave action, so they decreased in size. The remaining 11 appeared totally unchanged in size and shape. The identical shape of these individuals indicates that it is improbable that they were torn and then regenerated to the same size. So it appears that individuals do not necessarily grow constantly, but sometimes in intervals. Strangely, individuals may be very

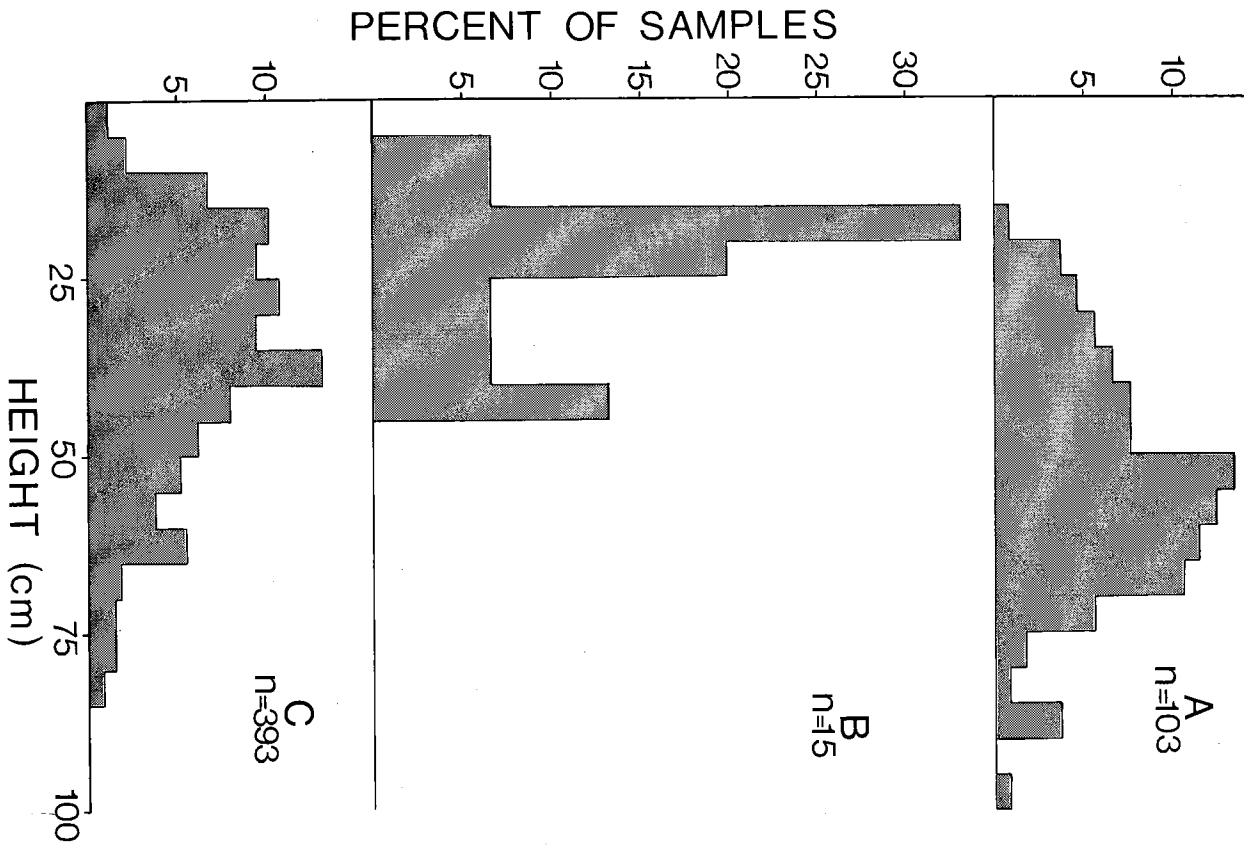

FIGURE 2. Size distributions of *G. ventalina* found washed up on the shore (A), those which disappeared from the population in 20 mapped quadrats of the surf zone (B) and the total population in the mapped quadrats (C).

TABLE 2

COMPARISON OF A POPULATION OF *Gorgonia ventalina* IN A SHALLOW REGION OF FREQUENT HEAVY SURF CONDITIONS WITH ONE IN A DEEPER, SHELTERED CALM-WATER AREA

Location	Depth	Number measured	Number of G. *ventalina*/m2	Height (cm) mean ± standard deviation	Range of height (cm)
Punta Galeta*	2 m	393	4.9	35.6 ± 12.6	2.3 to 85
Ogogpuquid†	6 to 9 m	63	0.6‡	88.3 ± 28.3	41 to 145

*Punta Galeta is on the exposed open coast of Panamá.

†Ogogpuquid is an island of the Holandes Cays of Islas San Blas, sheltered by an extensive barrier reef. These two specific populations were selected as representative of the two kinds of habitats.

‡Other tall, branching gorgonaceans were present at 3.3 per m² in this area.

TABLE 3

GROWTH INCREMENTS OF *Gorgonia ventalina* OVER A PERIOD OF 230 DAYS IN GENERALLY ROUGH SURF CONDITIONS*

		Surface area (cm²)			Height (cm)				
		slope of regression			slope of regression				
Location of *Gorgonia*	Number of measured *Gorgonia*	Δ area/230 days versus initial area	95% confidence limits	mean growth increment/ 230 days	95% confidence limits	Δ height/230 days versus initial height	95% confidence limits	mean growth increments/ 230 days	95% confidence limits
Control area	41	+0.0006	+0.0269 −0.0257	38.0	60.1 15.9	−0.0214	+0.0090 −0.0518	2.8	3.8 1.8
Cleared area (reduced abundance)	14	+0.0297	+0.0651 −0.0057	38.7	50.7 26.7	−0.0045	+0.0767 −0.0857	3.2	4.0 2.4
Combined data from both areas	55	+0.0035	+0.0208 −0.0138	38.4	50.2 26.6	−0.0265	+0.0037 −0.0565	2.6	1.9

*Individuals that did not produce measurable growth and those that were reduced in size are not included in these calculations.

similar in initial surface area, yet grow very differently. For instance, three *Gorgonia* colonies were initially 158 cm^2, 157 cm^2 and 138 cm^2 in surface area and grew 19 cm^2, 28 cm^2 and (apparently) not at all (respectively) in 230 days. However, the mean initial size of those that did not grow was 998.6 $\pm$ 165.7 cm^2 (standard error) while the mean size of those that grew was 407.2 $\pm$ 144.7 cm^2. Therefore, in spite of a nearly complete size overlap, there is a significantly greater probability of a large individual than a small individual not growing in a given period.

The recorded growth increment data for *Gorgonia ventalina* is summarized in Table 3. Only individuals which produced measurable growth were included in the analysis; those that did not grow and those reduced in size by tissue having been torn away were separated into different categories. The growth increments were analyzed for both surface area and height, but for the same individuals.

The slopes of the regression lines relating growth increment to initial size during the period of observation do not differ significantly from zero. Thus absolute growth rate does not change as a function of size. Although not significantly different from zero, the mean slopes of regression lines relating growth in height to initial size are negative while mean slopes of lines relating growth in surface area to initial size are positive. This implies that increase in width becomes relatively predominant to increase in height as the *G. ventalina* grows larger. The analysis of changes in surface area (2 dimensions) is probably more meaningful than changes in height (1 dimension). The 95 per cent confidence limits are more narrow and the data less variable for surface area measurements.

Since there was no evidence that growth rate is a function of size, simply the mean growth increment was calculated from the data. Assuming the rate of growth throughout the year is the same as in 230 days of generally rough surf conditions, the average increase in size would be 60 cm^2 in area or 4 cm in height per year. This is roughly 30 per cent lower than the rate of height increase obtained by Cary [1], perhaps due in part to the heavy surf conditions and turbid water throughout the present study.

No significant difference was found between the growth rates of *G. ventalina* in the control area (49 *G. ventalina*/m^2) and those in the area in which the population was reduced to a density of one per square meter. Student's *t* tests were performed on surface areas, heights and regression slopes, respectively, and no significant differences were found.

Two categories of recruitment were tallied in Table 1. "Regeneration" refers to instances in which an adult was almost entirely removed but a small patch of coenenchyme from the holdfast remained without a single polyp. A small fan actually arises from the patch of coenenchyme, presumably by regeneration of polyps but possibly from the settlement of a planula larva. "Regeneration" occurred in the control area in the mapped location of an adult *G. ventalina* which disappeared except for a small

piece of holdfast. The six in the cleared area were in the previous locations of *G. ventalina* removed in the clearing process.

"Reproduction" refers to tiny *G. ventalina* completely new to the location. A total of 12 appeared in the 80 m^2 control area and 8 appeared in the 20 m^2 cleared area. If we assumed the probability of recruitment was the same in each area, the expected values would be 16 in an 80 m^2 area and 4 in a 20 m^2 area. As the only known ecological difference in these two areas is the reduction of the population in the cleared area by 83 per cent, it tentatively appears that the recruitment of juveniles increases with the removal of adults. However, replication of this experiment is underway since there are small numbers in the data and there is a probability of about one in twenty that the results could be due to chance.

A net productivity of 147 cm^2/m^2/year (4 per cent of the standing crop) was estimated. The methods of calculating this estimate are given in Table 4. The role of grazing by several predators (mainly *Cyphoma gibbosum*) are not included in this study although grazing may have caused the deaths of 3 of the 4 *G. ventalina* that died but were still attached by their holdfasts. Generally, *C. gibbosum* will move to another *G. ventalina* (or another gorgonacean) before critically damaging an individual. But the removal of a small portion of the surface areas of many individuals by *C. gibbosum* may absorb much of the net productivity. Removal of tissue and individuals by wave shock removes about 43 per cent of the surface area produced.

Discussion

The largest living *Gorgonia ventalina* found in the surf zone population at a depth of 2 meters was 85 cm tall (although an individual 100 cm tall was found washed up on the beach). The largest in the surf zone was actually smaller than the mean size of *G. ventalina* in areas of greater depth (to 22 m) and regions of calmer water in Islas San Blas, in which individuals reached heights of up to 145 cm and surface areas roughly four times greater than the largest in the surf zone. It appears that wave action places a size limitation on *G. ventalina* (Table 2). However, the size distribution of *G. ventalina* washed up on the beach, significantly larger than the size distribution of living populations, does not provide an accurate estimate of the relative mortality of different size classes. The greatest rate of disappearance was in the smaller size range of *G. ventalina* (Fig. 2). Although a size limit does exist, the probability of survival of small *G. ventalina* is less than that of larger *G. ventalina*. I interpret this as indicating that increasing the reception of the force of wave shock by growing large in surface area accounts for less mortality than the inability of panulae to accurately select the portions of solid substratum which cannot be pulled loose by wave force on an attached fan.

The rate of growth does not change as a function of size within the size range present in the surf zone at 2 meters depth (Table 3). On this basis,

TABLE 4

ESTIMATED PRODUCTIVITY IN TERMS OF SURFACE AREA OF A *Gorgonia ventalina* POPULATION AT A DEPTH OF 3 METERS ON THE OPEN COAST OF PANAMÁ

	Source of estimation from data present in previous tables (1 and 3)	Surface area (cm^2/m^2)	% of standing crop surface aaer
standing crop	mean initial surface area × number of individuals per m^2	4018.4	100.0
a. growth of initial population	mean growth increment × 365 days/230 days × number of individuals/m^2 × per cent that grew	203.6	5.1
b. growth of new recruits	mean size of new individual × 365 days/230 days × number of new individuals/m^2	56.6	1.4
Total growth	a + b	260.2	6.5
c. tissue ripped away	mean amount ripped away × number of individuals per m^2 × % that decreased in size	102.7	2.5
d. individual ripped away	average surface area of individuals measured for area that were 23.3 to 24.3 cm tall (mean height of the 15 ripped away) × 15/393	10.8	0.3
Total decrease	c + d	113.5	2.8
Net gain	(a + b) − (c + d)	146.7	3.7

it was calculated that the force of wave shock ripped away 43 per cent of the surface area produced in the same period of time (Table 4). For general steady state conditions, roughly 57 per cent of the surface area produced (i.e., 147 cm^2/m^2) would be an appropriate amount for grazing by predators. During this period of generally rough wave action, nineteen individuals died while 13 recruited (Table 1). Thus wave shock is responsible for the removal of nearly half the tissue produced (43 per cent) and 80 per cent (15 of 19 deaths) of the individuals. The physical environment is far more predominant in terms of the removal of entire individuals than in general tissue. On the other hand, grazers such as *Cyphoma gibbosum* and *Hermodice carunculata* will graze away tissue but will most often move off to another prey individual before one is entirely killed. Grazing is more important in terms of tissue removed, while wave action is the predominant source of mortality in *G. ventalina*.

Recruitment was in the same order of magnitude as mortality in the natural population (13 to 19), while the recruitment may have increased when the population was thinned (0.15/m^2 to 0.4/m^2). I know of no record of mass recruitment anywhere. Although the physical rather than biological environment stresses account for most of the mortality, this particular population is as close to a steady state as one would expect in the real world.

This apparently increased rate of settlement is the only result we found due to the thinning of the adult population. The rate of growth of adults did not change significantly. Thus, intraspecific competition appears to be relevant only to attachment space and not to the acquisition of nutrients.

In shallow-water populations (depth of 2 meters) in an area of heavy surf, *Gorgonia ventalina* is a relatively short-lived, abundant and predominant species in contrast with deeper populations (Table 2). A comparison of size distributions in these two zones implies that the higher turnover rate, greater clearance for settling space and lower size (and probably age) limits are probably due to the harsh physical environmental wave shock. In deeper water, the lower abundance of *G. ventalina* and its smaller proportion of the gorgonacean species is probably due to increased competition for space with other species in a more stable physical environment. The amount of reproductive products produced are presumably proportional to surface area of the individual colony of polyps. Individuals that reach adulthood in deeper water are eventually more productive than individuals in the surf zone because they tend to grow larger and live longer (presumably reproducing each year after reaching adulthood). However, if the localities in Table 2 are fairly representative of the two environments, then the shallow-water population is more productive since deep-water individuals tend to be over twice as tall (and perhaps about four times as large) but only one eighth as abundant. In terms of reproductive products or biomass, deep-water individuals are more productive, but populations as a whole are more productive in shallow water. Although in contradiction to

consideration of the productivity of reproductive material of adult individuals, the numerical predominance of *G. ventalina* and productivity of the entire population indicates that it is a species more highly adapted to surf zone conditions than to competition in a more physically stable, biologically complex environment.

References

1. Cary L. R., 1914. Observations upon the growth-rate and oecology of gorgonians, *Carnegie Inst. Wash. Pub. 182:* 79-90.
2. Lever, J., A. Kessler, A. P. van Overbeeke and R. Thijssen, 1961. The "hole-effect": a second mode of sorting of lamellibranch valves on sandy beaches, *Neth. J. Sea Res. 1:* 339-358.
3. Lever, J., M. van den Bosch, H. Cook, T. van Dijk, A. F. H. Thiadens, S. J. Thijssen and R. Thijssen, 1964. Quantitative beach research III. An experiment with artificial valves of *Donax vittatus, Neth. J. Sea Res. 2:* 458-492.

INTOLERANCE PATTERNS IN PLEXAURA HOMOMALLA COLLECTORS: CASE REPORTS AND DIAGNOSTIC STUDIES

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Abstract

An intolerance syndrome affecting divers working with *Plexaura homomalla* is described in terms of case histories. Allergic mechanisms, toxins and pharmacologically active substances were evaluated. Two divers were medically monitored in the course of diving both for collecting *P. homomalla* and for control purposes. The results and interpretation of these studies are discussed.

The catalog of the sea's toxic hazards is long and by no means complete. In large scale harvesting of *Plexaura homomalla,* divers have been exposed to this gorgonian and its contents in unprecedented quantity. This triggered an intolerance syndrome which we describe here together with work done to elucidate its pathogenesis.

Case Reports

1. M. S. is a 33-year-old college graduate who has worked in or around the water all his adult life. He is an experienced diver. He enjoys good health and has had no previous serious illnesses. There is a history of mild respiratory allergy (rhinitis, sinusitis sometimes precipitated by dust exposure) and occasional abdominal pain which seems to be associated with periods of stress.

He had participated in coral collection efforts starting in December 1970, and had worked at this for 3 to 5 days every 4 to 6 weeks until symptoms of intolerance appeared about 9 months later. Typically a day's activity would include one or two hours of cutting coral in the water followed by several hours of chopping or grinding and bagging the cut material. During this time the only evidence of intolerance was repeated peeling of his palms —presumably the skin surface with greatest and most sustained contact with P. homomalla. *He experienced some headaches during collection sessions but only in retrospect associated with* P. homomalla *exposure.*

On September 6 and September 9, 1971, M. S. collected P. homomalla *using for the first time scuba equipment (all previous collections had been done with snorkel only). This allowed him to cut with more freedom and promised to make the harvesting operation more efficient. During and*

following these collecting sessions he experienced mild headache and dizziness. On the 13th he collected again and experienced headache and difficult breathing.

Following this, equipment failures interrupted collection activities for one week. On September 20 M. S., with several associates, worked with P. homomalla *in and out of the water for about 4-½ hours and in the process became quite sick. The symptoms experienced reflected multisystem involvement which included temperature elevation (101.4° maximum), headache, feeling of fullness in the head, dizziness or lightheadedness, difficulty breathing (which was relieved by a bronchodilator), pain in the chest, and throat irritation and tightness. He also was coughing and his nose was completely occluded. His heart rate was labile; by the patient's count the rate changed from 44 to 100 within one minute. In addition to the pain noted above, his back hurt severely and his testes were tender and possibly swollen.*

The different symptoms remitted gradually after varying intervals. Respiratory problems were much improved the next day. Headache and dizziness persisted over the succeeding week becoming progressively less severe. Backache and associated limitation of activity likewise remitted gradually. During all this M. S. did not consult a physician so we have no medical verification of the syndrome or report of physical findings.

Seven days after the beginning of these symptoms he was still mildly symptomatic. He had a slight backache and could not breathe deeply without coughing. There was a feeling of pressure in his head. This day he returned to the water for the first time since the onset of his symptoms, not collecting but marking areas for harvest and photographing colonies under special study. After about 30 minutes of this activity he began to feel dizzy and developed a headache, first in the occipital region and then generalized. He returned to shore and reported to the local hospital where he was examined. Pulse, blood pressure and temperature were normal and aside from peeling skin on the hands there were no abnormal findings. The examining physician felt that with the symptoms experienced, the seeming decreasing tolerance and exacerbation by relatively trivial exposure, an anaphylactoid reaction was a good possibility. He advised M. S. to stay out of the water until this could be explored.

M.S's symptoms gradually remitted over the succeeding several weeks.

At least 3 other workers have experienced symptoms of intolerance while working with *P. homomalla.* In no case were there symptoms which had not been experienced also by M.S. so these men will be reported but briefly.

2. J.H., a 24-year-old male, was employed briefly in the harvesting operations. He was not atopic and had enjoyed good health previously. On September 20 when M.S. developed the symptoms previously described, J.H. was working with him and experienced some of the same problems. Specifically he noted headache, light-headedness, cough, sore throat, tightness in the throat, sinus congestion and rhinitis, chest pain and difficult breathing. His symptoms were milder and briefer than those of M.S., being completely gone after 2 days.

One week later he experienced a recrudescence of symptoms (headache principally) while working with M.S. in the circumstances described above. On this occasion his symptoms were transient; he has experienced no sequelae.

3. A.S., a 23-year-old male, also worked briefly collecting P. homomalla *and was diving with M.S. and J.H. on September 20th. At this time he had headache, dizziness (lightheadedness), weakness, faintness, and pain on breathing. His symptoms remitted over the next two days. He did no further work in this project and had no further symptoms.*

4. L.H., a 31-year-old male, acted as barge captain during several collection operations. His principal activity, aside from handling the barge on which the cut P. homomalla *was transported to shore, was chopping coral and bagging the cut material. He did this with a machete and in the process encountered a good bit of splatter. Though he wore protective clothing (raincoat, face mask, gloves) some sap did get on his skin and he commented that the odor was strong. On two different days he noted dizziness and a "funny feeling in the back of his head." Once he also had a marked conjunctivitis—probably he had rubbed his eyes with a contaminated hand. The eye inflammation cleared quickly and the dizziness more slowly.*

Laboratory and Clinical Work-up

Our studies proceeded in three major areas. First, since the possibility of anaphylaxis had been raised and an anaphylactic reaction occurring during diving could be fatal, it was important that this be evaluated early. Second, if no anaphylactic or other allergic mechanism could be demonstrated, it seemed logical to look for toxins or other pharmacologically active substances in *P. homomalla* or in the water near a harvesting operation. An important adjunctive concern was to determine a route of entry for the offending substance. Finally, if the hazard seemed defined and acceptable when these studies were complete, we planned gradually to reintroduce M.S. to the water and then to the harvesting operation. The equipment and procedures were to be modified to ensure better isolation of the diver from surrounding water and the whole operation was to be monitored medically so that we could treat and study any problems which arose.

Evaluation of Allergic Mechanisms

Our early concern about an allergic or anaphylactic mechanism was based on certain of the symptoms which had occurred (tightness in the throat, cough, difficulty breathing) and on the recurrence of symptoms with seemingly trivial exposure. Several factors ruled against this mechanism—occurrence of symptoms in several divers at the same time, presence of symptoms not usually associated with an anaphylactic response, a 6-hour

delay in onset of tightness and wheezing in the chest, and absence of visible angioedema or hives. It was also possible that the syndrome represented a nonanaphylactic hypersensitivity response. Indeed, a good match between the syndrome M.S described and allergic alveolitis (farmer's lung in the special case of sensitivity to mold contaminants in hay [1]) existed. This disease is mediated by precipitins.

In order to examine more closely the above possibilities several different fractions were prepared from the coral. Figure 1 shows a rough schema of the fractionation. Fraction 1 should contain essentially all of the prostaglandins and fraction 4 the bulk of the protein and polysaccharide constituents. Fractions 2 and 5 are similar except that fraction 5 included emulsified lipids among which are prostaglandins. Fractions 2, 3 and 4 should be prostaglandin free. All fractions were reconstituted to the original volume of the extract. Table 1 summarizes the results of tests designed to detect reagins (IgE), precipitins (IgG or IgM) or delayed reactivity (IgX, cellular immunity). None of the divers showed a positive response in any of these tests.

When the above work was done, M.S. was seen in consultation by Dr. Kenneth Mathews (University of Michigan). Dr. Mathews found Mr. S. to be mildly atopic (history of rhinitis and sinusitis, positive skin reactions to house dust, feathers, mixed trees, and alternaria and mild persistent eosinophilia) but agreed that there was no evidence of allergy to a constituent of *P. homomalla.*

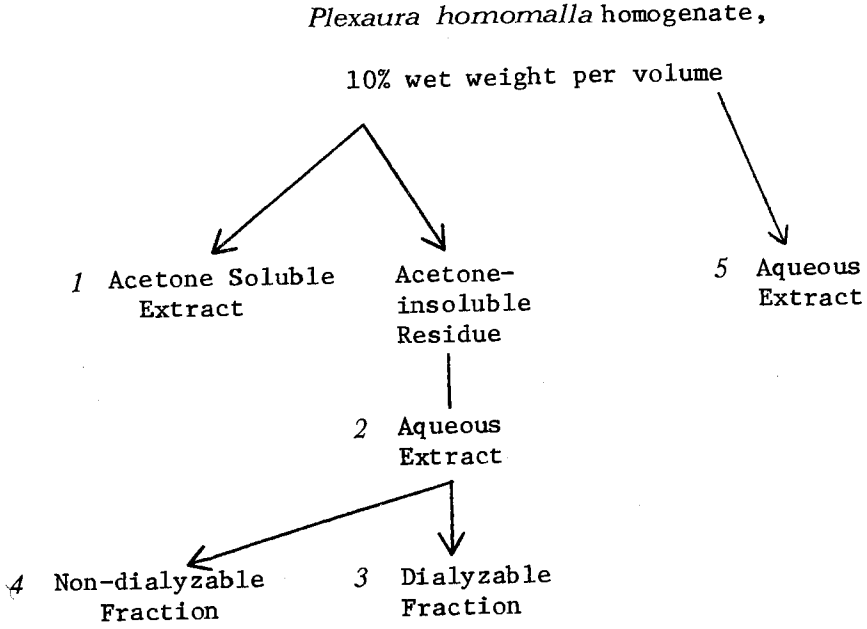

FIGURE 1. Fractionation of *P. homomalla* extract.

TABLE 1

TESTING OF DIVERS' SERA FOR REACTIVITY TO *P. homomalla* EXTRACT FRACTIONS

Extract Fractions	Reagin Mediated		Tests for Precipitins		Cellular Immunity
	Scratch Tests	Intradermal Tests* (titered)	Double Diffusion	Passive Hemagglutination	Patch Tests
Fraction 4 (Protein Containing, PG Free)	X	X	X	X	X
Whole Aqueous Extract					X

*An immediate wheal and flare reaction would indicate reagins; a reaction at 12 hours would suggest precipitins, and one at 48 hours, cellular immunity.

TABLE 2

AREAS OF BLUING FOLLOWING INJECTION OF FRACTIONS 4 AND 5 INTO SKIN OF RATS

	Full Strength	1/10	1/100
Saline	60	—	—
Fraction 4	91	72	53
Fraction 5	176	100	90

EVALUATION OF TOXINS OR PHARMACOLOGICALLY ACTIVE SUBSTANCES

The following activities were found in the fractions described above:

1. *In vitro hemolytic activity against human erythrocytes.* Both fractions 4 and 5 lysed human red cells. Lytic activity was concentration- and temperature-dependent. It was destroyed by boiling, was adsorbed by formalin-treated sheep red blood cells and inhibited by addition of lecithin to the reaction mixture. This activity had the characteristics of a phospholipase.

2. *Increased capillary permeability following injection of fractions 4 and 5* into the skin of Sprague-Dawley rats. Rats received Evans Blue dye at −2 minutes, the designated treatment at time 0 and were sacrificed at +2 hours. "Areas" of bluing (product of 2 diameters at right angles to each other) appear in Table 2.

3. *Aerosol Toxicity.* Rats received a 30 minute exposure to aerosolized full strength fractions 4 and 5 for six days with a two-day hiatus in the middle. None exhibited unusual behavior. Respiratory tracts were normal at autopsy.

4. *Intravenous Toxicity*. All 5 fractions were injected intravenously (1 ml of full strength solution). Fractions 2, 3, and 4 caused no detectable toxicity. Fraction 1 (acetone soluble material) caused cyanosis and spastic breathing but was not lethal. Fraction 5 caused death within 20 minutes. These animals had clinically manifest pulmonary edema. At autopsy they had heavy, wet lungs and, like recipients of fraction 1, petechiae in the mesenteric nodes and Peyer's patches. Centrifugation of fraction 5 at 12,000 × g for 30 minutes removed the lethal principle.

5. To evaluate the *quantity of prostaglandin potentially available in the ambient seawater* about a harvesting operation we placed a stalk of the gorgonian in a plastic bag and traumatized it to produce the characteristic "brown cloud." Coral, water and bag were then frozen in dry ice and returned to our laboratories for analysis. Assays done by Dr. E. G. Daniels appear in Table 3.

TABLE 3

ASSAYS OF PROSTAGLANDINS IN SEAWATER SURROUNDING TRAUMATIZED *P. homomalla*

	Concentration per Liter of Sea Water Sample	
	Sample #1	Sample #2
Wt. of Lipid Extract	89 mg	109 mg
PGE_2 and PGE_2 Me Ester	11.2 mg	28 mg
PGA_2 and PGA_2 Me Ester	6.1 mg	14 mg

MEDICAL MONITORING OF TRIAL COLLECTIONS

We proposed that collections be cautiously begun again with additional precautions to isolate the divers from their environment (full wet suit, closed, double-tube regulator, full face mask) and a physician in attendance to treat any problems and record any clinical findings associated with intolerance. Two divers were studied. One (M.S.) had had a substantial problem in the past. The other (S.A.) had had considerable past exposure to *P. homomalla* but never any symptoms or signs of intolerance. The endpoints monitored were selected on the basis of features of the intolerance previously reported, activities of the *P. homomalla* fractions demonstrated in the laboratory, and our estimate of what constituted reasonable monitoring of the men's health. Thus we looked for evidence of cardiac and respiratory dysfunction, chemical evidence of liver, kidney or muscle damage and signs of hemolysis, intravascular coagulation and bleeding tendency. Our selection of laboratory and clinical endpoints was tempered by the need to select techniques and tests simple and portable enough to carry to the site.

Our protocol called for a 30-minute exposure to water in an area where *P. homomalla* was uncommon, then a non-working dive of 30 minutes in one of the collection areas followed by 30-, 60-, and 120-minute collection efforts on succeeding days.

The endpoints monitored before and after dives included:

1. Vital signs (temperature, pulse, blood pressure)
2. Peak expiratory flow rate
3. Physical examination
4. Electrocardiogram
5. Hematology studies (stained blood film for cell morphology, platelet count, bleeding time)
6. Urinalysis
7. Blood chemistries
   Na, K, Cl, Ca, P
   SGPT, CPK, LDH
   Bilirubin
   Haptoglobin
8. Stool Guaiac (on all stools passed during test period)
9. Monitor symptoms/complaints

After the 60-minute collection effort M.S. noted a mild headache but it was evanescent and seemed of no consequence. Next day after 50 minutes of a scheduled 120-minute dive he returned to the boat complaining of a headache similar to those he had experienced before. The headache originated in the right temporal region and rapidly spread to involve the entire head. It was aggravated by movement of the head or hanging it dependent. The blood pressure had not changed from pre-dive values. His temporal arteries felt unusually full but otherwise there were no remarkable physical findings.

Questioning whether this was related to the collection or to the diving per se we had M.S. return to the water and engage in strenuous but sterile activity away from the *P. homomalla* beds. After 70 minutes of this he had no symptoms. Next day M.S. collected again but was obliged to stop after only 35 minutes because of recurrence of headache.

During all this the other diver, S.A., who had similar exposure had no complaints. The clinical and laboratory endpoints in both men were stable. Both had increased respiratory secretions after diving, both had drops in body temperature (oral) of 1 to 2 degrees and both became ketotic.

DISCUSSION AND CONCLUSIONS

Though the intolerance seen in divers collecting *P. homomalla* could not be demonstrated as an allergy in the usual sense of the word, there does seem to be substantial variation among divers as regards the amount of exposure required to cause symptoms. Further it appears that in a susceptible individual repeated exposure results in a decreasing threshold.

The route by which the divers were exposed to the offending substance(s) has never been shown with certainty. It seemed early that a likely mechanism was inhalation of a contaminated aerosol. Water leaking into the single-tube regulator valve made a fine aerosol when the valve was activated; the result tasted and smelled like *P. homomalla*. Residuae of the coral could be seen in valves that had been used during collection. Further, the fact that intolerance became a problem only after scuba gear was substituted for snorkel during the collection efforts seem to support aerosol entry of the offending substance.

The only other route of entry which seemed possible was across intact skin. Since M.S. developed symptoms while using a full face mask and double tube gear which seemed to preclude entry via the respiratory tract, significant penetration across the skin may occur.

Which of the active substances demonstrated in *P. homomalla* has caused intolerance is unsettled. The protein toxin with its lytic properties seems entirely capable of causing problems if present in sufficient quantity. Possibly the peeling of hands which several workers have experienced is due to this activity. We never found any evidence of hemolysis or intravascular coagulation in the divers. Thus a systemic effect of the toxin in the circumstances in which we observed these men was not evident. It could, however, facilitate activity of other toxic principles.

This leaves the prostaglandins themselves as the principal offenders among the known active constituents of *P. homomalla*. Certainly the gorgonian releases substantial quantities of both PGE and PGA into ambient seawater when traumatized. The quantities of prostaglandin assayed in our laboratory specimen may not exactly reflect the situation in the vicinity of the coral collection but we tried to make these samples seasonably representative. How much of the prostaglandin in the seawater ultimately gains entry to the exposed divers is entirely unknown but we could make some hypothetical calculations. If men were working in an environment containing concentrations similar to those assayed and reported above, they would be exposed to a solution containing around 25 μg/ml of free and esterified PGA and PGE. Jackson [2] reported that single 0.2-ml doses of solutions containing 100 μg/ml of PGE_1 were locally irritating in the nose. Herxheimer and Roetscher [3] reported cough and upper respiratory irritation after inhalation of PGE_1-containing solutions. Thus it seems possible that at least the cough and upper respiratory irritation reported by the divers could result from chronic exposure to a prostaglandin-containing aerosol and that the amounts of prostaglandin to which the divers are exposed are physiologically significant.

If one compares symptoms reported by the divers with lists of side effects accumulated during occupational exposure or clinical trials of prostaglandins given for several indications [4] one sees considerable overlap as shown in Table 4.

This is at best circumstantial and inconclusive proof of a relationship between prostaglandins and the divers' complaints. Nevertheless it is suggestive and is the best link we have between clinical report and laboratory observation.

TABLE 4

COMPARISON OF DIVERS' SYMPTOMS AND SIGNS, AND SIDE EFFECTS REPORTED IN CLINICAL TRIALS WITH PROSTAGLANDIN DRUG CANDIDATES

Symptoms/Signs

System Involved	Reported by Divers	Reported from Clinical Trials or Occupational Exposure
Skin	Peeling (hands, feet and lips)	Facial flushing
Eyes	Conjunctivitis	Eye irritation Photophobia Difficulty focusing Retrobulbar pain
CNS	Headache	Headache
	Pressure or fullness in head	
	Dizziness, Light-headedness	Dizziness
	Faintness	Faintness
Respiratory	Chest pain, pain on breathing	Respiratory distress
	Breathing difficulty (wheezing?)	
	Cough	Cough
	Pharyngitis; raw, sore throat	Pharyngeal pain, irritation
	Sinus congestion; rhinitis	Sinus fullness, sinusitis
	Tightness in throat	Tightness in throat
Cardio-vascular	Palpitations, labile rate	Tachycardia, bradycardia
Gastro-intestinal		Nausea, vomiting Diarrhea Abdominal pain
Genito-urinary	Testicular pain, swelling	
Musculo-skeletal	Weakness	Weakness
	Back pain	Muscular pain
General	Fever	Fever

Several points deserve special mention. First, gastrointestinal symptoms are frequently reported during systemic use of prostaglandins and none occurred in the affected divers. This would seem to make a prostaglandin etiology less credible; possibly route of entry had something to do with the notable absence of these complaints.

Second, headache was the problem which caused discontinuation of the medically supervised collection efforts. Certain featuers of the headache (feeling of fullness or pressure in the head, aggravation of symptoms when head was dependent, fullness of temporal arteries) suggest vasodilation as the basic mechanism. Both E and A prostaglandins are active vasodilators [5], and headache has been reported during infusion of small quantities of PGE_1 [6]. Thus M.S.'s headache and prostaglandin causation are compatible.

We conclude that hazards do exist in harvest of and exposure to large quantities of *Plexaura homomalla.* Individual tolerance appears to vary; in susceptible individuals repeated exposures leads to decrease in tolerance. The cause of symptoms in divers working with *P. homomalla* is not certain but could be prostaglandins or a tissue lytic toxin contained in the gorgonian, or both. Individuals who work with this coral should take measures to avoid excessive exposure to it or its water- or air-borne emanations.

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REFERENCES

1. PEPYS, JACK, 1971. Pulmonary aspergillosis, farmer's lung and related diseases. In: *Immunologic Diseases,* 2nd edition, Samter, Ed., Little, Brown, Boston.
2. JACKSON, R. T., 1970. Prostaglandin E_1 as a nasal constrictor in normal human volunteers, *Curr. Ther. Res. 12:* 711-717.
3. HERXHEIMER, H. AND I. ROETSCHER, 1971. Effects of prostaglandin E_1 on lung function in bronchial asthma, *Europ. J. Clin. Pharmacol. 3:* 123-125.
4. PATEL, N. C. AND E. M. SOUTHERN. Personal communication.
5. BERGSTRÖM, S., 1967. Prostaglandins: Members of a New Hormonal System, *Science 157:* 382-391.
6. CARLSON, L. A., 1967. In: *Prostaglandins,* Bergström, S. and B. Samuelsson, Eds., Nobel Symposium 2.

PANEL DISCUSSION

BIOCHEMISTRY, PHARMACOLOGY AND TOXICOLOGY

J. E. Pike, *Moderator*

DR. PIKE: Dr. Ciereszko and I have informally contacted some people who might give short discussions on some of the aspects of the biochemistry. Dr. Ciereszko has some comments, and I believe that Dr. Robley Light from Florida State University also has some interesting data on the very careful analysis of the content of *Plexaura homomalla* from Florida areas. Perhaps Dr. Ciereszko would like to begin.

DR. CIERESZKO: Yes, it happens that I have some slides to show you, not because I was prepared especially for this meeting, but because I gave a lecture at the West Indies Laboratory two weeks ago. I thought I would point out some of the problems that coelenterates have because they are large, conspicuous, sessile animals and need, at the larval stage, to find a place to settle where they can become adults. I would like to show a few slides, some of which are relevant to points that probably will be brought up in the next panel discussion.

The fate of a gorgonian is not necessarily to die of old age, but to be knocked over by moving water, and I show this slide of a fallen gorgonian to remind you of that fact. The next slide shows another one that has fallen over. These were not tampered with, but are in their natural state. One of the specimens happens to be *Plexaura homomalla,* as you probably recognized. In the next slide you see the skeletons of gorgonians which probably fell a couple of years ago and now are encrusted with various growths. I presume that ultimately these will disintegrate and be incorporated in the environment. In the next slide we see a specimen of *Cyphoma,* which was mentioned yesterday. I thought, however, I would remind you that one of the common predators of gorgonians is the flamingo tongue snail, *Cyphoma gibbosum.* We see a closeup of it in the next slide. It is a small snail that normally lives its life on gorgonians, which seem unable to protect themselves against this particular predator. We have looked at this in terms of some of the substances that we find in gorgonians, and although these will stun the snail so that it curls up and relaxes for a period of time, it will revive if you flush it out with clean sea water. In the next slide, I would like to call your attention to something that was mentioned yesterday by one of the discussants, and that is damage to a gorgonian: look at the

tips particularly, and the abrasion on some of the branches. Someone mentioned the bristle worm, and you sometimes see gorgonians which have been nipped off at the tip by these worms. I described this to my students as resembling the work of a child with a new pencil sharpener on his way to school. The worm in question is shown on the next slide. It is a rather large polychaete worm, *Hermodice carunculata,* which lives on *Zoanthus* as well as on other coelenterates. In the next slide, we see another *P. homomalla* in the foreground, but behind it is a gorgonian skeleton which is entirely covered with *Millepora,* the stinging coral. You can see it looks different from a living gorgonian in that there are short little branches off the main stem, and the tips are whitish. That is a fire coral which has taken over a gorgonian skeleton. Presumably, at some point the gorgonian was injured, the *Millepora* larva was able to settle in this bare spot, and then grew over the entire coral. It covers the gorgonian skeleton, and then has independently continued out from the very tips, beyond the skeleton of the gorgonian. These are problems that confront gorgonians.

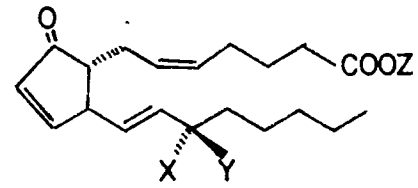
Now, I want to extend my remarks to other reef coelenterates. In the next slide we see a coral being overgrown by a zoanthid, a green mat-like material that is very abundant on many reefs. It tends to take over other corals, such as the brain coral at the center of the field. You see a similar thing in the next slide, which shows a massive brown coral covered on top by *Zoanthus,* a sort of green carpet; the brown disappears as it is covered by the zoanthid mass. This is very common in many places, where boulders are entirely covered by green carpet, acres of it, and you recognize the boulders as dead corals on which the overgrowth has occurred. In the next slide you see another zoanthid, this one a yellow *Palythoa,* growing on the green mat of *Zoanthus.* I don't know whether this is a real succession, but very often you see it where predation has occurred to interrupt the continuity of the mat, and something new starts to grow there. *Palythoa* is a particularly successful coelenterate that overgrows many other organisms and is very often found on dead coral. The next slide shows this to be the case. Here is a rather large brain coral, about 2½ or 3 feet in diameter, on which you see yellow patches of *Palythoa mammillosa.* Part of the surface of the coral is living, but it is gradually being killed. A similar situation is shown in the last slide, where you see another species of coral overgrown by another type of *Palythoa.*

I point these things out because I feel that one of the problems of coral reef organisms is not only defense against predation, for they seem to do rather well on this score, but is also a matter of competition for space. Sessile animals must have a hard substrate to which they can attach in the larval stage and on which they can proceed to adult life. I imagine that Dr. Lang will have something to say about this when we get to the next panel discussion, but I think that some of the biochemistry of these animals will reflect this need for defense against predators and protection against settling from above. One of the points that brought our attention to *Plexaura homomalla* some time ago was the accidental discovery that putting a group

of *Plexaura homomalla* into an aquarium that drained into a cement tank below, containing spiny lobsters, caused the lobsters to scurry about in an effort to get out of the tank. Something is secreted into the water, which affects divers and which disturbs many other organisms. It could be that *P. homomalla* secretes prostaglandins to prevent other organisms from interfering with them either by settling or by predation. One of the compounds that we have found in coelenterates is serotonin, but I do not know how this acts in terms of the human symptoms that were described. Serotonin (that is 5-hydroxytryptamine) occurs in some coelenterates in rather large concentrations.

Now, unless someone wants to ask questions, I think we should give Dr. Light a chance to speak.

DR. LIGHT: I will try to be very brief. During the summer and fall of 1970, it was my good fortune to leave the hot climate of Florida and spend some time in Sweden, where I worked with Professor Bengt Samuelsson

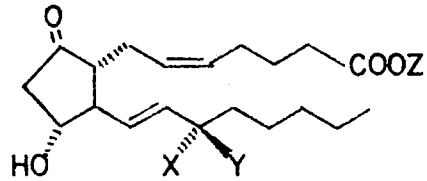


I Me Ac 15 (R) PGA_2,
X=H, Y= CH_3COO, Z= CH_3

II Me 15 (R) PGA_2,
X=H, Y=OH, Z= CH_3

III 15(R) PGA_2, X=H, Y=OH, Z=H

IV PGA_2, X=OH, Y=H, Z=H

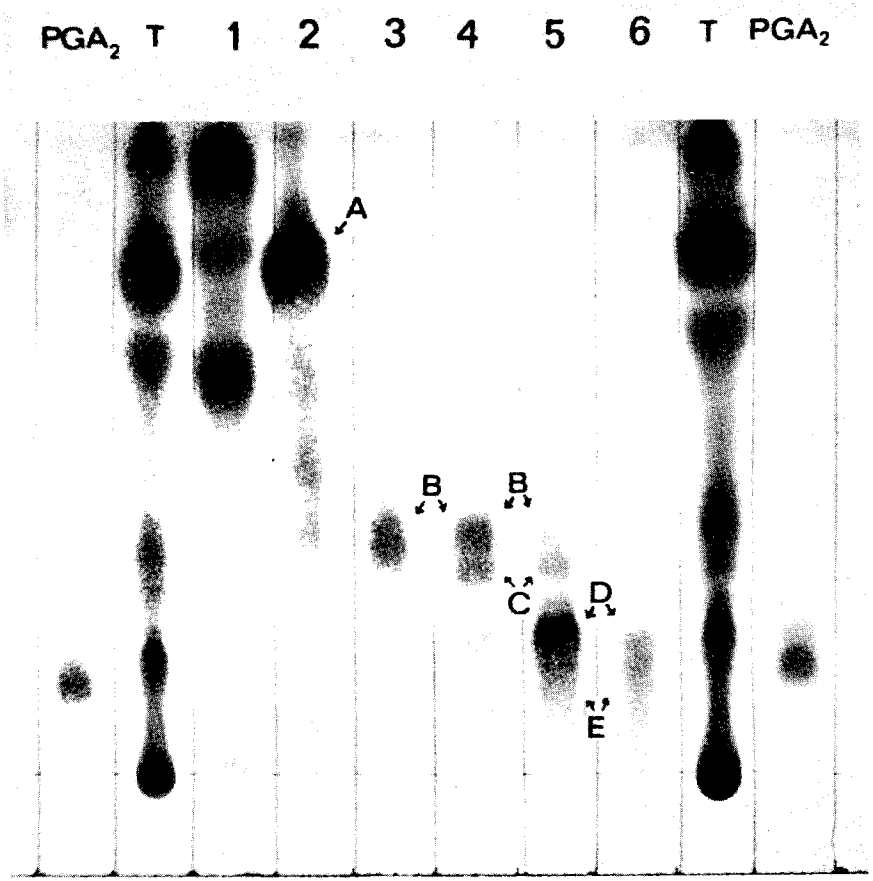


V 15(R) PGE_2, X=H, Y=OH, Z=H

VI Me 15 (R) PGE_2, X=H, Y=OH, Z= CH_3

VII PGE_2, X=OH, Y=H, Z=H

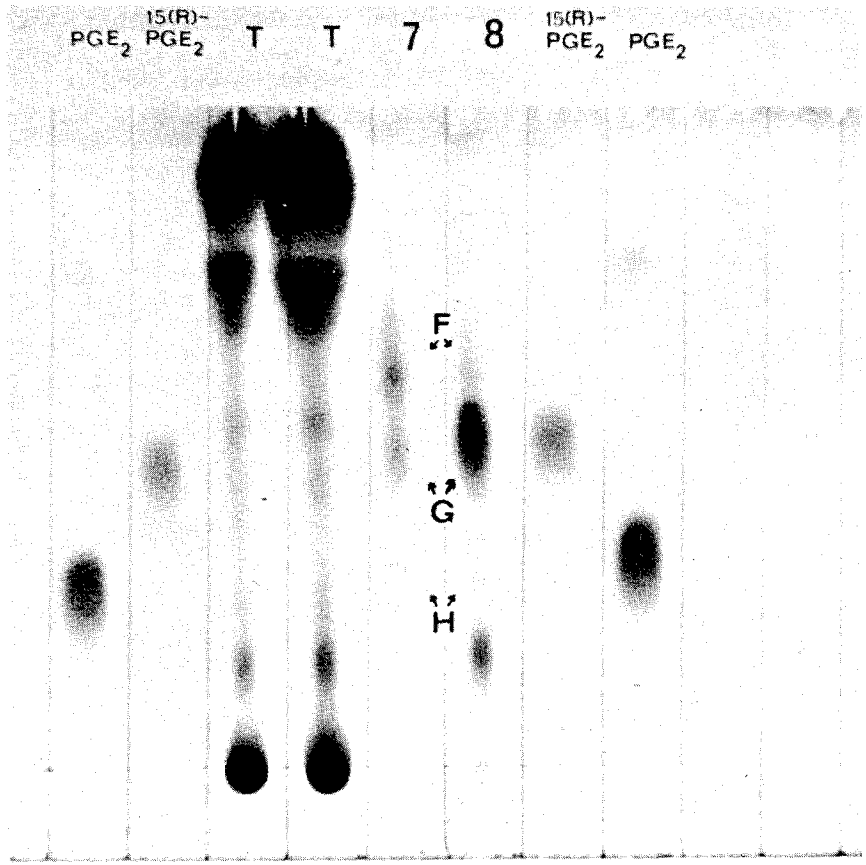
SLIDE 1.



SLIDE 2.

at the Royal Veterinary College in Stockholm. By coincidence, he happened to have in his freezer a few samples of the Florida coral that has been the subject of this symposium, and on which he was, at the time, attempting biosynthetic studies. I decided that it would be interesting to participate in that venture, and we did spend a good bit of effort with those frozen samples, trying to obtain extracts which would either convert arachidonic acid into a prostaglandin or would catalyze the metabolism of a prostaglandin. Most of that effort was without success. During this work, it became obvious that there was a more complex mixture of prostaglandins in *Plexaura homomalla* than the PGA derivatives by Weinheimer and Spraggins that had been reported. So, we thought we should take a more careful look just to see what compounds were there, because the composition might reveal something about the potential biosynthetic mechanism.

Just to remind you of the compounds again, I am showing you their structures on the first slide. The structure on the upper part of the slide

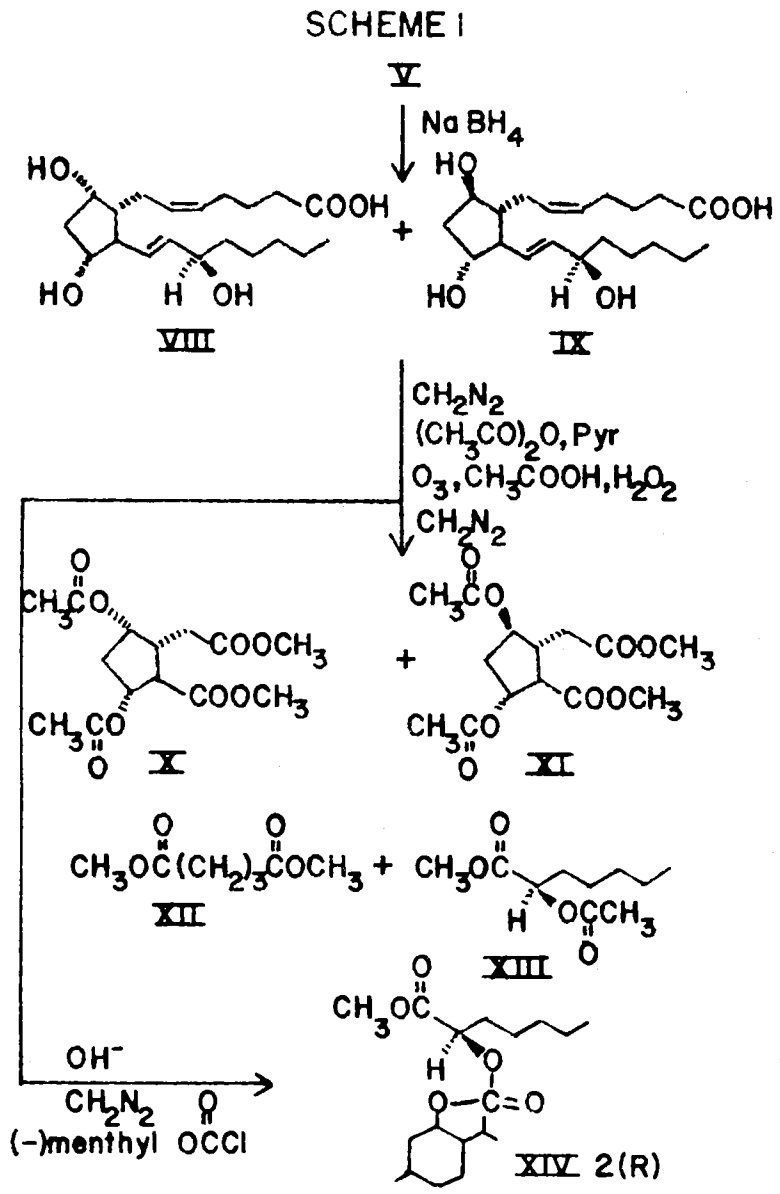


SLIDE 3.

represents prostaglandins of the A series which have a ketone group and a double bond in the cyclopentane ring; the lower structure represents prostaglandins of the E series which have a ketone group and a hydroxyl group in the cyclopentane ring. A major feature of interest here is the stereochemistry at the fifteenth carbon atom. Prostaglandins of the R series have a hydroxyl group facing up, corresponding to constituent Y in the figure. Prostaglandins of the S series have the hydroxyl group facing down, corresponding to constituent X in the figure.

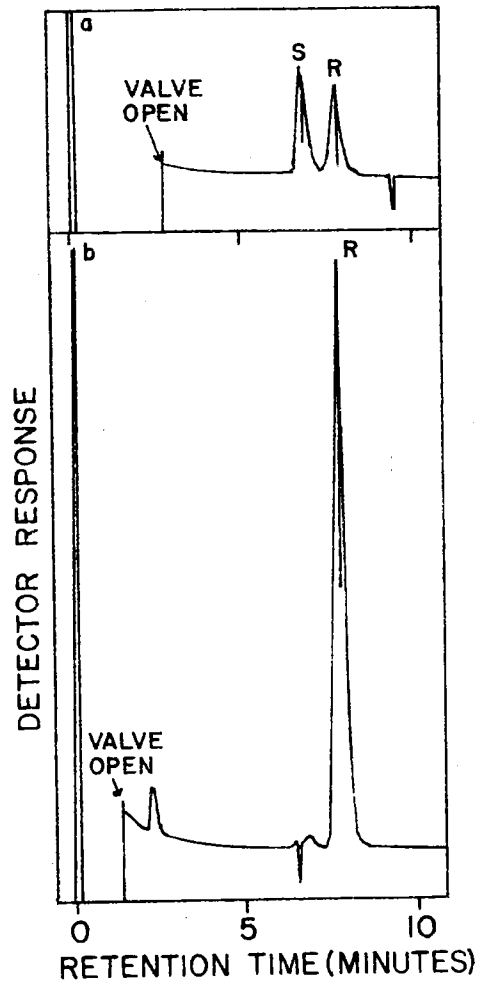
The next slide shows a thin-layer chromatogram of some silicic acid chromatographic fractions of a crude total lipid extract from *Plexaura homomalla*. The first column represents a three per cent ethyl acetate benzene elution which contains no prostaglandin-like material. The second one has one major spot, labeled here as compound A, which turned out to be the 15-acetyl, methyl ester derivative of 15(R)PGA, which was reported by Weinheimer and Spraggins. In fraction 5, compound D turned out

to be the free 15(R)PGA_2. There is some difference in polarity between the 15(R) and the 15(S) series, standard 15(S)PGA's being slightly more polar than the 15(R) series. We had a compound running in front of the 15(R)PGA_2 which we established was the methylester of 15(R)-PGA_2 (compound B). I'll come back to compounds C and E in just a



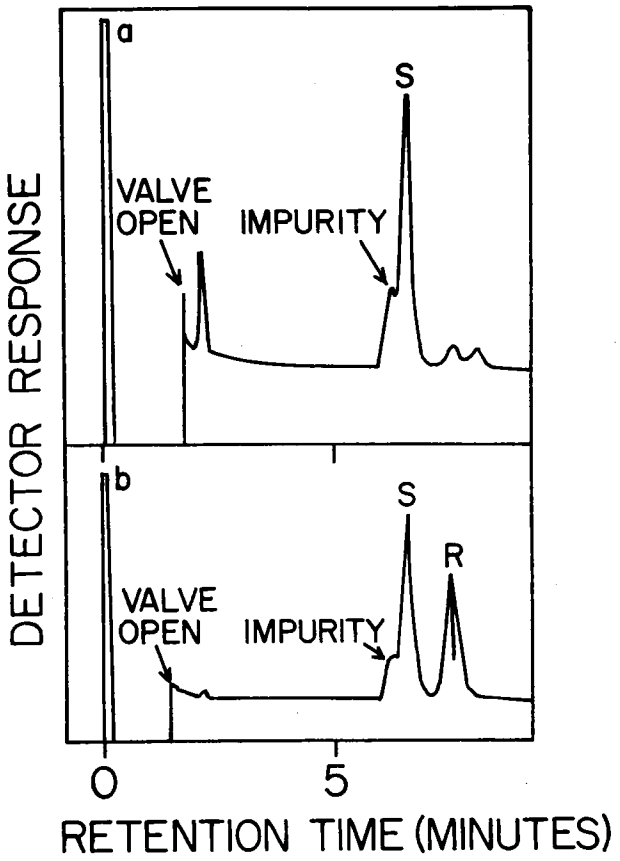
SLIDE 4.

moment. The next slide (3) shows more polar chromatographic fractions which were of most interest to us at that particular time because of the evidence for material chromatographing like 15(R)PGE_2, compound G in fraction 8. Compounds of the PGE series had not been reported from this organism. Derivatives of compound G chromatographed on gas liquid chromatography gave the same mass spectrum as derivatives of authentic 15(R)PGE_2. The mass spectrum of the derivatives established the carbon skeleton but not necessarily the stereochemistry. In order to establish the stereochemistry, we carried out a chemical degradation as shown in the next slide (4), in which the E compound was reduced to a mixture of the



SLIDE 5.

alpha and beta PGF_2 derivatives. The hydroxyl groups were acetylated and the compounds were ozonized, cleaving at the double bonds, to produce a mixture of the cyclopentane ring fragments which were established by gas liquid chromatography and mass spectrometry. The remainder of the molecule gives 2-hydroxyheptanoic acid. In order to establish the stereochemistry at the 2-hydroxy position (formerly the 15-hydroxy position of PGE_2), we adapted a procedure that has been published by Wesley and Halpern and which had been further developed by Dr. Hammerström in Professor Samuelsson's laboratory, in which the acid was reacted with methyl oxy-carbonyl chloride to produce a methylchloroformate derivative. This reaction introduces a second asymmetric center into the compound and causes it to be a diastereoisomer, and as such the 2(R) and 2(S) hydroxyheptanoates then can be separated by gas liquid chromatography as shown on the next slide (5). The top chromatogram shows an authentic mixture of the racemic 2(R) and 2(S) hydroxyheptanoates as their methyl



SLIDE 6.

chloroformate derivative, and the chromatogram on the bottom shows that the fragment isolated from the gorgonian PGE_2 did, indeed, have the R configuration. At this stage, though, we noticed a tiny peak, which was definitely there, which corresponded to the S isomer, and this raised in our minds the possibility of there being a small amount of the S prostaglandins, the configuration that is found naturally in mammalian systems. If we go back to the first slide 2, to point out that in the region referred to as compound E, there was, indeed, some material travelling at the same RF on thin layer as authentic 15(S)PGA_2. So then we took this fraction, fraction 6, through a similar chemical degradation. On slide 3, there was definitely some material in the region co-chromatographing with authentic 15(S)-PGE_2 (compound H), and we isolated this material and subjected it to the chemical degradation. The results are shown in slide 6. At the top we show the methylchloroformate derivative of 2-hydroxyheptanoate, derived from compound H in the PGE_2 region. You can see that it is predominantly the S compound. The one isolated from fraction 6, which would represent a mixture of 15(S) and 15(R)PGA_2 did indeed produce on degradation both the (S) and (R) 2-hydroxheptanoate derivatives.

Although time may be running short, I could raise some obvious questions, I think, about the biochemical interest in these compounds in *Plexaura*. If we're getting late and you want to move on, it is up to you, John.

DR. PIKE: I think it would be fine if you would like to speculate a little on the biosynthesis and how this may occur.

DR. LIGHT: As I say, most of these questions are probably obvious. Most of the interest that has been brought out at the meeting so far has been related to *P. homomalla* as a potential source of a pharmacological agent. But there is also an academic interest, I think, in the fact that this organism does make prostaglandins. One of the first questions has already been asked: whether or not the compound has any function in the *Plexaura*. That is a question that it is just not possible to answer, yet, until we have some more information. The second one has to do with the biosynthetic mechanism. Through the work of several laboratories, including that of Prof. Samuelsson, it has been established that arachidonic acid is a precursor in mammalian systems of PGE_2 and PGF_2-alpha, and that the biosynthetic enzymes produce either F_2-alpha or E_2, depending on conditions. PGA_2, on the other hand, is a further metabolic product of PGE_2. Just to remind you, arachidonic acid is a C_{20} polyunsaturated fatty acid with four double bonds in positions 5, 8, 11, and 14. The mechanism of synthesis involves an attack of an oxygen at the 11 position with the oxygen cyclizing around to introduce the oxygen function at the 9 position, and a second molecule of oxygen attacking at the 15 position. So one might ask, then, whether PGE_2 is the first product in *Plexaura,* with PGA_2's arising from PGE_2, or whether there is a different mechanism in the *Plexaura* in which the PGA_2 is the

initial product. The occurrence of both the R and the S isomers is a very intriguing observation, and one could ask how the R isomer arises, or how the two are interconverted. It seems to me there are two different possibilities. One would have to do with the S isomer being formed in the same way that it is in mammalian systems with some kind of isomerization reaction going on. Or, secondly, the possibility that there is a difference in the precursor acid, for example a C_{20} unsaturated fatty acid in which the 14, 15 double bond has the *trans*-configuration rather than the *cis*-configuration. In that case, the same mechanism could give rise to the R isomer instead of the S isomer. The Upjohn chemists have recently established that there is some 5-*trans* PGA_2 present in some of these species, raising the possibility of there being a 5-*trans* isomer of arachidonic acid.

I should point out, I think, that methyl esters are rather rare substances in biological systems, and there have been only a couple of places where the biosynthesis of methyl esters have been reported. They are certainly not as common as are the occurrences of acetylated hydroxy acids. There have been (in fact, we have done some work in this ourselves) some various yeast hydroxy acids in which the hydroxyl groups are acetylated. One could raise questions whether the acetyl and methyl groups are introduced very early during the biosynthetic process or whether they are introduced by some metabolic reaction after formation of the initial prostaglandin.

I was quite impressed by the slow growth of these gorgonians and was wondering at what stage the prostaglandins are made. Are they being made very slowly all the time, building up gradually, or are they made all in one stage of growth, to then remain metabolically inert? I think some attempts to answer that question would make it a little easier to know just what material one needed for biosynthetic studies. And, of course, the whole question of the relationship of the symbiotic algae to the biosynthesis and metabolism of the prostaglandins is a rather obvious one.

Finally, one of the most intriguing questions to me is the fact that these compounds seem to be found in just one species of *Plexaura*. Do the very closely related species make prostaglandins but also have enzymes which break them down? I think all of these biochemical questions can be tackled. The time is probably right to do it. I would hope that the marine biologists will work a little harder toward learning how to culture or maintain this in the laboratory so the biochemist can work with it more easily. Thank you, John.

DR. ANDERSON: I have been working with the gorgonians for some years now as a study of sesqueterpene and diterpene biosynthesis. Depending upon the species, it is fairly easy to isolate relatively pure zooxanthellae, and since zooxanthellae themselves are so tough, it is also quite easy to isolate what amounts to gorgonian fractions that are uncontaminated with zooxanthellae. A former graduate student of mine, Dr. Papastephanou at

Wisconsin, early clarified a soluble system derived from the zooxanthellae of *Pseudoplexaura porosa,* which is capable of transforming malonic acid all the way to a diterpene lactone crassin acetate. This is a macrocyclic diterpene lactone which Dr. Weinheimer briefly mentioned yesterday. So, obviously, the zooxanthellae once isolated have biosynthetic capabilities. Accompanying the diterpene lactone in this creature are some sesqueterpene hydrocarbons. Actually, this was our first interest in the gorgonians because we were interested in how the sesqueterpene hydrocardons were made. We were prejudiced that a hydrocarbon obviously should be made by a plant material, so we never looked seriously at anything except the zooxanthellae for this factor. Later on, a suggestion was made that we look at a sterol biosynthesis, and here we had no preconception as to where it might occur. Unfortunately, we found that the farnesyl paraphosphate which was used as a precursor did not go into sterols, but in the animal portion of the creature this farnesyl paraphosphate is transformed into sesqueterpene. This, of course, is highly unusual. I would not be at all surprised as to where the synthesis of prostaglandin takes place. It could be anywhere. It could be joint, it could be individual. I think that with the isolation of zooxanthellae and the gorgonian fractions from at least one species, we have tools to work with that could be very useful in the elucidation of the prostaglandin study. I must say, however, that *P. homomalla* is very difficult to work with as regards separating the zooxanthellae in reasonably good form. I recall making a separation like this and sending it to Upjohn in the hope of localizing the prostaglandin in the organism. I wonder if we could have the results on this.

DR. PIKE: I do recall those being sent to Kalamazoo, but I really forget the details of the analysis. There was a prostaglandin in the extract of the purified zooxanthellae. The quantities in the zooxanthellae relative to the amounts found in the total gorgonian were somewhat less. I think these data need reinvestigation, since you can see from what Dr. Light has said that one needs very careful analysis. Dr. Weinheimer, did you have a comment a moment ago?

DR. WEINHEIMER: I was going to ask Robley: You were looking at the methyl carbonyl and you saw a bit of the S form in the coral stump. Then you proceeded to compare this to E_2—the last slide in front of you. A standard would show some of the R, wouldn't it?

DR. LIGHT: There were two different fractions on the last slide. One was isolated from the (S) PGE regions of our chromatographic fractions; it showed predominantly the S isomer. The other one was isolated from chromatographic fractions that looked on thin-layer like a mixture of the (S) and the (R) PGA_2; it did show both S and R isomers. Our standard was obtained from authentic racemic 2-hydroxyheptanoate, and was shown on the next-to-last slide.

DR. LANG: I have a speculation to make regarding a possible biological importance of prostaglandins. Stony corals and gorgonians do come in association on reefs, as do the zoanthideans and stony corals that Dr. Ciereszko showed on the slides this morning. Quite commonly you do see stony corals growing around the bases or over the bases of gorgonians. In the laboratory, I have seen some stony corals digesting some gorgonians that happened to be near them in the same aquarium. The only gorgonians that I can remember the names of at the moment are species of *Pseudopterogorgia.* Now, I have never paid very much attention to *Plexaura homomalla:* it doesn't grow in the part of the reef where there are many stony corals, but I don't recall seeing it in close association with them, either. The mechanism whereby stony corals appear to recognize other coelenterates as a possible food source does appear to be associated with their ectodermal surfaces, that is, with the surface of their polyps. Over the last day, I have been reading that prostaglandin is probably active on cell surfaces. So, it occurs to me that quite possibly *Plexaura homomalla* could protect itself from attack by stony corals by secreting prostaglandin at its ectodermal surface. Since there are not very many stony corals in its habitat, this may not be very important, but it is the sort of biological role that you also might consider.

DR. ANDERSON: Either Dr. Weinheimer or Dr. Ciereszko should be speaking now, since they are the ones who have done this work. In their studies of the gorgonians, they found that almost anything might turn up in the gorgonian: crystalline diterpene lactone, which I have worked with; an enancepsenolid hydrocarbon with two lactone groups on each end; prostaglandins; butyl alcohols, etc. It seems as though every species of gorgonian, perhaps not without exception, has something that it makes and stores up, and this can be almost anything—usually derived from acetate, as most everything is.

DR. LIGHT: Just a brief response to that comment. I would point out that this phenomenon, often termed secondary metabolism, is quite common in plants and fungi. Perhaps this is the first case in an animal in which you find a secondary metabolite.

DR. ANDERSON: Well, it's a point. But "secondary" is wrong. We make too much of secondary metabolisms.

DR. LIGHT: So do plants make too much terpenes. So do fungi make too much of various sorts of compounds.

DR. BABCOCK: However, that might not be too much unlike the intestines of an animal making too much cholesterol, where the precursor sterol is useful in making vitamin D regulating calcium balance. We pile all that up where we don't need it. So it may be more common than just the plants and marine species.

DR. VOSS: I would like to ask Dr. Ciereszko if he has seen *Hermodice* actually eating alcyonarians, because the commonest place we find *Hermodice* here in Florida is in those parts of the reef where alcyonarians are not particularly abundant.

DR. CIERESZKO: If you get up early in the morning, and this is an unfortunate thing about *Hermodice,* you will see them crawling on gorgonians. During the next week or two I will be in the Caribbean and I will get up a little earlier than usual. I am going to try to spot them in their lair. You can see them, but they are hidden much of the day.

DR. VOSS: Have you seen them *feeding* on alcyonarians?

DR. CIERESZKO: No, they were just crawling.

DR. PIKE: I think perhaps we should call this session to a close. I believe that it is quite clear that there are many areas in the biochemistry and biology of *Plexaura homomalla* to be studied over the next few years.

PANEL DISCUSSION

ECOLOGY, HARVESTING, ENVIRONMENTAL IMPACT AND MARICULTURE POTENTIAL

J. W. Hinman, *Moderator*

DR. HINMAN: Let us get on with our final discussion panel and evaluation, entitled "Ecology, Harvesting, Environmental Impact and Mariculture Potential." We have a lot of territory to cover in less than an hour, I am afraid. All I intend to do is to announce the topic, and then turn the discussion over to those who are more knowledgeable about it. With that, I am going to consider my duties as moderator accomplished and hand the podium and the microphone over to Dr. Stoddart.

DR. STODDART: As Dr. Hinman said, this is an extremely wide topic of discussion, and to prevent it from degenerating into anarchy, it would be useful to have some guidelines for consideration in the discussion. I think one can look at the implications of the discovery of prostaglandins in *Plexaura* on a variety of levels. My perspective on this is very much influenced by the opportunity I had a few days ago of looking at the situation in the Cayman Islands, which has been described, where some of the harvesting has been carried on and some experimental work has been done on the possibility of harvesting. I think it is on these medium and small scales that it will be most profitable to talk about the implications of this discovery. Dr. Pike, in introducing the symposium yesterday, drew attention to some of the much wider implications when he spoke of world population growth and so forth, but obviously it would not be appropriate to consider the problem on that kind of scale during this discussion. In the medium scale, it seems to be useful to consider why *Plexaura* is concentrated in particular areas and what the implications of its exploitation are in such places. Particularly with reference to a situation such as at Cayman where—if, in fact, a substantial demand develops for a prostaglandin source—it could have substantial local implications. If, in fact, there is going to be substantial exploitation of *Plexaura,* would this in any way affect the longer-term potential of the Cayman Islands as a tourist resort? Whether one can overcome this by some kind of spatial segregation of the exploitation and conservation activities, or whether one can think in terms of multiple use. I think the pictures that we have seen so far, of regeneration on harvested colonies, suggest that many people may not even be aware that harvesting on the conservative basis that has been tried so far,

has in fact been carried out. I think the implications of this need to be looked at rather more. But even more than on this intermediate level, I think it is on the smaller scale—in terms of control of the distribution of *Plexaura* as an animal, of the implications of its removal upon the local ecosystem, of the effect also of cultivation and what effects will arise from this—that we are particularly concerned with. I think, biologically, we are interested in the controls on distribution, on the density, on the spatial segregation of the animals; we are concerned with population status and, of course, all the controls which affect that, especially in terms of harvesting and replacement.

In this discussion, I expect that we really ought to look at fundamental science requirements: what, in fact, we ought to do to find out more about this beast. I think that everyone must have been impressed by the fact that most of our information on *Plexaura* has often come from incidental observation, that it is obviously fragmentary, and that there are a great many suggestions that have been brought up by it, but we don't really know very much about it in any kind of detail so far. Secondly, and partly involved in this, quite clearly there are operational problems in studying *Plexaura* in the field, as was shown in the discussions yesterday, such as the purely technical problem of measuring growth rates. This is much more difficult than working with stony corals, simply because of the flexibility of *Plexaura.* I think the technical problems of getting basic measurements of biological importance are by no means solved, and I hope that people who worked on other gorgonians, such as *Gorgonia flabellum,* which is obviously a much simpler animal to deal with, can give an indication of this. Thirdly, the conservationist's side of this: the implications of harvesting or farming. One can immediately see that there are feedback linkages between all of these three major topics.

If we could have discussions starting primarily with science and then going on to more practical aspects, it might give this session some kind of form. Dr. Wickstead might like to lead off, if he could tell us where to go and say something about the kind of goals that might be involved in a scientific investigation in the context of possible exploitation of *P. homomalla* commercially.

DR. WICKSTEAD: Looking at this from the point of view of what has evolved from this symposium, there have emerged several quite clear-cut problems that must be decided at this early stage. Primarily, it seems there is no doubt that these prostaglandins are going to have great impact on the world, and the situation is such that people just cannot get enough of them. We must, therefore, increase production of prostaglandins to a very great extent. Speaking as a biologist and not as a biochemist, I would suggest that the ultimate objective is to analyze these compounds and, in the end, synthesize them completely in the laboratory in order to be independent of outside sources. I think this would have to be assessed by the Upjohn people, and we should try to get from them an estimate as to how far away they are from complete synthesis. If they think they are a matter of 10 or

20 years away from this, then they must look forward to getting the prostaglandins from naturally occurring organisms. The first thing to decide, which should emerge from this meeting about prostaglandin precursors, is: do they come from the algae, or do they come from the *Plexaura*? In my ignorance of the biochemistry, I would imagine that this would be a fairly easy one to resolve. If we find that they come from the algae, I think it would simplify matters in the sense that basic techniques of culture have been devised. Recently there has been a breakthrough in the culture of these organisms, the Gymnodiniums, at the Allan Hancock Foundation, and they are going ahead actively there. If we find it comes from the *Plexaura,* then I think it is now time to start considering long-term farming, for which *Plexaura* seems to be an almost ideal organism.

Much of the basic work has been done by Dr. Schroeder and the people in Cayman; it seems to me quite feasible, quite possible and, I would say, highly desirable to try to raft-culture these creatures as oysters are done. One can control the environment, one can control the predators, one can keep the rafts clean, and so on. If you try culturing these things on the bottom, you are merely dealing in one dimension. If you have three-dimensional, multi-layered rafts that you can sink or float in the sea, stacked so that the light can get to the bottom layers, then you are dealing with the possibility of having *Plexaura* in ten dimensions, so to speak, and harvesting would be simple. You would get so much more production per given square meter of sea bottom and, as I said, it seems that *Plexaura* is an ideal organism for farming. I would suggest that these are the priorities.

If these prostaglandins do have to come from natural organisms, now is the time to start thinking of long-term policies. I know from my experience at Plymouth that biochemists get terribly impatient with biologists. They ask questions, and they ask if it can be done in two months, or three; but I think one must think in terms of years, a 10-year project. One will have to look ahead, and I think that now is the time to start considering this long-term farming situation. The basic knowledge is there, due to work by biologists in the field. Now I think one should start considering the farming.

DR. STODDART: Thank you very much. That is a good look at the animal to begin with, and what such needs are in elucidating further the controls on distribution, growth and so forth.

DR. LANG: I would like to ask why you don't consider doing the equivalent of force-feeding experiments. The nutritional requirements of gorgonians are not well worked out; the relative importance of light for algal photosynthesis, of dissolved nutrients and, possibly, of zooplankton are not known. It seems to me that you should begin in the laboratory with controlled experiments in which you carefully evaluate the physical and chemical environment, find out what quanta of light, at which wavelength for how many hours a day, cause the greatest growth of *Plexaura* tissues, and establish whether or not they require dissolved nutrients or some form of particulate organic matter such as particular zooplankton species or

Artemia—whatever it takes in the surrounding medium, with how much of each. I do think that you should try to find out how to maximize the growth of *Plexaura* tissues. At least for scleractinian corals, David Barnes has shown that a regular alternation of day and night cycles is needed for skeletal growth. Conceivably, you might find, for example, that if you give a gorgonian a longer than normal daylight regime it puts less energy into skeleton formation and more into tissue growth. These are the kinds of questions for which it will take a very long time to get answers if you wait to find out what happens to *Plexaura* in the lagoon at Cayman Island after addition of manure, or in areas of strong or weak water circulation. I imagine that these kinds of nutritional-requirement experiments could be done in a long trough through which water is flowing rather rapidly so that you are giving the gorgonians an adequate supply of oxygen and removing their metabolic wastes. Then, possible sources of nutrition could be sequentially added to the trough. I may say that this technique is, at the moment, being used very successfully in the cultivation of fresh-water fishes. You can pack a large number of fish into what is really a small volume if you keep the water flowing fast, and if you add nutrients to it. To my knowledge, it is not now being used commercially for any marine organisms, but an Israeli mariculture company is evidently constructing such a trough, in which they intend to raise marine fish. At the moment, they have populations of this fish in cages out on the reef, which they are giving a standard quantity of fish-food every day. These cages are now attached to floats, so that you can haul a cage up to the surface and throw in the fish-food—you don't even have to get wet, if you don't want to. Within these cages, the fish are growing something like two or three times more quickly than the surrounding resident fish populations. What they are planning to do with the troughs is to see whether or not these fish will grow even faster than in a more rigorously controlled environment. I think that you can also play this kind of game with *Plexaura,* both in the laboratory and in the lagoon, but I think you will get answers on what they require to grow much faster in the laboratory.

DR. STODDART: Yes, it has been very noticeable in the symposium that there were only two mentions of *homomalla* in aquaria under laboratory conditions, both by someone this morning. Very little work seems to have been done on this.

MR. GOLDBERG: I would like to point out that in the order of priorities for mariculture of gorgonians, feeding may be secondary to survival *per se.* It seems to me that gorgonians are very easily raised in an aquarium. At least, I have had no difficulty in keeping many species alive, either in closed- or open-system aquaria, but *Plexaura homomalla* seems to be an exception. I have had a tremendous amount of trouble keeping *P. homomalla* alive. For example, when one collects gorgonians on the reef, one normally takes them out of the water and places them in the boat. The brief exposure to air that ensues is no problem with most gorgonians, but

apparently with *Plexaura homomalla* it is. I have found that if they were exposed to air for a brief period they did not survive very well and, in fact, were completely decomposed by the following day—which, in itself, is unusual. So, as far as culturing techniques are concerned, the first order of priority should be what affects their survival. It may not be primarily feeding, because many gorgonians when observed to feed do not do so at regular intervals, so it seems to be a short-term phenomenon; I think we are dealing with immediate survival rather than with feeding *per se*. They can go a long time without actively feeding, as far as I can see. I don't know about dissolved organics, which may be a major nutrient source to them.

DR. VOSS: I am always just a trifle skeptical when people start talking about mariculture as the final answer, because I have been concerned with various kinds of mariculture here and elsewhere, and a tremendous amount of basic research must be done before mariculture ever becomes successful. One group that has been worked on longer than many others is shrimps, yet there has not been a commercial crop of shrimp produced so far that has been able to compete with the natural crop from the ocean, despite all the work that has been done and the millions of dollars that have been spent both in this country and abroad, especially in Japan. Regarding the matter of cage culture and trough culture and so on, this already has been brought to a very high peak of development by the Japanese, who have been the leaders in the field. They have been producing a number of different products from the sea using this method, some of which are proving to be quite successful, especially using cage culture. Of course, rack culture has been used, but we are dealing here, I believe, with an entirely different type of organism. We are dealing with an organism that does not take crowding very well. It has a distinct spatial relationship, as far as we know, in nature, which occurs only in fairly clear, open waters. It has certain requirements which we are far from knowing at the present time, none of which indicates that *Plexaura* would be susceptible to this kind of culture. I think this is the thing that we should be looking toward, and I would urge you not to put all your eggs in one basket. Do not go all-out for culturing, but start on both fronts. Continue your experimental studies in the field of natural harvesting, accompanied by well-planned experimental laboratory work so that if one doesn't work out, the other may. We need to know a great deal more about *P. homomalla*. We need a great deal of experimental work, but I feel that this will have its greatest result in the open farming of *Plexaura* in the reef and back-reef habitat. I am not saying that it cannot be cultured, but I think it is going to take a long time to develop techniques, and that we should not forget other possibilities. There are several that could be considered for harvesting in the open ocean or on the reef. We have had explained to us three different ways of cropping or or clipping *Plexaura,* of which the medium height is apparently the most successful. We do not know the amount of axial build-up in the terminal portions. Perhaps they are too new, and a little bit longer resting period

would be desirable. I believe that one of the aspects that could be considered for natural farming would be, as someone suggested, that we take four or five or six areas of sufficient size to yield the crop required, and harvest one each year, going on to the next one next year, and so on. This has been done for many types of farming, and is commonly practiced in some areas of sponge fishery. In this way, you would not come back to the first place harvested for about five years. You then should have complete terminal regrowth, which should have been there long enough for the prostaglandins to accumulate to their original levels. Also, you would only have one time, quite shortly after the first crop was taken, when there is any apparent but minor disfigurement to the reef. As far as the skin-diver and the tourist viewers are concerned, I don't think they are going to care about it anyway. At least you would have taken this precaution and the reef would be back in pretty much its natural state within a year. Thank you.

DR. WICKSTEAD: When I mentioned things like raft culture, I was thinking in terms of the natural environment, not in back-waters where, of course, the problems could be rather great. As you say, this sort of thing has been tried with shrimps and what have you, but nothing has ever proved completely successful. I was, in fact, thinking of growing *Plexaura* where the adults occur so they actually would be in their natural environment. Another thing I meant to say is that when you have growth under culture conditions you can, to some extent, control the parent from which your cuttings come. I would suggest that it might be useful at this stage to take sample clippings from different individuals and to have a look at the type and amount of prostaglandin precursors in them. No doubt you will find that this will vary quite a bit from colony to colony. It might well be possible to tag a colony which is a very high yielding one, re-examining it, of course, at regular intervals to see that it stays a high-yielding colony, and then you could use this as a parent plant from which to take your cuttings to transplant to raft cultures. In this way, you might insure a much higher yield per unit length of *Plexaura*.

DR. BIRKELAND: This symposium has been successful in making concrete decisions regarding the most efficient method of cropping gorgonaceans by considering the growth characteristics that we have learned. We seem to have decided that pruning would be more productive than reaping on a long-term basis. The growth characteristics leading to the conclusion are that regeneration is dependable and perhaps more productive than growth in gorgonaceans, and that recruitment is sparse. Dennis Opresko's observations and my data indicate that recruitment of gorgonaceans is fairly sparse, but a few settle each year. If I remember correctly, Bob Kinzie found that there were no mass settlements except occasionally, in patches, by *Pseudopterogorgia*. The purpose of this symposium was to consider the possible environmental impact of a program of harvesting on the coral reef, and we never really have approached this question. However, our conclusions concerning harvesting methods may have obviated the whole problem.

When attempting to bring temperate farming methods to the tropical forests, man is finding that optimizing production on a long-term basis by pruning is best for complex "stable" systems in which symbiotic relationships are prevalent and nutrient recycling is important, while in simpler temperate systems predominated by short-lived, fast-growing species and nutrient input, reaping of crops in monoculture may be more efficient. This suggests that we follow the examples of the natural predators of gorgonaceans on complex coral reefs, such as *Cyphoma* and *Hermodice,* and browse or prune rather than graze or reap.

I realize that, as a general rule of exploitation, the maximum yield is obtained from populations at less than maximum density. However, the degree to which the population is harvested in order to obtain maximum yield depends upon the life-history characteristics of the exploited species. Gorgonaceans do not frequently saturate the environment with offspring in the manner of barnacles, oysters or bryozoans. The independent studies by Opresko and myself each conclude that gorgonaceans are in about as much of a steady-state system as one would ever expect to see in the real world. The exploitation rule is more applicable to oysters than to gorgonaceans in the same way that it is more applicable to quail than to condors. We should prune gorgonaceans as we would a tropical forest rather than reap it as we would a grain crop.

DR. KINZIE: I think that there probably is a difference and we don't know which it is we are faced with. There probably is a difference between the natural production under natural circumstances and the maximum sustainable yield under harvesting situations. Certainly in a lot of situations where there is a stress put on a population, generally in terms of a fishing stress, the amount of production that the fish population is capable of is much greater than the production under normal circumstances. I think that one of the major problems is the time factor. If an experiment is done, such as the kind of experiments that have been going on in Grand Cayman and the kind of experiments that Bob Schroeder has been doing, the results won't be seen for one season or, in some cases, probably two, three or four seasons. I think the idea of using more than one approach at once is mandatory in a situation where you don't know what is going to happen for one, two or three years. I think the kinds of laboratory experiments that Judy Lang was talking about are necessary if there is some plan for a long-term production, improvement of the stock, and improvement of the rates of production of material. On the other hand, I think that a number of farming-type experiments such as Bob Schroeder was talking about, and a continuation of the moderately large-scale cropping or harvesting operation, should be done simultaneously. The reason is that none will show useful results now or next year or probably the year after that. But until the work has been done in all of these approaches, you can't even begin to expect useful results.

DR. SCHROEDER: I agree entirely with Dr. Kinzie. Furthermore, I would like to say that I hope no one is deluding himself that we have avoided the ecological problems with this thing simply by going out and planting cuttings. We don't know what the effect of increasing the number of colonies in this area a hundred or a thousand or more per cent is going to be. Specificially, I think that the settlement of planulae may be correlated with the behavior of some of the benthic organisms. These populations are bound to be changed. If you start placing slabs of concrete in areas of soft bottom, you will change the populations there. We don't know what the effect will be of increasing the number of corals putting substances into the water—and they obviously put some, if runoff from an aquarium containing them chases lobsters out of the aquarium. We don't know what this will do to the reef environment, and we had better find out before we put too much faith in it. I took this approach because I thought it was the most plausible approach to getting a harvest soon, but let's not delude ourselves that it will not create ecological problems. It will, and we had better know about it. We need population studies of many other things, some of them rather hard to study, such as sand-bottom organisms.

DR. STODDART: Have you got any indication of pathological conditions with concentrations of the gorgonians?

DR. SCHROEDER: Not with a total of only 368 cuttings. I think that we can't guess; it is too complicated. We will have to actually plant some area—a large area, several acres anyway, maybe more—densely, and then some other places in different densities, and then compare them. I think that in each case we are going to find that we will get different results, because of differences in the areas, differences in what we are doing. We may soon get some indications of what happens when you plant on a large scale, but unless you plant on a large scale, you are not going to find out.

DR. BUNT: There appear to be two distinct areas of potential interest. One appears not so much ecological as simply cultural, that is, how to encourage the growth of *P. homomalla* in the shallow waters and often more or less bare sediments of Grand Cayman. As a more broadly ecological question, it would be reasonable to inquire into the community influences of organisms synthesizing quantities of substances with high biological activity.

MR. GOLDBERG: It seems to me that when we talk about the natural regulation of coral reef populations, that is to say, a complex community, and we are changing the structure of the community, then we are in fact changing the balance of the structure. I am reminded of the efforts to grow *Hevea brasiliensis,* the Brazilian rubber tree, in a complex rainforest ecosystem of Brazil where the naturally occurring rubber trees were relatively rare. Areas were cleared for plantations, or areas not inhabited by anything were planted, and suddenly a disease broke out and nearly wiped out the entire Brazilian rubber-tree industry, at least for a time. This kind of

natural regulation of populations by predators when one kind of organism becomes too numerous is something that we see very often in complex ecosystems, and I think we should be aware of it.

DR. THEODOR: I think that one important point of the discussion is that, first of all, an estimation of the early requirements of this substance should be made. Fortunately for the inhabitants of the Cayman Islands, this is not the only place where habitats of *Plexaura* are found. There are the Bahama Islands; there is plenty of *Plexaura* there. I have seen *Plexaura* in Haiti, and around many other islands. I think that some estimation of the standing crop could be made. Some of the specialists here who have been diving in these regions—Dr. Bayer, Dr. Kinzie and others—could tell us if 10^3, 10^4, or 10^5 tons of dry material would be found. It is suggested that after five to seven years, there would be enough regrowth in a given harvested area to allow a second harvesting. If this harvesting could be made in these various places, it might not be necessary to look for any other method, such as transplantation or tissue culture.

DR. WICKSTEAD: I am going on a little from Dr. Theodor's remarks. I wonder if we could get some ideas of quantities involved. I would like to know from the Upjohn people if we take, say, a gram of administerable prostaglandin, what is the wet weight of *Plexaura* needed to produce that gram? And can you give us any idea of your projected research—how many grams of prostaglandin will you need, say in the future six months? Just to get an idea of the quantities you have in mind for harvesting.

DR. BABCOCK: It is one of our objectives here to be as frank and open about everything we have done as is possible. That, however, is one of the figures that we are not at liberty to discuss in detail, and I would rather put it this way: if we would talk about five per cent of the *Plexaura* that is present in North Sound, for example, for a year's harvest, that would not be an unreasonable figure to hope for from our standpoint. Similarly, if we had several other such areas that we could plan to harvest as Dr. Voss has suggested, moving not every five years but perhaps every ten or 15 years, back to the original spot, I think that would probably be a reasonable estimate of the amount of material that might be required for the commercial use of this natural resource.

DR. SCHROEDER: Since we are not to have a figure as to how much the Upjohn Company actually needs, I hope they are taking into account possible expansion. I am sure they are. I suppose if this indeed becomes the answer to the birth control problems of the world, or something of the kind, no doubt their needs will be much greater. I understand that less and less prostaglandin is needed experimentally all the time. I wonder if Dr. Hinman would have any comments on this.

DR. HINMAN: One of the reasons this is difficult to talk about is that we do not know solid figures ourselves and we would simply be speculating. We think it is too touchy a point to speculate on. In terms of order of magnitude, I said earlier that we could clearly see that if we used the earlier method of making prostaglandin by the utilization of the enzymes from sheep seminal vesicles and the unsaturated fatty acid precursors, we could calculate that the world supply of sheep seminal vesicles would be grossly inadequate to meet the needs that we could foresee. Looking at it from the same gross point of view, I think we can clearly state at this time that the South Atlantic and Caribbean has more than an ample supply of *homomalla,* so that it would take much less than five per cent of it per year to meet the needs that we can see arising in the future, even though I can't attach a definite figure to it.

DR. SCHROEDER: Another thing interests me: We don't yet have any data, and won't for a while, on what happens on secondary and tertiary croppings of the same colony. If a significant number of the colonies in the area are cropped, the question arises, how does this interfere with the sexual reproductive cycle of the organism? Is the *Plexaura* that grows back equivalent to what existed before, and how does this affect the survivability of the colony in terms of being blown down? What grows back will be a colony that has been clipped and regrown; it will not be a new colony.

DR. VOSS: There are a couple of comments I would like to make about this. I know that Sid Anderson has covered the Caribbean pretty well in looking at the supply of *Plexaura homomalla* and its distribution, but I would suspect that probably the greatest supply will be found on the greatest bank of shallow water that occurs in the Caribbean, and that is in the Bahamas. I am wondering, if actually this becomes a major requirement for world population control, as is possible, whether we are not then looking primarily toward the Bahama Bank as one of the major sources of *homomalla.* If we are, then I believe that Grand Cayman is not the best place to carry out studies of the ecology and biology of this organism, because it is a very small bank with a limited area of deep, clear water and with oceanic water exchange continually occurring. It is entirely different from the area at Margot Fish Shoal, which Dennis Opresko described, that is sitting far inside the reef, with somewhat cloudy water and an entirely different type of ecological habitat surrounding it. It is also very different from that which is found in the Bahama Bank, and where there also are a number of fisheries. I would suggest that, although the program in Grand Cayman is apparently in an ideal place for continuing a good deal of the investigations that need to be done, this will never suffice. If prostaglandin proves to be a very successful substance, Grand Cayman will never meet the demand, and you had better start doing ecological studies in the areas where you will get your major supply. I suggest that field studies be set up in areas set aside for the purpose. The *Plexaura* beds in the Bahamas and Florida are surrounded by other types of reef organisms in very large concentrations, nothing like that in the Grand Cayman region. It would be interesting to crowd the *Plexaura* in test areas to see what effect it has upon itself, and to set this up in areas adjacent to different ecological habitats to see what

effect it will have upon them. Would it have an effect upon the crawfish fishery, which could be very important? I have always been impressed with *Plexaura* in large beds. There is not a great deal else around it, and it seems to set itself aside to a certain extent in this way. In the matter of farming, trimming and so on, I am continually reminded of the sponge fishery, in which a particular portion of it is harvested. The shallow part has always been set aside for hooking, so divers wouldn't kill the young sponges with their lead shoes. In the Bahamas, I think there are closed areas and open areas, and these are worked at various times. There have been plantings, but plantings never have been really successful as there are all sorts of things that influence them. Nobody has looked at the ecology to find out why they weren't successful. I think that this is the route, aside from the experimental approach in the laboratory, that we need to follow. I think that consideration should be given to planning a large-scale study of trimming, of planting, of overcrowding and, especially, of the ecological requirements. We just don't know them. I know of no studies of the illumination levels required, or whether crowding affects the illumination level, as it certainly must. Is this one of the factors that keeps *Plexaura* from resettling in the area? Perhaps reduced illumination is the reason you don't find it crowded in with other types of alcyonarians. Is it true that *Plexaura* can get along without much plankton, and you can furnish them with other food? This has been discussed but we have nothing, either experimentally or in the field, designed to tell us this. I think that the sooner we look to the matter of natural predation, of natural growth, of damage to the area, and particularly of the food-light-substrate requirements in nature, the sooner we will be taking care of some of the problems facing us here.

DR. STODDART: There is the point, is there not, that the prostaglandin precursor from Cayman *homomalla* differs from those from the Bahamas?

DR. HINMAN: As far as we know, it doesn't differ from that in the Bahamas, but we haven't had much information to go on.

MR. GREINER: As I listened to this discussion, I thought it might be of value to pose the question differently and yet reasonably specifically. We have long-term interests, medium-term interests and short-term interests, and my particular short-term interest is to get, in the reasonable future—like part of this year and part of next year—a reasonable amount of *P. homomalla*. I think that the short-term picture, insofar as I can see it right now—this year and next year—could be expressed in two ways. One is: could we get on the order of a ton a month, 10 tons a year, of *P. homomalla* without damage to the area from which it would be harvested? Another way I would like to pose the question is in percentage, particularly as I have heard five per cent mentioned in the discussion. What is the likelihood of roughly a one per cent harvesting in terms of pros and cons concerning the ecology and other factors? I think that neither one per cent, nor ten per cent, nor one-tenth of one per cent is, in my view, outside the

scope of our interest. I would think that something on the order of one per cent of the estimated *P. homomalla* in various areas would be a significant amount for our near-future visible needs, if it were to amount to the order of magnitude of 10^1 tons per year, to put it in terms of exponents.

DR. THEODOR: A few minutes ago someone said that *Plexaura* is usually not surrounded by many other species. Was that correct? But I have observed many *Plexaura* grounds where this was not the case. From what I have seen, in those areas where there are accompanying gorgonians, it is not so much a question of the presence or absence of *Plexaura* as a question of well-being. On the grounds where Opresko found the *Plexaura* not so large, my guess is that this might be related to, for instance, a higher turbidity. Everywhere that I have found *Plexaura homomalla,* the water was very clear, very shallow, and with little or no sediment, as on a rocky bottom far from sandy areas. I have located, on detailed maps, areas that correspond to that description and later *Plexaura* was found to be there. Another point: a few weeks ago, I made an estimate of how many *Plexaura* there were in a certain region. The result was, for whatever it is worth, 1000 tons or more of dried *Plexaura.*

DR. STODDART: What was the extent of the area?

DR. BIRKELAND: Was it an acre? A square mile?

DR. THEODOR: No, it was a vast area. It would correspond to a region, and I suggest that there are as many tons of *Plexaura* in the other regions that I have not visited.

DR. STODDART: Are you thinking of something like the Bahama Bank?

DR. THEODOR: That is your guess.

MR. OPRESKO: By comparing the standing crop of *P. homomalla* on the patch reef at Bache Shoal with the figures given here a little while ago, it is possible to calculate the approximate amount of reef area that would have to be utilized to fulfill Upjohn's needs. It was said that the company might require ten tons of *P. homomalla* per year. Ten tons, wet weight, would be equivalent to about 6×10^6 g dry weight (the dry weight of gorgonians ranges from 50 per cent to 80 per cent of the wet weight, depending upon species and size of colonies). The standing crop of *P. homomalla* at Bache Shoal in 1962 was 338 g/m^2; thus, 1.6×10^4 m^2, or about 4 acres, would be needed to yield ten tons of coral. If the colonies were not completely removed from the substrate, but rather were selectively pruned to allow for rapid regrowth, then the yield per square meter would drop considerably and the amount of reef area needed might increase tenfold or more.

The survey area at Bache Shoal was a patch reef and thus was isolated from other such patch reefs by grass beds and sandy bottom. It would be useful to compare the population density, standing crop, and growth rates of *P. homomalla* at the study area in the Caymans with those at Bache Shoal. The reef areas around the Caymans are better suited for harvesting

gorgonians, and it would be interesting to know how much area was used by Upjohn, and what the average yield was. Having all the necessary information, it then would be possible to select a number of similar localities around the Caribbean, to harvest the *P. homomalla* from one area each year, and then to allow a sufficiently long time for regrowth of the gorgonians before the area is worked again.

DR. HINMAN: I don't have the data here, Dennis, but I think this is entirely feasible. I know that the area we harvested would be a tiny figure in acres. I think we have the information, so we could make a comparison on the basis that you suggest. Thinking back to what Dr. Voss said about the desirability of studies in another area, particularly the Bahamas, another reason that makes this important is that we really have no guarantee how long the waters in our study in Grand Cayman are going to support *P. homomalla,* because if development continues there and dredging goes on as it is, I am afraid that our studies will come to an abrupt halt before many years. For that reason, in addition to the ones that Dr. Voss mentioned, we really should have another study area to develop.

DR. CHESHER: Keeping in mind that we are dealing with a fishery, I think Dr. Voss' analogy of the *P. homomalla* fishery to the sponge fishery is a very appropriate one. Examination of records of sponge fisheries does not show any profound ecological effect even though sponges have been fished for over 100 years. There may have been undetected ecological changes in sponge fishing areas but the biota, including the sponges, survived the fishing pressure. Sponges are probably more important, ecologically, than *P. homomalla.* A number of organisms live in the channels of sponges. Stone crabs, for example, spend a good portion of the juvenile stage in sponges. In the 1930's, commercial sponges suffered a blight that severely depleted the sponge population. The sponges were infested with a fungal parasite called *Spongiophagia.* The epicenter for this plague was, reportedly, at a sponge farm in the Bahamas. Perhaps the farming operation, by changing the population density of sponges, also changed the ecology of the parasites. Changing the ecological condition of parasites and predators is something that is unavoidable when farming anything. Plagues, which begin in farms—whether sponge farms or, as Walter Goldberg pointed out, rubber-tree plantations—not only affect the farm but can have far-reaching consequences. The sponge blight of the 1930's spread throughout the Caribbean, from the Virgin Islands to Florida. Aquaculture of *P. homomalla* should, therefore, be closely monitored. Harvesting existing *P. homomalla* (i.e., creating another fishery) would probably have negligible environmental effect, particularly if good sponge-bed practices (closed areas, rotational crops, etc.) are instituted as Dr. Voss suggested. I think it is slightly unrealistic to limit harvesting of *P. homomalla* in any area to one per cent of the total population. It would cost a fortune to find out what that one per cent of the total population was and there would be no satisfactory means of enforcing such a regulation.

DR. ANDERSON: Chemical synthesis of the prostaglandins was briefly alluded to earlier. We haven't heard much about whether this is continuing at an active pace, presumably then to supplant any requirements for *homomalla* as far as prostaglandins are concerned. This is obviously in the area of a trade secret, but can we have any kind of assurance from Upjohn that they will continue their efforts in this direction?

DR. BABCOCK: That is not a trade secret. There have been a number of total syntheses of prostaglandins accomplished so far, a number of them commercially useful syntheses. We reported about two months ago, to the chemical community, the discovery of the variant of *P. homomalla* (or symbiont) that produces the S form; we reported to the chemical community then, too, about the trans isomer, and we reported there a synthesis of the useful prostaglandins from the PGA's found in those gorgonians. We have not reported on our total synthesis, although there are several excellent ones that have been developed by E. J. Corey and which are in use in various areas, including other pharmaceutical industries. We are about to report, probably within the week, the synthesis of a new synthetic prostaglandin that is up to 400 times more potent than the natural ones. These compounds are extraordinarily interesting, and I think they will be extremely important to worldwide medicine. So, I don't see the need for *Plexaura* continuing indefinitely into the future. Instead, I see an important immediate need lasting several years into the future. I think that, if anything, the demand for this substance has the potential of decreasing as more potent compounds become available. But, alternatively, we have to keep in mind the possibility, in fact the real probability, of other indications and of other uses (such as in the area of cancer, and a number of the the other areas that were alluded to). Currently, there are, in fact, quite promising potential applications in several research areas for the prostaglandins. There may be new needs. And I think, for these reasons, we are considering the ocean as a potentially valuable natural resource and one which should not be overlooked at this time but which must be handled responsibly and in such a way that it will be preserved for potential future use. But I wouldn't want you to think that there is no total synthesis. There is; it is commercially feasible, and it is not unreasonable in cost relative to the source from *Plexaura homomalla.* So, in a sense, it may be a toss of the coin at this point.

DR. KINZIE: I would like to point out one interesting thing as we are drawing to a close. People who have worked on coral reefs have always prided themselves on working in a fairly esoteric, certainly very complex, and generally rather useless field. Now they are being asked questions of a very practical and quite immediate nature, and they are being asked to give answers in terms they had not thought of. One of the basic problems here is how people who have worked on generally "useless" sorts of things can find answers when confronted with practical problems. I think that we

certainly don't have very many answers, but some of the things that have been suggested here are ways of getting at them, and I think that this is one of the major results that may have emerged from this symposium.

DR. STODDART: Yes, I think it certainly is true that the kinds of suggestions that have been made this morning are going to involve a great deal more organization of research into *Plexaura* than has been possible hitherto. I wonder if it might be possible for the people who have worked on *Plexaura* and related gorgonians to give some advice to those down at Grand Cayman who are measuring growth rates and so forth. We saw some of these problems applied yesterday, of branch-identification, height measurement and such: growth increments. Is there any kind of advice that could come out of the work you have done? You chose a rather simple kind of gorgonian for this, but it seems to me that the problems are intractable with the kind of techniques new being used.

DR. TAYLOR: I think that, in terms of measuring growth rates of these organisms, it is essentially a question that already has been answered by forestry biologists who are looking at the growth of arborescent organisms, and it is an area that is quite firmly based in experimental work—measurement of growth rates and that sort of thing. I should think it would be advisable to go to that literature to get your techniques. It is a very straightforward question as far as I can see. Looking at the growth-rate data that has been presented here, I must say that I am not particularly impressed. The question has been raised as to what happens after the second and third cutting. This is a very important point that needs investigation. Also, since *Plexaura* is capable of laying out flat sheets of tissue, what are the possibilities of introducing artificial axial skeletons for it to grow around, perhaps PVC rods, and setting up an artificial culture system to harvest just tissue? What proportion of that tissue is prostaglandin under these kinds of growth conditions? I would agree with Dr. Voss that it is a fishery problem. I don't think that I would agree with him that you will be confronted with the same biological consequences that the shrimp mariculturists have. It is my impression that most gorgonians have very strong antimicrobial properties, and a lot of them are disease resistant in that sense. A lot of problems that you have in other areas of mariculture may not occur here. In fact, most of the gorgonian pests that you do find are macroscopic pests that quite easily could be dealt with by divers in a simple cultivation system.

DR. STODDART: Are there any other contributions this morning? If not, as time is pressing on, I shall hand the microphone back to Dr. Hinman.

DR. HINMAN: Yes, and now I will hand the microphone to Dr. Voss for his summary.

DR. VOSS: Let me say first that I don't believe in summarizing a meeting like this one by parroting what the participants have already said, so I won't give a summary. A broad diversity of information and of viewpoint has been presented at this symposium, and from that standpoint I think it has been excellent, but it really is only the beginning. After hearing the papers yesterday and the discussions today, I am impressed above all by the difficulties that confront us in meeting the present demand for *Plexaura homomalla* as a source of experimental and pharmaceutical prostaglandin. The development of the mariculture techniques necessary to farm *P. homomalla* will be enormously expensive in terms of both time and money. This is clear from the experience that many have had in developing culture methods for seafood items. One by one they have failed, or exist only to fill the demands of a high-priced luxury market. Successes on the practical, commercial, moderate-cost level have been few, if there have been any at all. It seems to me that work must continue on two broad fronts. First, we need more research on the techniques of harvesting procedures so that we will know what kind of regrowth characteristics to expect when natural stands of *Plexaura* are cropped, in terms of both time and chemical quality. Second, we need to continue investigating techniques of artificially culturing *Plexaura* on a relatively small scale. This involves studies of the food-light-substrate requirements in order to find out the optimum density at which artificial stands can be grown, how they can be made to grow at the fastest rate and produce the highest yield. Until the results of such research have been evaluated and put into practical use, it appears that natural stands of *P. homomalla* are adequate to meet medical and research needs for prostaglandins. Here I think it is mandatory to employ good agricultural rotation technique, harvesting one of perhaps five wild stands each year and returning to the first after five years, as has been done successfully in the Bahama sponge fishery. Similar techniques have been used in the management of the crawfish and conch fisheries, and they seem to work. This is a basic fishery problem and you should treat it as such, but let me stress that a great deal more research must be done on it before it can be managed successfully and efficiently.