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THE INFLUENCE OF LIGHT ON ASEXUAL REPRODUCTION IN GREEN AND APOSYMBIOTIC HYDRA

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The occurrence of symbiotic algae in certain hydra has been known since the description of the first green hydra from southern Russia by Peter Simon Pallas over 200 years ago, but despite numerous studies on green hydra and its algae relatively little is known about the nature of the relationship between the algae and their hosts.

Early studies on the nature of this relationship suggested some contribution of the algae to the nutrition of the hydra, but were carried out with crude culture conditions and generally lacked quantitation (*e.g.*, Whitney, 1907, 1908; Goetsch, 1924; and Haffner, 1925). Recent studies using more effectively controlled culture conditions and improved quantitative techniques have provided more definite evidence of algal contribution to the nutrition of the host hydra (Muscatine, 1965; Muscatine and Lenhoff, 1963, 1965; Muscatine, Karakashian and Karakashian, 1967; Stiven, 1965; and Park, Greenblatt, Mattern and Merrill, 1967). All of these investigations have indicated that the algae in green hydra make no significant contribution to the nutrition of well-fed animals. Most have further indicated that under conditions of starvation the algae may make a positive contribution to the nutrition of the host, although two of the reports (Whitney, 1907; and Park *et al.*, 1967) have shown that green hydra experimentally deprived of their algae (aposymbiotic hydra) reproduce as well as or better than normal green hydra during starvation. As Park *et al.* (1967) have stated, the variety of experimental conditions and strains of animals used in these various studies renders difficult the interpretation of these contradictory results.

Several previous studies on the effects of light have dealt with the effects of light on the behavior of hydra (Singer, Rushforth and Burnett, 1963; Rushforth, 1966), but little attention has been devoted to the effects of light on growth and reproduction. Also, several previous workers have investigated the effects of population density in hydra, but most of these studies have focused on sexual differentiation (Loomis, 1954; Park, Mecca and Ortmeyer, 1961) and floating behavior (Lomnicki and Slobodkin, 1966). Davis (1966) reported a diffusible substance, possibly protein in nature, released into the medium of crowded *Chlorohydra viridissima* and *Hydra littoralis* which influences budding and regeneration of animals cultured in such media.

Two distinct species of green hydra have been described, *Chlorohydra viridissima* (Pallas, 1766) and *Chlorohydra hadleyi* Forrest, 1959. Nearly all the previous studies on the role of symbiotic algae in hydra have been conducted

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with hydra usually designated in recent years as *Chlorohydra viridissima,* but often inadequately described. Until Forrest's recent description of *Chlorohydra hadleyi,* all green hydra were generally assigned to a single species usually called either *Chlorohydra viridissima* or *Hydra viridis.*

As noted previously, certain of the earlier studies have led to some apparently contradictory results. Some of these contradictions may well be due to differences between strains or species of hydra used. The recent work of Park *et al.* (1967) comparing designated strains of the two described species points out a number of distinct morphological and physiological differences between these two green hydras and clearly suggests the possibility of significant differences in the role of algae in the different species. Both Park *et al.,* 1967, and Oschman (1967) provide evidence of morphological differences between the algae of different strains of green hydra.

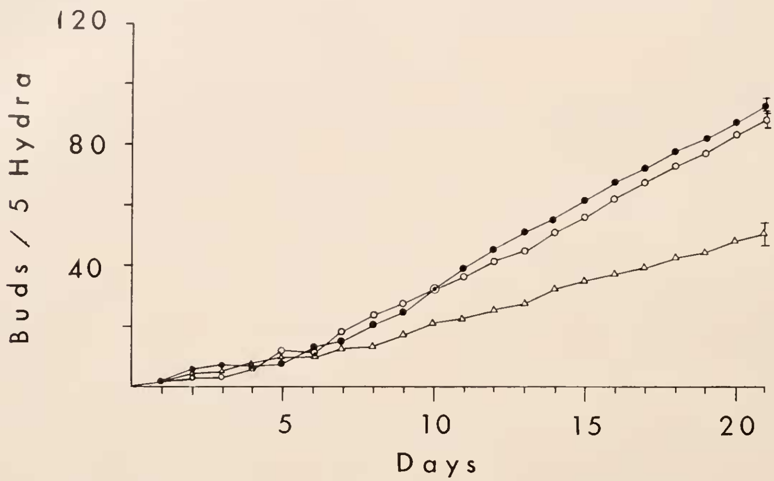

FIGURE 1. Effect of continuous darkness on asexual reproduction of Burnett Green (triangles), Kenilworth Green (closed circles), and Kenilworth Albino hydra (open circles).

The purpose of the present study is to investigate further the interactions between the symbiotic algae and their hosts through an analysis of the effects of light and other factors on asexual reproduction in designated strains of *Chlorohydra viridissima* and *Chlorohydra hadleyi.*

MATERIALS AND METHODS

Two species of green hydra were used in this study: *Chlorohydra viridissima* (Pallas 1766) (Burnett Strain) and *Chlorohydra hadleyi* Forrest, 1959 (Kenilworth Strain). Initial stocks of both strains were obtained from Dr. Helen D. Park of the National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland. Clones were subsequently developed from a single individual of each strain and an aposymbiotic clone was developed from our Kenilworth clone by culturing the green hydra in 0.5% glycerine medium for several weeks (Whitney,

1907) to induce expulsion of the algae. Despite repeated attempts in our laboratory, we were unable to develop a viable culture of aposymbiotic Burnett hydra using glycerine, chloramphenicol, or culture in darkness.

The culture medium (BVS) (modified from Loomis and Lenhoff, 1956) consisted of 100 mg $NaHCO_3$ and 50 mg disodiumethelene-diaminetetraacetate (versene) in a liter of filtered spring water. Stock cultures of several hundred hydra were maintained in three-inch Carolina Culture Dishes containing 50 ml culture medium and kept in foil-lined light tight boxes. Illumination was provided continuously for twelve hours each day (5:00 AM–5:00 PM) by a single fifteen watt fluorescent light (Sylvania Cool-white) placed 10 inches from the top of the culture dishes providing a uniform light intensity of approximately

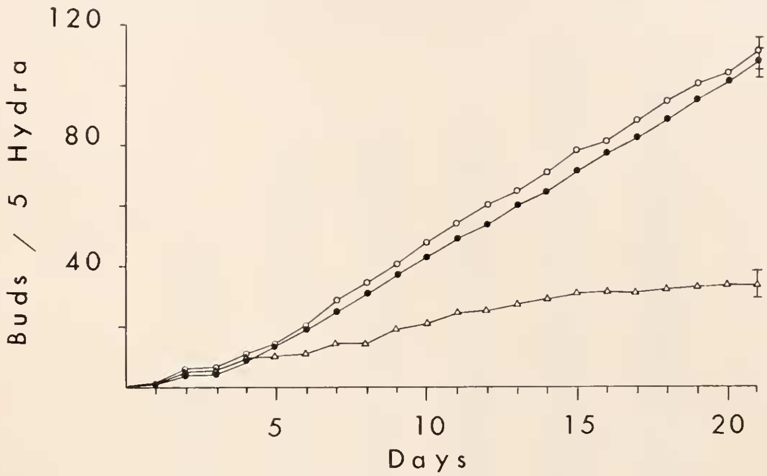

FIGURE 2. Effect of 12 hours light/12 hours darkness on asexual reproduction of Burnett Green (triangles), Kenilworth Green (closed circles), and Kenilworth Albino hydra (open circles).

250 foot candles at the level of the cultures. Temperature was maintained at 20° C ±1°.

Cultures were provided with freshly-hatched specimens of *Artemia* nauplii for a period of one hour on alternate days, allowing the hydra to feed to repletion. The cultures were then rinsed and fresh culture medium provided. On non-feeding days the culture solution was changed, and once each week the culture dishes were cleaned to minimize bacterial contamination. These methods provided an environment under which hydra reproduced exclusively by asexual budding during the course of experimentation. Prior to the experiments cultures of all three strains were maintained for several months under these conditions.

Experimental cultures consisted of five uniform hydra selected from stock cultures; uniform hydra defined by Lenhoff and Bovaird (1961) as animals having a single bud in early stages of development and having been starved for one day. Culture conditions for experimental cultures except as specified in particular experiments were as previously stated for stock cultures with two notable exceptions: Culture medium in those maintained under starvation conditions was changed daily, and the period of illumination in the photoperiod experiment was altered manually. Cultures referred to in the results as having been maintained in continuous darkness actually received 10 minutes of ambient laboratory light per day due to the need for light during feeding and counting.

Reproduction was measured each day by counting and removing buds dropped from the five parent hydra in each culture dish.

Results

Effects of light on budding

Three replicate cultures each of Kenilworth Green, Kenilworth Albino, and Burnett Green hydra were cultured under conditions of continuous darkness,

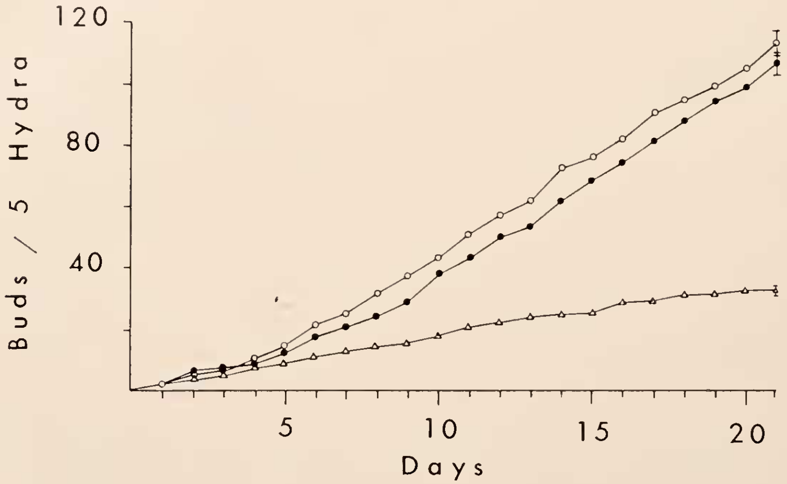

Figure 3. Effect of continuous light on asexual reproduction of Burnett Green (triangles), Kenilworth Green (closed circles), and Kenilworth Albino hydra (open circles).

12 hours light/12 hours darkness, and continuous light for a period of 21 days to investigate the effects of light on budding. Light intensities were maintained at 250 foot candles. As illustrated in Figures 1–3, the Kenilworth Green and Albino hydra produced more buds under all three lighting conditions than the Burnett Green hydra ($P < 0.01$). There was no significant difference in the number of buds produced by the Kenilworth Green and Albino hydra under any of the light regimes ($P > 0.05$). Both produced significantly more buds under continuous illumination and under 12 hours of light and 12 hours of darkness than under continuous darkness ($P < 0.01$).

The Burnett Green hydra, in contrast, produced significantly more buds in continuous darkness than under either illumination condition ($P < 0.01$), but there was no significant difference in the number of buds produced under continuous light and 12 hours of light/12 hours of darkness ($P > 0.05$). The data from this experiment, therefore, do not demonstrate any positive relationship between the algae contained in the gastrodermal cells of the hydra and budding in either the Kenilworth Green or Burnett Green hydra. The increased budding observed in the Kenilworth Green hydra under illumination cannot be attributed to the presence of endodermal algae since the Kenilworth Albino hydra exhibited a similar increase. These results are in basic agreement with those of Muscatine (1961), Muscatine and Lenhoff (1965), Stiven (1965), and Park *et al.* (1967), indicating that there was no difference in the budding of green and aposymbiotic hydra under constant illumination when food was not a limiting factor.

Effect of photoperiod on budding

Since the Kenilworth and Burnett Green hydra were differently affected by exposure to light and darkness, another experiment was undertaken to determine

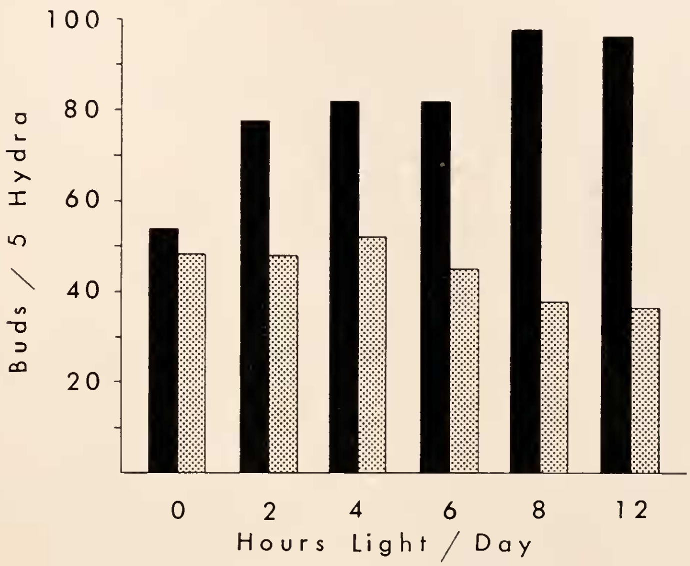

FIGURE 4. Effect of photoperiod on budding in Kenilworth Albino (solid bars) and Burnett Green hydra (stippled bars).

the effects of different photoperiods on budding in the two species. Three replicate cultures each of Burnett Green and Kenilworth Albino hydra were grown under continuous darkness and with 2 hours light (11:00 AM–1:00 PM), 4 hours light (11:00 AM–3:00 PM), 8 hours light (5:00 AM–1:00 PM), and 12 hours light (5:00 AM–5:00 PM) per day at an intensity of 250 foot candles for a period of 21 days.

The Burnett Green hydra produced the greatest number of buds from 0–4 hours of light per day and a significantly smaller number of buds from 6–12 hours of light per day (Fig. 4, Table I). The Kenilworth Albino hydra produced the greatest number of buds with the longest photoperiods employed (8 and 12 hours of light) and produced the smallest number of buds under constant darkness (Fig. 4, Table II). No significant difference in budding was observed between

Table I

Summary of Duncan's Multiple Range Test comparing budding of Burnett Green hydra under different photoperiods

Hours light	0	2	4	6	8	12
0	—	ns	ns	ns	1	1
2	ns	—	ns	ns	1	1
4	ns	ns	—	5	1	1
6	ns	ns	5	—	1	1
8	1	1	1	1	—	ns
12	1	1	1	1	ns	—

1—$P < 0.01$
5—$P < 0.05$
ns—not significant

Table II

Summary of Duncan's Multiple Range Test comparing budding of Kenilworth Albino hydra under different photoperiods

Hours light	0	2	4	6	8	12
0	—	1	1	1	1	1
2	1	—	ns	ns	1	1
4	1	ns	—	ns	1	1
6	1	ns	ns	—	1	1
8	1	1	1	1	—	ns
12	1	1	1	1	ns	—

1—$P < 0.01$
5—$P < 0.05$
ns—not significant

2–6 hours of light with the Kenilworth Albino hydra. Thus the results of this experiment tend to confirm the results of the previous experiment and furthermore, these results demonstrate that budding tends to decrease with increasing exposure to light in the Burnett Green hydra but tends to increase with increasing light in the Kenilworth Albino hydra.

Effects of light intensity on budding

After it was found that Kenilworth Green hydra produced more buds and the Burnett Green hydra produced fewer buds with longer photoperiods, we designed two experiments to investigate the influence of increased light intensity on budding in the two species. In the first experiment three replicate cultures of Burnett Green, Kenilworth Green, and Kenilworth Albino hydra were maintained under constant illumination of 3200 foot candles provided by two 15-watt Sylvania Cool-white fluorescent bulbs located four inches from the top of the culture dishes. The culture dishes were placed in a foil-lined cardboard box to increase the uniformity of illumination and increased ventilation was provided to prevent temperature increase due to the proximity of the lights. Temperature was maintained at 20 ±1° C as in the other experiments.

The results of this increased light intensity indicated a further reduction in the budding of the Burnett Green hydra which produced a total of only eight buds per culture, all of which were produced during the first five days of the 21-day experiment (Fig. 5). The Kenilworth Green and Albino hydra produced 99.3 ± 3.33 hydra per culture and 95.0 ± 3.41 hydra per culture, respectively, during the 21-day period and continued budding at a relatively constant rate throughout the experiment. The difference in budding between the Kenilworth Green and Albino hydra was found not to be statistically significant ($P > 0.05$).

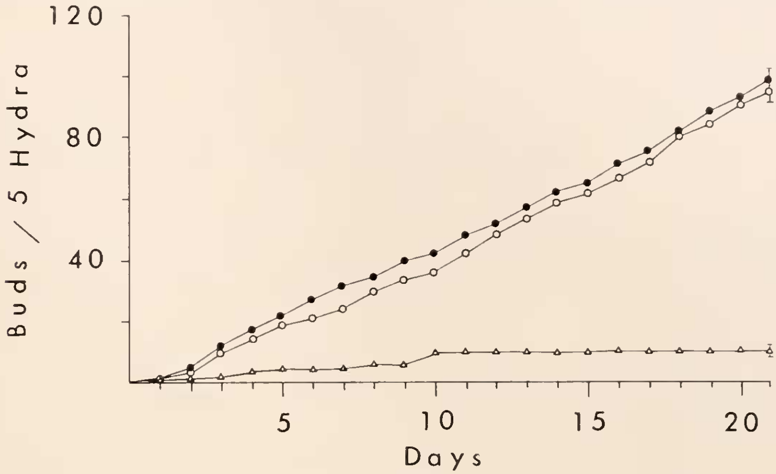

FIGURE 5. Effect of continuous light of 3200 foot candles on asexual reproduction in Burnett Green (triangles), Kenilworth Green (closed circles), and Kenilworth Albino hydra (open circles).

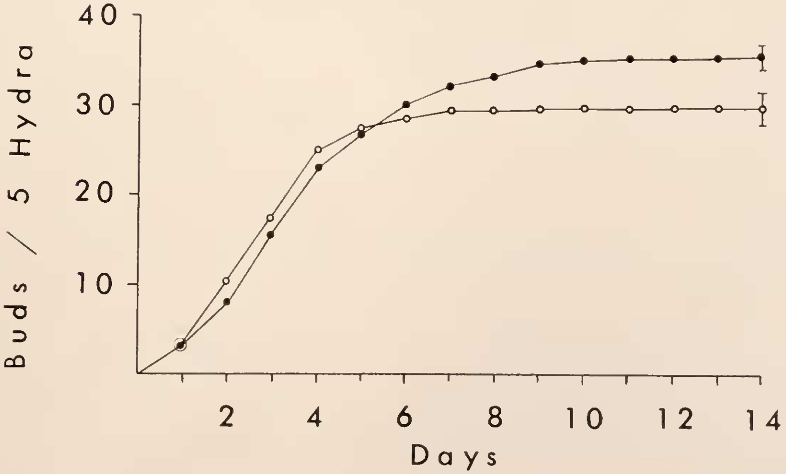

FIGURE 6. Effect of continuous light of 3200 foot candles on asexual reproduction in Kenilworth Green (closed circles) and Kenilworth Albino hydra (open circles) during starvation.

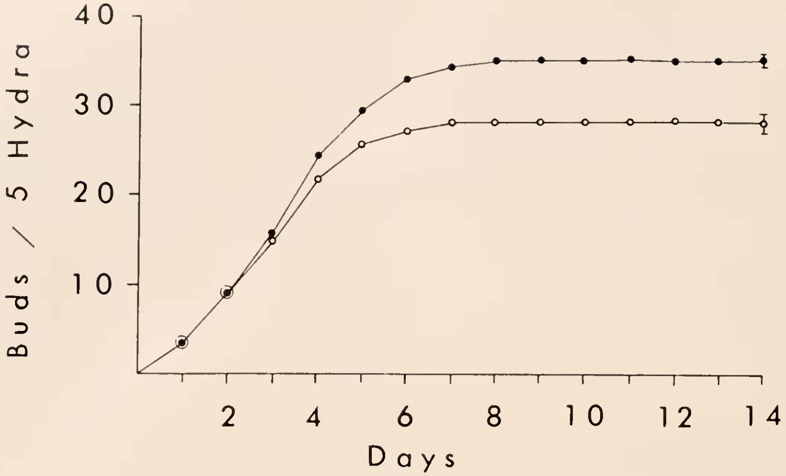

FIGURE 7. Effect of continuous light of 250 foot candles on asexual reproduction in Kenilworth Green (closed circles) and Kenilworth Albino hydra (open circles) during starvation.

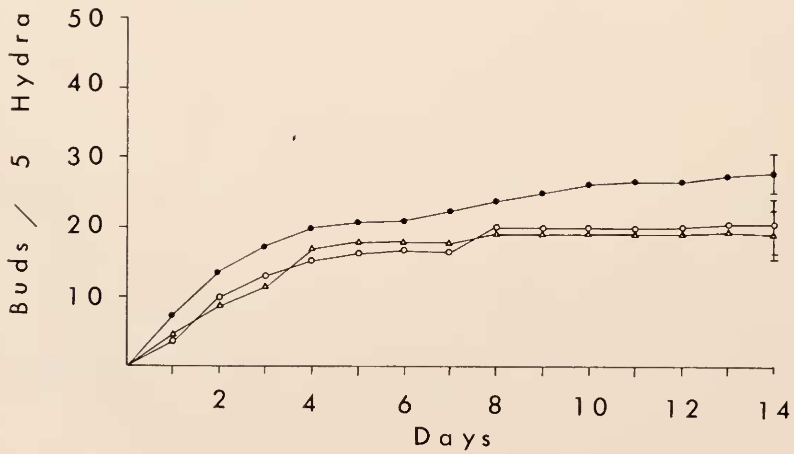

FIGURE 8. Effect of continuous darkness on Burnett Green (triangles), Kenilworth Green (closed circles), and Kenilworth Albino hydra (open circles) during starvation after previous exposure to uncrowded conditions.

Another experiment designed to test the effects of light intensity on budding in green versus albino hydra was conducted with Kenilworth Green and Kenilworth Albino hydra under conditions of starvation for a 14-day period. Three replicate cultures of each strain were cultured under 250 foot candles (Fig. 6) and 3200 foot candles (Fig. 7) of fluorescent illumination. These hydra exhibited a significant difference in the number of buds produced by the green and albino hydra at both intensities ($P < 0.05$), but no significant difference in the number of buds produced by corresponding hydra at the two light intensities ($P > 0.05$).

Therefore these results indicated that under starvation conditions algae make a positive contribution to budding, but that there was no significant difference in the algal contribution in hydra exposed to 250 and 3200 foot candles.

Effect of light and starvation on budding

Since previous results had demonstrated that budding is influenced by both light and starvation, an experiment was conducted to explore the effects of different light regimes under starvation conditions in the three strains of hydra. Three

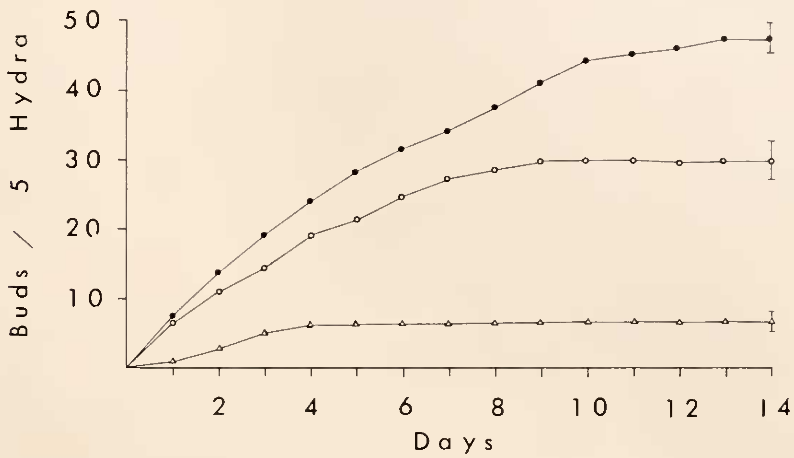

FIGURE 9. Effect of 12 hours light/12 hours darkness on asexual reproduction in Burnett Green (triangles), Kenilworth Green (closed circles), and Kenilworth Albino hydra (open circles) during starvation after previous exposure to uncrowded conditions.

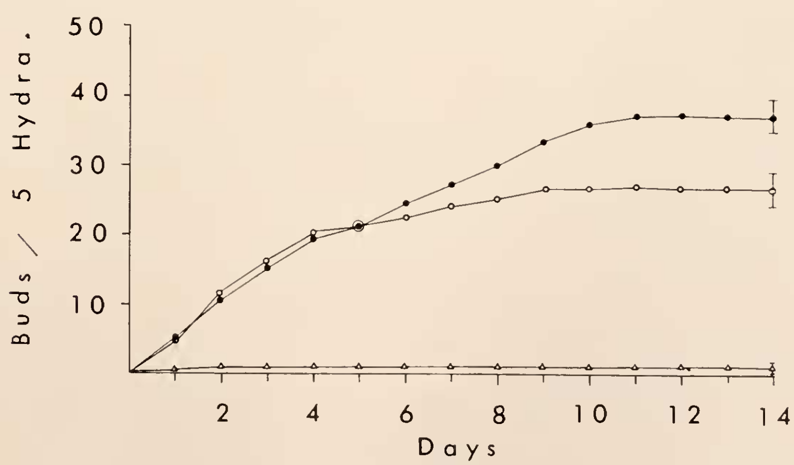

FIGURE 10. Effect of continuous light on asexual reproduction in Burnett Green (closed circles), and Kenilworth Albino hydra (open circles) during starvation after previous exposure to uncrowded conditions.

replicate cultures each of Burnett Green, Kenilworth Green, and Kenilworth Albino hydra were maintained under conditions of continuous darkness, 12 hours of light/12 hours of darkness, and continuous light, and fed on alternate days for 21 days. At the end of this period feeding was discontinued and observations made of the survival and bud production in each of the three strains. Observations were continued for 14 days.

Survival of 100% was observed in all three strains, but significant differences in budding were recorded as shown in Figures 8–10. Under 12 hours light/12 hours darkness the Kenilworth Green and Kenilworth Albino hydra exhibited the greatest budding, producing 47.0 ± 2.20 buds per culture and 29.0 ± 2.99 buds per culture respectively, and continued to produce buds over the longest period of time. Under continuous darkness the inverse was observed. With continuous light the Kenilworth Green and Albino hydra produced fewer buds, but under all three light regimes budding by the Kenilworth Green hydra was significantly higher than that of the Kenilworth Albino hydra (Table III, $P < 0.01$).

Budding in the Burnett Green hydra was greatest in continuous darkness where 19.0 ± 2.69 buds per culture were produced, and was the least under continuous light where only 1.6 ± 0.33 buds per culture were obtained. Under 12 hours

TABLE III

Summary of Duncan's Multiple Range Test comparing budding of three strains of hydra from crowded and uncrowded cultures during starvation and under three different light regimes

		12 hrs light/12 hrs dark						Continuous light						Continuous dark					
		B	G	A	b	g	a	B	G	A	b	g	a	B	G	A	b	g	a
12 hrs light/12 hrs dark	B	—	1	1	1	1	5	5	1	1	1	1	1	1	1	1	ns	1	1
	G	1	—	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	A	1	1	—	1	ns	1	1	1	ns	1	ns	1	1	ns	1	1	1	1
	b	1	1	1	—	1	ns	1	1	1	ns	1	ns	ns	1	5	1	ns	ns
	g	1	1	ns	1	—	1	1	1	ns	1	ns	1	1	ns	5	1	1	1
	a	5	1	1	ns	1	—	1	1	1	ns	1	ns	5	1	1	ns	ns	ns
Continuous light	B	5	1	1	1	1	1	—	1	1	1	1	1	1	1	1	1	1	1
	G	1	1	1	1	1	1	1	—	1	1	1	1	1	1	1	1	1	1
	A	1	1	ns	1	ns	1	1	1	—	1	ns	1	1	ns	5	1	1	1
	b	1	1	1	ns	1	ns	1	1	1	—	1	ns	5	1	1	5	ns	ns
	g	1	1	ns	1	ns	1	1	1	ns	1	—	1	1	ns	1	1	1	1
	a	1	1	1	ns	1	ns	1	1	1	ns	1	—	ns	1	5	1	ns	ns
Continuous dark	B	1	1	1	ns	1	5	1	1	1	5	1	ns	—	1	ns	1	ns	ns
	G	1	1	ns	1	ns	1	1	1	1	1	ns	1	1	—	ns	1	1	1
	A	1	1	1	5	5	1	1	1	5	1	1	5	ns	1	—	1	ns	5
	b	ns	1	ns	1	1	ns	1	1	1	5	1	1	1	1	1	—	1	5
	g	1	1	1	ns	1	ns	1	1	1	1	ns	ns	ns	1	ns	1	—	ns
	a	1	1	1	ns	1	ns	1	1	1	ns	1	ns	ns	1	5	5	ns	—

Crowded hydra
B — Burnett Green strain
G — Kenilworth Green strain
A — Kenilworth Albino strain

Uncrowded hydra
b — Burnett Green strain
g — Kenilworth Green strain
a — Kenilworth Albino strain

5 — $P < 0.05$
1 — $P < 0.01$
ns — not significant

light/12 hours darkness the Burnett Green hydra produced 6.6 ± 1.65 buds per culture.

Effects of crowding on budding

Since the animals in the previous experiment had been subjected to different light regimes prior to the period of starvation by nature of the experimental design, a similar experiment was conducted with animals having a common exposure to light during the period immediately prior to starvation. Three replicate cultures of Burnett Green, Kenilworth Green, and Kenilworth Albino hydra were set up using animals taken directly from stock cultures and grown under three

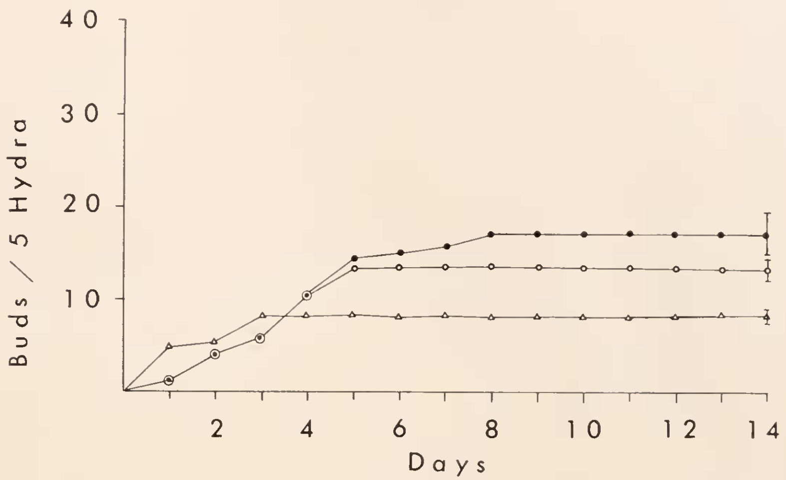

FIGURE 11. Effect of continuous darkness on asexual reproduction in Burnett Green (triangles), Kenilworth Green (closed circles), and Kenilworth Albino hydra (open circles) during starvation after previous exposure to crowded conditions.

different light regimes: constant darkness, 12 hours light/12 hours darkness, and continuous light. Bud production was recorded during a period of 14 days of starvation. These cultures therefore differed in two principal ways from those used in the previous experiment: (1) They had a common history of light exposure for several weeks prior to starvation (12 hours light/12 hours darkness), and (2) they came from stock cultures with a relatively high population density (several hundred hydra per three-inch Carolina Culture Dish) rather than from cultures with a lower population density (five hydra per dish).

The results of this experiment, illustrated in Figures 11–13, indicate that during the 14-day starvation period the Kenilworth Green hydra produced the greatest number of buds in each environment. Budding was significantly higher under continuous light and 12 hours light/12 hours darkness than under continuous darkness (Table III, $P < 0.01$), but no significant difference in budding was observed between constant light and 12 hours light/12 hours darkness ($P > 0.05$). The Kenilworth Albino hydra showed no significant difference in the number of buds produced under any of the three light regimes ($P > 0.05$).

The Burnett Green hydra produced fewest buds under continuous darkness but significantly more buds under both conditions of illumination (Table III, $P < 0.05$). There was no significant difference in budding between the animals grown under continuous light and under 12 hours of light/12 hours darkness ($P > 0.05$). The Burnett Green hydra therefore exhibited a different pattern of budding than that observed in the previous experiments where budding was greatest under constant darkness.

These data indicate that previous exposure to high population densities (crowding) affects the asexual reproduction of green hydra exposed to starvation. Comparing the data from this and the previous starvation experiments, the corresponding Kenilworth Green and Albino hydra produced fewer buds (Table III, $P < 0.05$) subsequent to exposure to crowding conditions than when grown in low density cultures. This decreased budding in hydra exposed to crowding conditions suggests the presence of a lowered metabolic reserve available for budding in these animals. The absence of a significant difference in the budding of Kenilworth Green and Albino hydra starved in continuous darkness indicated the presence of similar levels of metabolic reserves available for budding (Fig. 11). Since the animals were maintained in darkness during the period of experiment, the algae were producing no photosynthetic products which might contribute to budding. In the cultures exposed to light, however, the budding of both types of green hydra, Burnett Green and Kenilworth Green, was significantly greater than under constant darkness (Table III, $P < 0.05$). Furthermore, the budding of the Kenilworth Green hydra was approximately double that of the Kenilworth Albino hydra (Figs. 11–13).

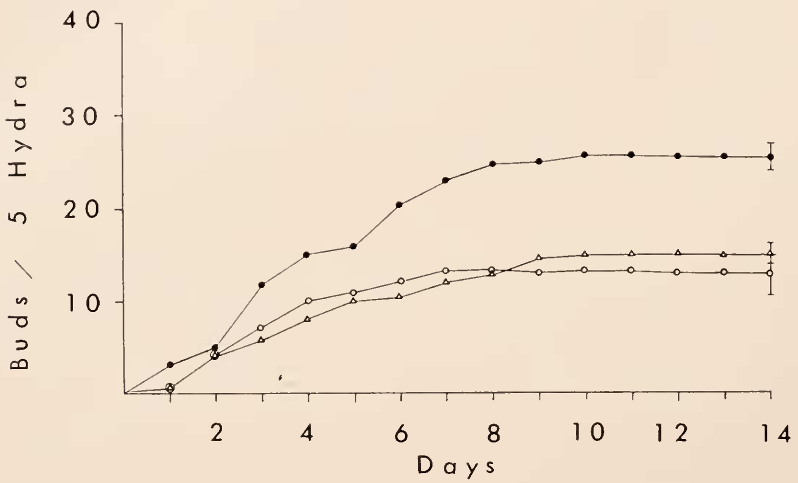

FIGURE 12. Effect of 12 hours light/12 hours darkness on asexual reproduction in Burnett Green (triangles), Kenilworth Green (closed circles), and Kenilworth Albino hydra (open circles) during starvation after previous exposure to crowded conditions.

Exposure to the crowding in the Burnett Green hydra had an effect similar to that on the Kenilworth Green hydra; budding was reduced in animals maintained in the dark as compared to animals under either lighted regime.

Discussion

The results of these experiments demonstrate that light exerts an important influence on budding in both green and albino hydra and that the influence of light is not restricted to its effect on the symbiotic algae of the green hydra. These experiments have shown that budding is the same in both Kenilworth Green and Albino hydra under a wide range of lighting conditions, and that both

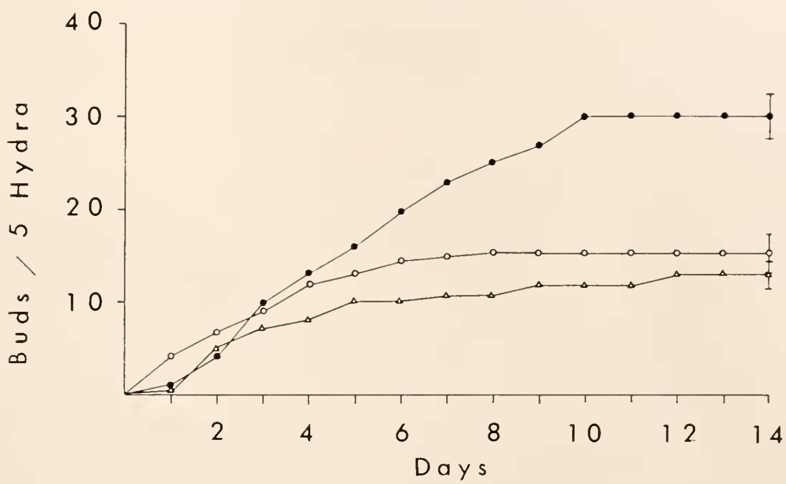

FIGURE 13. Effect of continuous light on asexual reproduction in Burnett Green (triangles), Kenilworth Green (closed circles), and Kenilworth Albino hydra (open circles) during starvation after previous exposure to crowded conditions.

green and albino hydra exhibit large increases in bud production in lighted cultures (Figs. 1–3). This observation clearly indicates that some factor other than symbiotic algae stimulates budding in the albino hydra and obviously suggests that a similar mechanism may also contribute to the increased budding of green hydra exposed to light.

The Burnett Green hydra consistently exhibited lower bud production than the Kenilworth Green or Albino under corresponding conditions, indicating a lower reproductive capacity in this hydra. The most striking difference between the Burnett and Kenilworth hydras is the pronounced difference in the influence of light on budding in the two hydras. Increasing light exposure invariably stimulated budding in the Kenilworth Green and Albino hydras; in contrast, increasing light generally had an inhibitory effect on budding in the Burnett Green hydra.

Well-fed Burnett Green hydra produced fewer buds in constant light and under 12 hours light/12 hours darkness than in constant darkness (Figs. 1–3). Increasing the light intensity from 250 to 3200 foot candles stopped budding in the Burnett Green hydra after about five days while the Kenilworth Green and Albino hydra continued budding at a rapid rate to the end of 21 days (Fig. 5). Photoperiods from 4–12 hours resulted in a significant reduction in budding in the Burnett Green hydra, but the Kenilworth Albino hydra showed increasing bud production with longer photoperiods (Fig. 4).

Experiments with starved animals in lighted cultures demonstrated an increased budding in the Kenilworth Green hydra over that of the Kenilworth Albino hydra in accord with the results of several previous workers, including Goetsch (1924), Muscatine and Lenhoff (1965), and Stiven (1965). These previous investigators have all interpreted the greater budding of the green animals as evidence for a contribution of the symbiotic algae to the nutrition of the host; data from the present experiments show that light may also stimulate budding in starved albino hydra (Figs. 8–10). Albino hydra previously exposed to crowded culture conditions, however, showed no significant difference in budding rates with varied illumination (Figs. 11–13).

Crowding also was found to affect the response of Burnett Green hydra to light. Light exposure inhibited budding in starved Burnett Green hydra from uncrowded cultures but resulted in a stimulation of budding in animals from crowded cultures. Davis (1966) demonstrated the accumulation of a diffusible substance, possibly protein in nature, in the medium of crowded *Chlorohydra viridissima* and *Hydra littoralis*. This substance was found to decrease both the rate of budding and the rate and extent of regeneration of experimental animals.

Evidence from the present experiments suggests that budding may be regulated by the available supply of some critical metabolite(s) in the hydra tissues. The hypothesized metabolite appears to be derived both from feeding and from the photosynthetic activity of the symbiotic algae since well-fed Kenilworth Green and Albino hydra show no difference in reproduction (Figs. 1–3) and since light stimulates budding in starved Kenilworth Green hydra (Figs. 8–10). The decreased budding of starved Kenilworth Green and Albino hydra exposed to crowding as compared to Kenilworth Green and Albino hydra from uncrowded cultures (Table III) further suggests that crowding affects the pool size of this hypothesized metabolite, possibly via the diffusable inhibitor demonstrated by Davis (1966) in the medium of crowded cultures. Starved Kenilworth Green animals, with smaller pools of the metabolite, demonstrate the release of the metabolite by the algae through their increased budding in lighted cultures (Figs. 11–13).

The Burnett Green hydra appear to maintain lower pool sizes of the hypothesized metabolite(s) than Kenilworth Green hydra exposed to similar environmental conditions, as reflected in their lower bud production. Furthermore, Burnett Green hydra appear to be very susceptible to increased levels of the metabolite since most of the experiments showed an inhibitory effect of light on budding in this hydra; perhaps in such experiments the combined contribution of the metabolite from feeding and from algal photosynthesis resulted in an effect resembling substrate inhibition.

Burnett Green hydra from crowded cultures, like the Kenilworth Green hydra from crowded cultures, showed evidence of a lowered pool size of the metabolite.

In the case of the Burnett Green hydra, however, this decreased pool size resulted in an apparent reversal in the effect of light on budding of the crowded animals. Thus we postulate that the mechanism of budding and the role of the algae in green hydra in the two strains of green hydra as revealed in these experiments is explainable on a common basis, assuming only quantitative differences in pool sizes and possibly differences in inhibitory levels of the critical metabolites.

Our experiments were not designed to provide evidence regarding the chemical nature of the critical metabolites. The work of Muscatine (1965) and Muscatine *et al.* (1967), however, on algae isolated from other strains of green hydra (Florida Strain 1961, Carolina Strain 1960, and Lenhoff's mutant strain) has demonstrated that these algae liberate significant amounts of maltose and/or glucose into the incubating medium. These results therefore offer one suggestion as to the possible nature of the hypothesized metabolites responsible for the observed differences in budding in our experiments.

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Summary

1. Experiments were conducted with three strains of green and aposymbiotic hydra to study the effects of light on asexual reproduction. Animals used were the Kenilworth Green and Kenilworth Albino (aposymbiotic) strains of *Chlorohydra hadleyi* and the Burnett Green strain of *Chlorohydra viridissima.*

2. Budding of both the Kenilworth Green hydra and the Kenilworth Albino hydra was significantly greater in constant light and with 12 hours light/12 hours darkness than under constant darkness, indicating that some factor other than the green algae promotes budding of albino hydra in lighted cultures.

3. Budding was less in Burnett Green hydra in constant light and 12 hours light/12 hours darkness than under continuous darkness.

4. Budding increased with increasing photoperiods in the Kenilworth Albino hydra but decreased with increasing photoperiods in the Burnett Green hydra.

5. No difference was observed in the budding of Kenilworth Green and Albino hydra at 250 and 3200 foot candles, but budding was greatly reduced in Burnett Green hydra at 3200 foot candles.

6. Kenilworth Green hydra produced more buds during starvation when exposed to constant darkness, 12 hours light/12 hours darkness, and constant light than Kenilworth Albino hydra.

7. Prior exposure to crowding conditions resulted in decreased budding in starved Kenilworth Green and Albino hydra grown in constant darkness, 12 hours light/12 hours darkness, and constant light. Similarly treated Burnett Green hydra, however, showed increased budding under all three light regimes.

8. The results suggest that budding in green and albino hydra is regulated by levels of some undefined critical metabolite which can be derived both from food

and symbiotic algae. Burnett Green hydra appear to maintain smaller metabolic pools of this substance than Kenilworth Green and Albino hydra, and excessive levels of this metabolite tend to inhibit budding in this strain.

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