Meetings

Coelenterate Biology: Experimental Research

A pilot program to train students for experimental research with tropical marine animals was initiated at the Hawaii Institute of Marine Biology during the summer of 1967. A group of 15 graduate students and 5 instructors spent 3 months using some relatively simple biochemical techniques to investigate extensively animals of a single phylum, the Coelenterata. Rather than concentrating on one aspect of coelenterate biology, the group investigated a wide range of problems. The results of their research were presented 5 and 6 September. Their findings covered such diverse areas as collagen biosynthesis, chemoreception, symbiosis, calcification, and carbohydrate metabolism.

The in vivo formation of protocollagen and its hydroxylation to collagen was demonstrated by John Gosline (Duke University). He showed that the sea anemone *Aiptasia,* given a pulse of C^{14}-proline, produced a labeled protein, presumably protocollagen, which was soluble on autoclaving and which had a high ratio of proline to hydroxyproline. Within a few hours after this protein formed, the relative amount of radioactive hydroxyproline increased until the ratio of the two labeled amino acids was like that of unlabeled *Aiptasia* collagen. The labeled collagen most likely originated from the fibrous acellular mesoglea separating the two main epithelia of *Aiptasia.*

The sea anemone *Aiptasia* has been shown to contain another collagen making up part of the capsule wall of its nematocysts (*1*). Nematocysts, used for feeding and defense, are highly complex intracellular capsules containing an eversible harpoon-like thread. The nematocyst collagen is unusual because it is linked by disulfide bonds, and does not solubilize on autoclaving (*1*). R. Mariscal (visiting instructor, University of Miami) showed that nematocysts from 12 different species of Hawaiian coelenterates were solubilized by dithioerythritol, and hence probably also contained disulfide-linked collagens.

Some kinds of nematocysts contain powerful toxins. R. Pardy (University of Arizona) examined the nematocysts of the colonial hydroid *Pennaria tiarella.* He found that although *Pennaria* bears two sizes of the toxin-carrying nematocysts, the small stenoteles (capsules 20 microns long) on its elongated filiform tentacles, and large stenoteles (capsules 70 microns long) on the club-like, capitate tentacles, it was the large stenoteles that functioned to immediately paralyze captured prey. No doubt these nematocysts, which are about the size of those found on the Portuguese man-of-war, are the ones responsible for the painful stinging of humans by *Pennaria.*

Specific chemical activators, released from prey penetrated by the nematocyst threads, have previously been demonstrated to control the specific feeding movement of some coelenterates. The best known cases are the glutathione control of feeding in hydra discovered by Loomis (*2*) and the proline control of feeding in *Cordylophora* shown by Fulton (*3*). At Hawaii, the feeding behavior of three coelenterates, representing three different orders, was shown to be under chemical control. *Pennaria tiarella,* a colonial gymnoblastic hydroid as is *Cordylophora,* was shown to respond to proline and its analog pipecolic acid (R. Pardy). The swimming anemone *Boloceroides* was shown to respond to the branched amino acid valine (K. J. Lindstedt, University of Southern California). Unlike the response of other coelenterates to a specific chemical, however, a feeding response in *Boloceroides* could not be observed unless an inert solid material was present at the same time. Because isoleucine, and not leucine, proved to be an effective competitive inhibitor, it appears that *Boloceroides* has a receptor specific for an α-amino-*n*-butyric acid with a branch point at the β carbon.

The third coelenterate found to respond to specific chemical activators was the coral *Cyphastrea ocellina.* This coral was able to respond not only to proline and its analog pipecolic acid (both at $10^{-7}M$) but also to reduced glutathione and its nonreducing analog *S*-methyl glutathione (R. Mariscal).

The existence and role of alcohol-soluble proteins in coelenterates was discovered by G. Murdock (Duke University) while studying the fate of labeled food ingested by the sea anemone *Aiptasia.* In a kinetic study he demonstrated that, while in the food vacuoles, labeled food protein is partially degraded into large peptides soluble in alcohol (yet precipitable in trichloroacetic acid). Within one day after the alcohol-soluble protein intermediates were formed, they broke down and the animal tissue (*Aiptasia*) formed new protein. Thus, Murdock was able to show the intracellular breakdown of food protein and the simultaneous synthesis of cell protein from the food protein, a phenomenon impossible to demonstrate through use of standard protein measurements.

Another approach to following the fate of ingested food was taken by J. Rees (University of Puerto Rico). Using labeled mouse tissue as food, he measured the rates and direction of food distribution in laboratory-raised colonies of the colonial hydroid *Pennaria tiarella.* He first fed the labeled food to a specific hydranth, waited a prescribed interval, fixed the animal, and counted the various parts of the colony. By such a technique he mapped out the paths of food distribution under various conditions. He showed growing tips especially active in taking up the label. The lateral stolon stems seemed more active than their attached hydranths in taking up label from food ingested by distant hydranths. Radioautographs of the intact fixed colonies agreed with data obtained by counting for radioactivity.

Some biochemical methods were used to attack problems of coelenterate digestion that have broad ecological significance. L. DiSalvo (University of North Carolina) was interested in determining whether corals can ingest marine bacteria that become entrapped

in coral mucus, and whether the ingested bacteria are assimilated by corals. He covered coral with a suspension of S^{35}-labeled bacteria, stimulated the corals to ingest their mucus (by adding crushed plankton), and subsequently compared the distribution of radioactivity in the labeled coral and in labeled bacteria not ingested. His results indicated that once the bacteria were ingested they were hydrolyzed and assimilated by the corals.

Another type of nutritional problem was posed from observations made on the zoanthid *Zoanthus sandwichensis*. This organism has never been reported as ingesting particulate food (nor was it observed to do so in our summer investigations) or to exhibit a feeding response to food extracts. A. Reimer (University of Southern California) was able to demonstrate the *Zoanthus* could take up more than half of the C^{14}-glycine presented to it in the sea water. Once, in the *Zoanthus* tissue, a significant amount of the label was found in glycerol, and eventually was found in the *Zoanthus* lipids.

Some coelenterates are known to supplement their exogenous food supply with nutrients derived from symbiotic algae living within coelenterate cells. To examine the extent of the algal contribution, two students used *Chlorohydra viridissima*. A. Szmant (Scripps Institute of Oceanography) found that the rate of photosynthetic fixation of $C^{14}O_2$ by the endosymbiotic algae, rather than being relatively constant, increased dramatically and temporarily between 14 and 20 hours after the feeding of the hydra. The reason for this burst of activity is not yet understood, but might be a secondary effect of the cell division occurring in the cells housing the algae. Whatever the explanation, this work points out that in experiments on algal endosymbiosis it is necessary to control the time at which the hosts are fed, and the time following feeding of the hosts that the experiments are conducted.

Once the $C^{14}O_2$ was fixed by the symbiotic algae, the transfer of C^{14}-labeled algal products to the host tissue was measured. E. Eisenstadt (Washington University) helped devise a method for separating the algae from the endoderm and hence was able to get a good index of transfer from algae to animal. Whereas previously only 12 percent of the fixed carbon had been shown to have been transferred to the algae-free ectoderm (*4*), in a kinetic study using $C^{14}O_2$, Eisenstadt found that within an hour the animal contained 41 to 49 percent of the label originally fixed by the algae.

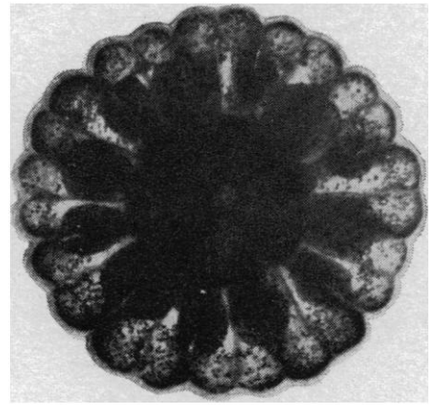

Fig. 1. Polyp (2 mm in diameter) of the Hawaiian colonial coral *Pocillopora damicornis*, Dana, 24 hours after settling of the planula larva. Around the periphery, the clearer ectoderm can be distinguished from the endoderm containing symbiotic intracellular algae, zooxanthellae. Shadowy outlines of primary and secondary skeletal septa are also visible. [S. Arthur Reed, Michigan State University, East Lansing]

Whereas to date most biochemical investigations on algal-animal symbiosis have been concerned with the transfer of material from the algae to the animal tissue, C. Cook (Duke University) investigated the contribution of animal metabolism to the algae. Within a few hours after feeding S^{35}-labeled mouse tissue to the symbiotic sea anemone *Aiptasia*, he was able to show that a significant portion of S^{35} was in the algal protein.

Another approach for studying the transfer of nutrient from the animal tissue to the algae was taken by R. Gorkin (University of California, Irvine). Rather than follow the path of metabolites from ingested solid food, he gave free C^{14}-glycine and C^{14}-proline to the coral *Pocillopora damicornis* and found that: (i) the coral was efficient in taking up the label from the environment, as 90 percent of the amino acids was found in coral tissue within 4 hours after the incubation began; (ii) the amino acid pool of the coral could be tapped by the endosymbiotic zooxanthellae; and (iii) the pattern of proline uptake by the free zooxanthellae was like that of the algae *in situ*.

Symbiotic algae are thought by some to play a role in the calcification process of corals, because reef-forming corals contain endosymbiotic algae. It had been demonstrated previously that environmental carbonate contributes to the formation of $CaCO_3$ in the coral skeleton. V. Buchsbaum (Stanford University) was able to provide direct evidence that significant skeletal carbonate originated from the CO_2 produced by coral metabolism. She did this by feeding the coral polyps mouse tissue labeled with C^{14}-amino acids and, after varying intervals, measuring for label in the coral skeleton carbonate.

Three other approaches toward understanding the calcification process were undertaken. One, by S. Young (University of California, Los Angeles), concerned an analysis of the matrices of different decalcified coral skeletons. Such work is especially interesting because some biologists believe that the matrix plays an important role in controlling the types of calcification that take place. His results with one coral, *Pocillopora*, agreed with previous findings that chitin is a major component of the matrix. He did not find chitin in any of the other five corals examined, however. Instead, the components appeared to be proteinaceous; the matrices of two corals, *Pavona* and *Cyphastrea*, showed only a single prominent amino acid on two-dimensional chromatography.

To determine the early patterns of the initial skeleton laid down, A. Reed (visiting instructor, Michigan State University) developed methods for rearing polyps from the settling of *Pocillopora damicornis* planulae (Fig. 1). Application and modification of his methods may also provide means for investigating the uptake of calcium-45 in stages of *Pocillopora* before and during calcification, and for obtaining matrix free both of tissue and of calcium deposits.

The studies of C. Clausen (Loma Linda University) may aid our understanding of the calcification process, as well as provide experimental evidence to help explain why luxuriant reef formations usually occur in areas where the water temperature is 18°C or higher. After working out a simple way for relating calcification rate to the mass of coral tissue, Clausen showed that calcification rate (uptake of calcium-45) increased logarithmically between 15° and 28°C. The activation energy was unusually high, 33,000 to 43,000 calories per mole. As might be expected, the calcification rate declined at a temperature over 30°C. But, most significantly, the rate at 12° and 15°C was extremely low and the coral tissue began to disintegrate after a few hours at those temperatures. Hence, such experiments show that one of the factors which may account for the unique geo-

graphical distribution of coral reefs is the high activation energy of the calcification process; a slight increase in temperature results in a great increase in calcification rate. The relatively high temperature for "cold death" of coral tissue may be a result of the association of the tissue with the calcified skeleton, or may be a secondary adaptation to warmer climes.

The unusual distribution among the coelenterates of two enzymes of carbohydrate metabolism was the subject of the final report (D. Powers, University of Kansas). Following the recent discovery in hydra by C. Rutherford (University of Miami) that glucose-6-phosphate dehydrogenase activity (G6PDH) is abundant and that 6-phosphogluconate dehydrogenase activity (6PGDH) is absent, Powers surveyed over 20 marine coelenterates for these two enzyme activities. He found that extracts of all hydroids tested (marine or fresh-water) had G6PDH, but lacked 6PGDH. Extracts of all other coelenterates tested, except the scyphozoans, had both activities. Two of three scyphozoans which were tested lacked detectable 6PGDH. Other enzyme activities that might be involved in the metabolism of 6-phosphogluconate (6PG) or of gluconic acid were assayed and found to be absent in hydroids. The hydroids and scyphozoans could degrade 6PG, however, presumably by the 6PG phosphatase activity described in hydra by Rutherford (in preparation).

The results presented at the formal and final session of the training program gave promise that more intensive application of a wider variety of experimental techniques to marine organisms will open new avenues for research in many virtually unexplored areas of experimental marine biology.

This program was supported by a grant (GB-6134) from the Facilities and Special Programs Section of the National Science Foundation awarded to Philip Helfrich and Vernon Brock of the Hawaii Institute of Marine Biology. The instructors were the undersigned. Visiting instructors were R. Mariscal (University of Miami) and A. Reed (Michigan State University). Lectures on Hawaiian lore were given by Kaupena Wong (Bishop Museum). Summary of the research will be published in a monograph, *Experimental Coelenterate Biology*, by University of Hawaii Press. The program for the summer of 1968 will be in molluscan biology, and the instructors will be M.

Wells (Cambridge University, England), V. Fretter (Reading University, England), A. Kohn (University of Washington), and A. H. Kay (University of Hawaii).

HOWARD M. LENHOFF
Laboratory for Quantitative Biology, University of Miami, Coral Gables, Florida 33124

LEONARD MUSCATINE
Department of Zoology, University of California, Los Angeles 90024

LARY V. DAVIS
Department of Zoology, University of Hawaii, Honolulu 96822

References and Notes

1. R. Blanquet and H. M. Lenhoff, *Science* **154**, 152 (1966).
2. W. F. Loomis, *Ann. N.Y. Acad. Sci.* **62**, 209 (1955).
3. C. Fulton, *J. Gen. Physiol.* **46**, 823 (1963).
4. L. Muscatine and H. M. Lenhoff, *Science* **142**, 956 (1963).
5. Contribution No. 288 of the Hawaii Institute of Marine Biology.

Calendar of Events

National Meetings

June

16–20. American Soc. of **Ichthyologists and Herpetologists**, Ann Arbor, Mich. (D. Tinkle, Museum of Zoology, Univ. of Michigan, Ann Arbor 48104)

16–21. American Soc. of **Parasitologists**, 43rd annual, Madison, Wis. (G. W. Hunter, Dept. of Microbiology, Univ. of Florida Medical School, Gainesville 32601)

16–21. **Weights and Measures**, 53rd natl. conf., Washington, D.C. (M. W. Jensen, National Bureau of Standards, Washington, D.C. 20234)

17–19. American **Marketing** Assoc., Philadelphia, Pa. (The Association, 230 N. Michigan Ave., Chicago, Ill. 60601)

17–19. American **Neurological** Assoc., Washington, D.C. (M. D. Yarh, 710 W. 168 St., New York 10032)

17–19. **Biomedical Engineering**, San Diego, Calif. (D. L. Franklin, Scripps Clinic and Research Foundation, La Jolla, Calif.)

17–19. American **Physical** Soc., Los Alamos, N.M. (W. W. Havens, 528 W. 120 St., New York 10027)

17–20. American **Dairy Science** Assoc., Columbus, Ohio. (C. Cruse, Executive Secretary, 903 Fairview Ave., Urbana, Ill. 61801)

17–21. **Automating and Miniaturizing Government Records**, Washington, D.C. (Director, Center for Technology and Administration, American Univ., 2000 G St., NW, Washington, D.C. 20006)

18–21. American Soc. of **Agriculture Engineers**, 61st annual, Logan, Utah. (The Society, P.O. Box 229, St. Joseph, Mich. 49085)

19–21. **Analytical Chemistry**, University Park, Pa. (A. T. Winstead, National Meet-