The chemical nature of the binding between actinomycin D and thyroxine (or that between actinomycin D and deoxyguanosine) is not known. The absorption spectrum of actinomycin D maximally bound to deoxyguanosine was not altered by the addition of thyroxine. This suggests that actinomycin D binds thyroxine and deoxyguanosine in a similar manner. Spectrophotometry indicates that there is no interaction between thyroxine and deoxyguanosine.

The spectrophotometric data support the observation that thyroxine can reverse the growth-inhibitory effect of actinomycin D on *Bacillus subtilis*. Actinomycin D (0.1 μg/ml) inhibited the growth of bacteria completely, whereas the culture inoculated with medium containing thyroxine (200 μg/ml) and actinomycin D (0.1 μg/ml) grew normally (Fig. 3). However, the same concentration of thyroxine had no effect when we used 0.25 μg of the antibiotic per milliliter. No further significant changes were observed in the
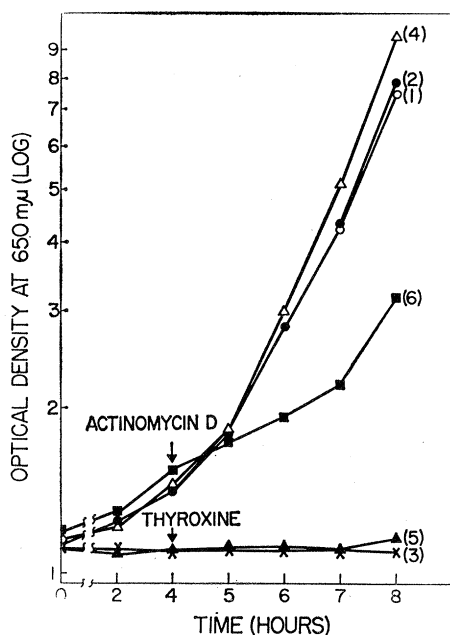

Fig. 3. Effect of actinomycin D and thyroxine on the growth of *Bacillus subtilis*, which was grown (at 35°C in a shaker) to 1 optical-density unit at 650 mμ in a minimal medium composed of salts and glucose. Stock culture (2 ml each) was then inoculated into the following media, and the growth followed. Curve 1, minimal medium; curve 2, thyroxine (200 μg/ml) in minimal medium; curve 3, actinomycin D (0.1 μg/ml) in minimal medium; curve 4, actinomycin D and thyroxine in minimal medium; curve 5, actinomycin D in minimal medium (thyroxine added after 1 hour of incubation of the culture); curve 6, thyroxine in minimal medium (actinomycin D added after 1 hour of incubation of the culture).

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spectrum of actinomycin D solution at a molar ratio (approximately 50) of thyroxine to actinomycin D. The reason or reasons for the requirement of a molar ratio greater than 50 to reverse the growth-inhibitory effect of actinomycin D are not clear, although the binding to other compounds and degradation of thyroxine by bacteria are probably major factors. Delayed administration of actinomycin D to a thyroxine-treated culture produced a lesser degree of inhibition. Thyroxine had no effect on cultures treated with actinomycin D. This agrees with the observation that DNA binds actinomycin D about 10 to 20 times more effectively than it binds deoxyguanosine (1). Therefore, any actinomycin D bound to cellular DNA is not available for binding with thyroxine. Thyroxine exerts no effect on the growth of this bacterium. Apparently, the complex of actinomycin D and thyroxine is unable to penetrate the cell membrane.

Our study may explain the observation that uptake of I^{125}-L-thyroxine by human kidney epithelial cells (T-1) was decreased by actinomycin D (8). Hence, it is important to examine the degree of interaction between thyroxine and actinomycin D in such a system.

A major portion of the labeled actinomycin D administered to tadpoles was associated with the nuclei, and most of the measurable activity was recovered in the chromatin fraction. The binding of actinomycin D to tadpole liver chromatin in vitro was not inhibited by thyroxine, or vice versa. This suggests that their binding sites are not the same (9); thyroxine binds mainly with the nuclear membrane fraction, whereas actinomycin D binds to DNA (10).

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Heliacus (Gastropoda: Architectonicidae) Symbiotic with Zoanthiniaria (Coelenterata)

Abstract. *Eight species of* Heliacus *are obligate symbionts with* Zoanthus *and* Palythoa, *colonial zoanthid sea anemones. The gastropods live among* Zoanthus *polyps and under the edges of* Palythoa *incrustations, on which they feed and to which they and their egg masses are attached with sturdy but elastic mucous threads.*

Eight species in the marine gastropod genus *Heliacus* are symbiotic with *Zoanthus* and *Palythoa*, colonial zoanthiniarian sea anemones of the family Zoanthidae. *Heliacus* comprises about 30 Recent tropical and warm temperate species in the Architectonicidae (1, 2), a family of mesogastropod prosobranchs with opisthobranch affinities. Hitherto, obligate symbiosis was unknown in the Architectonicidae. Other prosobranch symbionts with coelenterates are the families Epitoniidae, Janthinidae, Ovulidae, and Coralliophilidae, whose post-larval stages all live with and feed on coelenterates (3). Little is known as to why these prosobranchs are not deterred by nematocysts.

In the tropics, *Zoanthus* and *Palythoa* are abundant, sessile animals on rock substrata in shallow water. *Palythoa* lives where wave action is strong, such as reefs. At some places, *Palythoa* incrusts as much as 90 to 95 percent of the substratum (4). Architectonicids are seldom observed in situ, and population densities of *Heliacus* with zoanthids are usually low (Table 1). The abundance of *Heliacus* must be regulated by factors other than the abundance of zoanthids because *Heliacus* can be scarce where zoanthids are luxuriant. Also, the symbiosis with *Zoanthus* and *Palythoa* cannot occur in all species of *Heliacus* because there are several that live in the Mediterranean (2), where the Zoanthidae do not occur but where other zoanthiniarian families do (5).

Biologically, architectonicids are not well known. They have a very long, invaginable, acrembolic proboscis, with the true mouth at its everted tip; they also have jaws and a radula in a small buccal mass, large salivary glands, and a peculiar excretory organ (6). There are no data on the feeding habits of such genera as *Architectonica* and *Philippia*, whose radulae differ greatly from those of *Heliacus*.

In the literature on *Heliacus* there are nine observations on the habitats of six species. Four observations mention associations with zoophytes and corals, vague designations that include zoanthids. Long ago in the West Indies, *H. cylindricus* (Gmelin) was found "inserted in slimy zoophytes on rocks about low-water mark" (7). In the Hawaiian Islands, *H. variegatus* (Gmelin) and *H. mighelsi* (Philippi) were "found almost invariably on branched coral, . . . suspend[ing] themselves by strong gelatinous threads" (8). At Boynton Beach, southeast Florida, *H. bisulcatus* (Orbigny) was "characteristically . . . closely associated with living coral polyps in shallow water" (9). Two observations mention a *Heliacus* with "ascidians." At Carmen Island and near Guaymas, Gulf of California, *H. bicanaliculatus* (Valenciennes) has twice been reported occurring intertidally with a short, olive-green, compound ascidian (10). *Zoanthus* resembles some ascidians, and, as expected and already noted in a paper by Robertson and Merrill on abnormal coiling of *Heliacus* (11), *H. bicanaliculatus* does live with *Zoanthus*. Two observations do not mention other animals. At Cap Vert, Senegal, *H. perrieri* (Rochebrune) was in spray and foam on boulders (12). In the Florida Keys, *H. cylindricus* was mainly upside down in a muddy habitat (13).

The new data (Table 1) show that eight species of *Heliacus*, including all those mentioned above except *H. bisulcatus*, occur either with the genus *Zoanthus* or with *Palythoa* (14). There is no indication that postlarval *Heliacus* ever lives far from zoanthiniarians, although in the Hawaiian Islands several *H. trochoides* and *H. variegatus* have been found about a meter from the nearest zoanthid. The observations in Table 1 were made in all months of the year except September through November. The symbiosis occurs intertidally and to a depth of at least 9 m.

Host specificity between *Heliacus* and the various species of *Zoanthus* and *Palythoa* is low. Three or four *Heliacus* species are known to occur with more than one zoanthid species, including *H. cylindricus* with one species of *Zoanthus* and two of *Palythoa*. Some of the shell variability of *H. cylindricus* correlates with the generic identity of the host: large-shelled specimens (up to 14 mm high) that are abraded and brownish have been found only with *Zoanthus*, while those found with *Palythoa* are all smaller (less than 7 mm high), unabraded, and patterned with cream-white and brown. The different growth habits of *Zoanthus* and *Palythoa* account partly for these differences. *Zoanthus* has projecting polyps and a thin basal coenenchyme, while the shorter polyps of *Palythoa* scarcely project above a thicker coenenchyme. With *Zoanthus*, *Heliacus* lives among the projecting polyps where the shells are exposed to abrasive wave action; with *Palythoa*, *Heliacus* is protected under the incrustations in restricted spaces.

The following observations pertain to postlarval *Heliacus cylindricus* and *H. trochoides* with *Palythoa* colonies. *Heliacus* occurs in widely spaced clusters of two or three different-sized animals under the edges of loosely attached (old?) incrustations. Occasionally, *Heliacus* occurs singly, or on top of a colony, or nearby. *Heliacus* animals are fairly sedentary and usually are attached to their hosts and to the substratum with remarkably sturdy but elastic mucous threads. When *Heliacus* does crawl, the conical operculum projects into the umbilicus of the shell.

Table 1. Data on *Heliacus* symbiotic with zoanthids.

Heliacus specimens (No.)	Zoanthid hosts (14)	U.S.N.M. catalog No. (hosts)	Localities
		Heliacus bicanaliculatus (Valenciennes)	
7+	*Zoanthus danai* (Le Conte)	52285	South side Bahía San Luis Gonzaga, Gulf of California
		Heliacus cylindricus (Gmelin)	
5	*Palythoa caribaeorum* Duchass. and Mich.	51029	Entrance Point, North Bimini, Bahama Islands
1	*Palythoa caribaeorum* Duchass. and Mich.		North Rabbit Cay, south of southwest end South Bimini, Bahama Islands
4	*Palythoa caribaeorum* Duchass. and Mich.	51681	Bache Shoal, 3 km east of north end Elliott Key, southeast Florida
1	*Palythoa mammillosa* (Ellis and Solander)	52293	Reef east of south end Cay Corker, British Honduras
Many	*Zoanthus pulchellus* (Duchass. and Mich.)	52700	Arasji, northwest Aruba, Netherlands Antilles
		Heliacus discoideus sterkii (Pilsbry and Vanatta)	
5	*Palythoa vestitus* (Verrill)		Reef off Ala Moana Park, Honolulu, Oahu, Hawaiian Islands
		Heliacus implexus (Mighels)	
2*	*Zoanthus confertus* Verrill		4 km south of Koloa, Kauai, Hawaiian Islands
		Heliacus mighelsi (Philippi)	
15	*Zoanthus confertus* Verrill, plus *P. vestitus*		4 km south of Koloa, Kauai, Hawaiian Islands
		Heliacus perrieri (Rochebrune) (12)	
2+	*Zoanthus pulchellus* (Duchass. and Mich.)		Arasji, northwest Aruba, Netherlands Antilles
		Heliacus trochoides (Deshayes)	
4	*Palythoa vestitus* (Verrill)		Reef off Kaoio Point, Oahu, Hawaiian Islands
2	*Palythoa tuberculosa* (Esper)	52606	Reef southeast of Imma Island, North Male Atoll, Maldive Islands
3†	*Palythoa tuberculosa* (Esper)	52607	North end Ongu Island, North Malosmadulu Atoll, Maldive Islands
1	*Palythoa tuberculosa* (Esper)	52608	Northwest end Gan Island, Addu Atoll, Maldive Islands
		Heliacus variegatus (Gmelin)	
Many	*Palythoa vestitus* (Verrill)	52387 & 52388	Reef off Ala Moana Park, Honolulu, Oahu, Hawaiian Islands
4	*Palythoa vestitus* (Verrill)		Reef off Kaoio Point, Oahu, Hawaiian Islands
6	*Zoanthus confertus* Verrill		4 km south of Koloa, Kauai, Hawaiian Islands

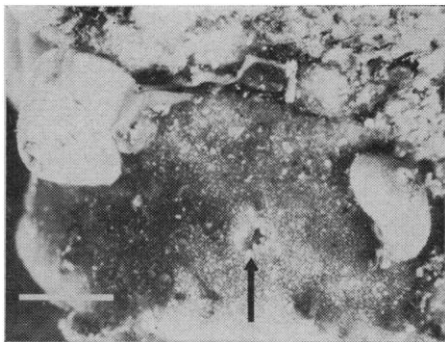*Including one empty shell *in situ*.   †Including two empty shells *in situ*.

Fig. 1. Pair of live *Heliacus trochoides* on under surface of *Palythoa tuberculosa* colony, with hole where one has fed (arrow). North Male Atoll, Maldive Islands. Scale, 3 mm.

When *in situ* the animals are in various positions, including umbilical side up. In the laboratory, feeding and spawning occurred only at night, and no direct observations were made of either activity. However, *Heliacus* does eat *Palythoa* body tissues; 3- to 4-mm deep holes were made overnight in the surface of the colonies (Fig. 1, arrow). Also, the feces of *Heliacus*, which are uncompacted, are the same pale yellowish brown color as *Palythoa* and contain sand grains derived from the sand-containing *Palythoa* tissues. The spawn of *H. cylindricus* is a U-shaped jelly mass that is attached with mucous threads to the host colony.

This case of symbiosis I consider to be ectoparasitism verging on commensalism. *Heliacus* does not harm its host much (the injured tissues heal speedily), and, relative to a host colony, even a full-grown *Heliacus* is small [rarely larger than a single zoanthid polyp (*15*)].

The larval ecology of *Heliacus* is noteworthy because there is at least occasional transoceanic larval transport by ocean currents. Architectonicid larvae can live in the plankton more than 3 months (possibly 7 months or more), and the West Indian architectonicids (including several species of *Heliacus*) occur in shallow water across the entire tropical Atlantic (*16*). The observations in this paper imply that contact with benthic zoanthiniarians is essential for successful settlement and metamorphosis of these *Heliacus* larvae and for their subsequent growth and attainment of sexual maturity.

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14. The species identifications of the zoanthids are provisional because the taxonomy is in disorder (according to C. E. Cutress, who identified them). The catalog numbers of the host specimens at the United States National Museum (U.S.N.M.), Washington, D.C., are listed to aid eventual revision.
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17. My field work was supported by various organizations: in the Bahamas, by the Society of the Sigma Xi and the Lerner Marine Laboratory of the American Museum of Natural History; in Florida, by the Marine Laboratory, University of Miami; in British Honduras, by the Humble Oil and Refining Company in a grant to E. G. Purdy (Rice University); and, in the Maldives, by the National Science Foundation as a part of the U.S. Program in Biology, International Indian Ocean Expedition. For the field observations and specimens from the Hawaiian Islands, I thank Mrs. E. Gage, Mrs. A. M. Harrison, and A. E. Kay; from the Gulf of California, D. R. Shasky; from Aruba, R. T. and S. D. Abbott, and Mrs. J. O. Johnson. For other information, I thank C. E. Cutress, A. M. Keen, T. L. McGinty, A. S. Merrill, and R. S. Scheltema.

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p-Chlorophenylalanine-Induced Chemical Manifestations of Phenylketonuria in Rats

Abstract. p-*Chlorophenylalanine, a potent inhibitor of phenylalanine hydroxylase in vivo, has been used to simulate phenylketonuria in rats. This inhibitor, when administered with phenylalanine, produces marked elevation of blood and tissue phenylalanine without an increase in tyrosine. Disproportionate ratios of phenylalanine to tyrosine are characteristic of phenylketonuria in humans. The use of p-chlorophenylalanine permits the production of this characteristic amino acid imbalance in experimental animals.*

Human phenylketonuria (PKU) is characterized by a genetically linked deficiency of phenylalanine hydroxylase in the liver (*1*). The immediate consequence of this deficiency is the inability of the subject to convert ingested phenylalanine to tyrosine. This is reflected in a markedly increased content of phenylalanine in the blood (*2*) with normal or slightly diminished amounts of tyrosine (*3*). Other consequences of this metabolic defect, such as the elevation of phenylpyruvic acid in blood and urine (*4*) and the lowering of platelet serotonin and urinary 5-hydroxyindole acetic acid (*5*), are apparently secondary to the primary defect.

The need for an experimental model of PKU for metabolic and behavioral studies in laboratory animals is obvious.

However, the production of the condition in animals has met with only limited success. Two techniques have been used: (i) the inclusion of phenylalanine (5 to 7 percent) in the diet (*6*), and (ii) the use of a genetic strain of mice with decreased phenylalanine hydroxylase activity (*7*). More recently it has been shown that hamsters have a much lower level of phenylalanine hydroxylase activity than rats have (*8*) and develop hyperphenylalaninemia when fed diets containing excess phenylalanine [for a detailed review of the various methods see (*9*)].

Although these methods lead to conditions resembling human PKU in that they produce hyperphenylalaninemia, abnormal phenylalanine tolerance curves, and some of the appropri-