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# Analysis of Symbiosis in Hydra Viridis

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The green bodies found in Hydra viridis and other animals have long been a puzzling problem to biologists. The study of the phenomenon dates back as far as 1830. This history of the problem and its progress toward definite conclusions is of much interest. D. D. Whitney has given us a detailed sketch from its beginning up to his own work in the introduction to his paper entitled, "Artificial Removal of the Green Bodies of Hydra Viridis." ('07) It will then be unnecessary, in this paper, to give a detailed discussion of this history. It will suffice only to mention some of its more salient features.

Before 1890 there was a great variety of opinions as to the nature and the relation between the animal and green bodies. Some considered them to be a part of the animal organism. With this inference in mind, they concluded that the green bodies must be chloroplasts of the same nature as those found in green plant tissues. Others-more observant-considered them to be separate organisms, and most likely some form of alga. This inference was followed up by two opinions as to the relation of the animal to the alga. One group considered that the alga was parasytic upon the animal, while the

other group considered the two organisms to be living in symbiotic relationship.

Beyerinck, in 1890, showed the animal and the green bodies to be separate organisms, and further showed that the green bodies were able to live outside of the animal's body in an artificial medium. He identified them to be algae-specifically zoochlorellae. On the other hand Whitney has shown that Hydra viridis is able to live without the algae being present within its cells. He did this by removing the algae by means of small percentages of glycerine.

The means whereby the symbiosis between the two organisms is affected has been the subject of a variety of speculations. Some have maintained that there is a mutual exchange of carbon dioxide and oxygen between the two organisms. Others maintained that the animal cells use surplus photosynthetic foods of the algae. Beyerinck and others held that the plant cells themselves may be absorbed in cases of great need on the part of the animal. In respect to food, Whitney has shown that the algae do not furnish sufficient quantities for asexual reproduction of Hydra viridis.

Hence, to summarize the work up thru Whitney, it can be said, concerning Hydra viridis and the green bodies within its cells, that the green bodies and the hydra in which they live are seperate organisms; that each symbiont can be made to live alone by artificial means; that the

green bodies are algae-specifically zoochlorellae; and that since neither seem to be visibly injured by the other, they must be living in some sort of mutual relationship.

In a paper recently published by Professor Kepner and myself entitled, "Reactions of Hydra to Chloretone", we have been able to show that the Hydra viridis is aided in some manner by the algae. We found that green hydras placed in 0.33-1/3% chloretone were more resistant to this percentage of chloretone than were brown and gray hydras placed in the same percentage of the substance. The green were found to elongate and would eventually recover, while the brown and gray would contract and eject a mass of endodermal cells, but would never recover. It was definitely shown that the difference was due to the algae by placing brown, gray and green hydras in complete darkness for two, three, four and five days and then testing them again with 0.33-1/3% chloretone. The green weakened considerably, while the brown and gray showed no change of reactions. In this, therefore, a true symbiosis is indicated.

In attempting to carry the problem beyond the work of Professor Kepner and myself, certain complications were encountered. Some of these seemed to yield results that were contradictory to the conclusions drawn by us. These contradictory results demanded analysis.

It was found in April and May that the green hydra was no longer able to resist chloretone as it had previously

done in March. Instead of elongating in a 0.33-1/3% solution, it would contract and in five to fifteen minutes its ectodermal cells would begin to slough slough off. Some could be found which would first elongate slightly, but eventually the ectodermal cells would sluff off. In no case during this period would the green hydras recover.

This variation of the green hydra in its reactions to chloretone from that observed in the earlier months of the year, was due to a lack of carbon dioxide on the part of the alga. This season of the year, spring, is the part of the year most suitable for the growth of aquatic plants. Therefore, the alga within the cells of the hydra are under a handicap in competing with the other aquatic plants for the limited supply of carbon dioxide. The pond from which hydras were taken for this work was literally green with the various species of aquatic plants.

This being the case, the question was raised; will a sufficient amount of carbon dioxide dissolved in water, in which are green hydras, enable them to regain their former resistance to chloretone? To answer this, carbon dioxide was passed thru water containing green hydras. Surprising results were obtained. Green hydras fresh from the pond were placed in an aquarium of about 6"x 6"x 10". Just prior to starting the experiment several of the green hydras were placed in 0.33-1/3% chloretone, and it was found that the ectodermal cells sluffed off in a few minutes. Then at 11:50 A. M. the experiment was begun. Carbon dioxide was started bubbling thru the water in the aquarium at a moderate rate. This was - - - - - - - - -

allowed to continue for two hours. At the end of two hours different ones of the green in the aquarium were taken out and tested with chloretone. Their resistance to chloretone was so enhanced that no hydras could be found in the aquarium that had not attained their former level of resistance to chloretone--a 0.33-1/3% solution. All tested with .5% chloretone elongated as did green hydras in the earlier months of the year, when placed in a 0.33-1/3% solution. Some tested with 1% chloretone elongated and showed considerably better resistance to this percentage than any had shown to 0.33-1/3% chloretone before the experiment was begun. Hence, by simply supplying carbon dioxide, green hydras in May were made as resistant to chloretone as green hydras in March. In fact they appeared to be slightly more resistant.

This resistance of Hydra viridis to chloretone is very variable. From the facts presented above, it is seen that in seasons of the year when aquatic plants are scarce, the hydra will be in prime condition to resist the action of chloretone. In seasons of great abundance of aquatic plants this resistance will be proportionately diminished. That is-the resistance of Hydra viridis varies with the amount of available carbon dioxide in the water. Beginning with January 1922 and up to January 1923, Hydra viridis has been tested with chloretone off and on for an entire year. Variations were found to be in accord with the above statements. The curve for the seasons would be something as follows:

Winter--resistance to chloretone at the maximum.
Spring--resistance at the minimum.
Summer--high.
Fall--slightly lower than in summer.

An excess of carbon dioxide is injurious to the hydra. It has a suffocating effect upon the animal, that is-the ectodermal cells swell up and burst, which results eventually in death. In December green and brown hydras were placed in a hanging droplet, which was placed in a container thru which it was possible to observe with a microscope the conditions of the different hydras, and thru which carbon dioxide could pass at the same time. After the hydras were placed in this container, carbon dioxide was started thru it under slight pressure. The process was allowed to continue for some time until conditions of the hydras indicated that they were dead. The results observed thru the microscope were typical. The brown hydra showed signs of disintegration. The cells of the ectoderm became swollen, bursted, and eventually sluffed away. About three quarters of an hour after the brown hydras began to show these signs of disintegration, the green began to show the same symptoms. After two hours both kinds were in such a state of disintegration that they were considered to be dead and the experiment was discontinued. Hence, it is shown that the green hydra could use carbon dioxide to its advantage and stand a concentrated amount of it better than the brown, but that it was not able to stand a high concentration indefinitely.

While a high concentration of carbon dioxide is

injurious to a green hydra, a fairly high concentration is required to tide them over unfavorable conditions as are obtained when a number of hydras are placed a test tube filled with spring water, sealed air tight and allowed to stand for two days. Under these conditions green hydras show no superiority over gray or brown hydras. But if carbon dioxide is dissolved in the water until a fairly high concentration is secured, the green shows a decided superiority . To determine this-test tubes were filled with water in which carbon dioxide was dissolved to varying concentrations. In each tube was placed a number of green and brown hydras. Then each tube was corked in such a manner that no air bubbles remained , and sealed air-tight. They were allowed to stand for two days in medium light. The following table indicates the manner of filling the tubes and results:

| Tube No. | No. C.C of $CO_2$ passed thru the water | Position of Hydra in tube | Hydras alive at end of two days. |
|---|---|---|---|
| 1. | 0 | | none |
| 2. | 1 | | " |
| 3. | 2 | | " |
| 4. | 3 | | " |
| 5. | 4 | side-near top | 1 green |
| 6. | 5 | " " " | 3 " |
| 7. | 6 | " " " | 2 " |
| 8. | 7 | " " " | All " |
| 9. | 8 | " " " | " " |

At the end of two days all brown hydras were dead in all tubes. All green were dead in tubes No. one, two, three and four, in tubes No. five, six, and seven, part of the green were dead and the rest were alive; in tubes No. eight and nine.

all green hydras were alive. Here again it is definitely shown that carbon dioxide is a definite aid to the green hydra.

The green hydra does not merely receive products of photosynthesis of the zoochlorellae, but has adapted itself so as to receive a maximum supply of these products. That is-it has learned that to locate itself in situations that are advantageous to the photosynthesis of the alga, means a better supply of these photosynthetic products. It goes in the direction of light, and toward the surface of the water where living conditions are more favorable. This last statement is true in natural environment, but if hydras are placed in vessels offering no light and no air surface, they will still go against gravity as if the air surface was still present. It appears that the green hydra does not perceive this air supply, but has learned that a negative geotropic reaction will result in the desired air supplies.

The presence of the algae in the green hydra is the one dominating physiological difference between the green hydra and the brown or gray hydra. If the two kinds, green and greenless are observed to react differently to certain stimuli, then these differences must be attributed to the presence of the zoochlorellae within the green hydra. Especially-this is true if the reactions of the green hydras, that are different from those of the brown or gray, are typical plant reactions.

In natural environment, the mean position of the green hydras is much nearer to the surface of the water, than

the mean position of the brown or gray hydras. Green hydras are often found on top of leaves or stones in full light, while brown and gray ones are never found in positions thus exposed. The green hydras are better able to stand direct light because the chlorophyll of the algae absorbs the light for use in photosynthesis. This absorption of the light rays by the algae not only may protect the hydra from their harmful effects, but also is beneficial to it. The photosynthetic products of the algae that the hydra uses, are produced in greater quantities. It is possible tho, for the light to be too strong for the green hydra, since these algae living within the endodermal cells do not entirely protect the protoplasm of the hydra.

It has been repeatedly noticed that green hydras live longer than other hydras in aquaria that are allowed to become stagnant. If green and brown or gray hydras are placed in aquaria, and the water in the aquaria left unaerated, it will be noticed that the green hydras will congregate around the edge of the water. Further, they will congregate more thickly on the sides of the aquaria which receive the best light. The brown and gray hydras on the other hand do not rise to the surface, they avoid the sides of the aquaria where the light is strong.

To observe this difference of reaction between green hydras and the brown or gray, an aquarium of about two liters capacity was filled one half full of spring water, then leaves with countless green and brown attached were placed in the water. This aquarium was then placed in medium light and left

undisturbed. In two days the green hydras were fairly well congregated around the edge of the water, and most thickly on the side where the light was strongest. The brown had not come to the surface of the water. The only reaction observed on the part of the brown hydra, was that none were to be seen on the side where the light was strongest. As time passed the green become entirely congregated around the edge of the water, and the brown remained as noted above. But eventually conditions in the bottom parts of the aquarium became unfavorable to the life of the hydras, and the brown began to die off. By the end of two weeks no brown hydras remained alive, while the green still were as numerous as ever. Here the difference between the light reactions of the two kinds of hydras is clearly indicated. The green hydra is positive to very strong light, while the brown is negative to anything but very diffuse light. The green went not only toward the light, but also in the direction of the surface where it could obtain the photosynthetic products, and at the same time gain a situation more favorable to its life processes.

It seems that in a moderate need of the desired photosynthetic functions, the green hydra will react positively to light, then if maximum light is gained and still a lack of these functions is felt, it will then react negatively to gravity, which it has learned will result in more favorable conditions. An aquarium was filled three fourths full of water and numerous

green and brown hydras were placed in it. It was allowed to stand undisturbed for a day until the green hydras had gathered around the edge of the water. Then the top of the aquarium was covered with opaque paper in such a manner that light could only gain access to the interior by entering the bottom, and about one half inch of the lower parts of the sides. It was suspended in medium light and aerated regularly. Within one day the green hydras had appeared at the bottom where the light was strongest. Aeration was continued for three days until all of the green hydras were at the bottom, then was discontinued. In two days after the discontinuation of aeration, the green hydras had left the lighted bottom of the aquarium and were found located around the edge of the water, in the darkened portions. It was noticed, too, that all of them had their oral ends directed toward the lighted bottom of the aquarium. The brown hydras on the other hand were not affected in this manner. The only definite reaction shown by the brown in this aquarium, was to move away from the most strongly lighted region.

This last reaction seems to be a reaction to air supplies. But this is not the case. Altho these may be gained, the reaction is simply a negative geotropic one. Conditions became unfavorable in the bottom of the aquarium, when aeration was stopped. The green hydra then reacted negatively to gravity, which, under ordinary or normal circumstances, leads to more favorable conditions. Hydras, green and brown, were placed in a flask of two liters capacity and sealed air-tight. No air bubbles were left in the - - - - - - - -

flask. The contents were solidly all water. The flask was then placed in complete darkness. At the end of one day no results were noticed. At the end of two days several green hydras had reacted negatively to gravity. This was indicated by the fact that they were distributed about evenly all over the upper surfaces of the flask-on the upper part of the bulge of the flask as well as in the neck. At the end of two days all brown hydras were dead-at the end of three days all green hydras were dead, such as had not reacted negatively geotropic. At the end of four days all were dead.

In the table previously given showing the results of the experiment using varying concentrations of carbon dioxide, it will be remembered that it was also indicated that the green hydras which remained alive at the end of two days were all located on the sides of the tube near the tops. They had encountered unfavorable conditions, and had reacted negatively geotropic.

There is one further detail that I would add with reference to Hydra viridis, and the algae living within it. Mosse in his work on photosynthesis of proteins by marine algae ('21), finds that these algae are able to fix nitrogen, and more so in light than in darkness. He also finds that the fresh water algae are not able to fix nitrogen in ordinary surroundings, but if they are placed in alkaline water they are able to fix nitrogen in the same manner as marine algae. He concluded then, that fresh water algae do not fix nitrogen photosynthetically because of the absence of alkaline in the water. Then if it is alkalinity

that is lacking for the photosynthesis of proteins by fresh water algae, it is highly probable that in the case of the zoochlorellae in Hydra viridis, we have this photosynthetic fixings of nitrogen. Since all animal cytoplasm is alkaline as over against the acidity of nuclear material. It is then likely that we have an important means whereby the hydra aids the algae, that is, in supplying an alkaline medium, which will make possible the photosynthesis of proteids.

## SUMMARY

1. Hydra viridis in April, May and June are less resistant to chloretone than are Hydra viridis in January, February or March.

2. Hydra viridis in April, May and June may be made as resistant to chloretone as Hydra viridis in January, February or March by placing them in water of a fairly high concentration of $CO_2$ for two hours or more.

3. So important is $CO_2$ to this animal, Hydra viridis, that when air contact is taken away from the water, they live longest in water with a fairly high concentration of $CO_2$.

4. There is, therefore, a true symbiosis in Hydra viridis. This symbiosis will help to explain how it is that Hydra fusca and Hydra grisia react negatively to light of certain intensities to which Hydra viridis reacts positively.

5. When unfavorable conditions are encountered, Hydra viridis will react negatively geotropic, while Hydra fusca and Hydra grisia will not.

## LITERATURE


Beyerinck, M. W.

1890 Kulturversuche mit Zoolchlorellen, Lichengonidien, u. anderen Algen. Botan. Zeit., Jahrg. 48.

Brandt, K.

1882 Ueber die morphologische u. physiologische Bedeutung des Chlorophyll bei Tieren. 1. Arch.d. Anat. u. Phys.

Entz, G.

1981 Ueber die Natur der Chlorophyllkorperchen niederer Tiere. Biol. Cebtralbl. Vol. 1.

Graff, L.

1884 Zur Kenntnis der physiologischen Funktion des Chlorophylls im Tierreich., Zool. Anzeiger Vol. 7.

Kepner and Hopkins

1923 Reactions of Hydra to Chloretone. In hands of Editorial Board Jour. Exp. Zool.

Moore, Benjamin

1921 Photosynthetic Processes in the Air, upon the Land, and in the Sea in Relation to the Origin and Continuance of Life on Earth. Jour. Chem. Soc. (London) 119.

Whitney, D. D.

1907 Artificial Removal of the Green Bodies of Hydra Viridis. Biol. Bul. Vol. 13.

1908 Further Studies on the Elimination of the Green Bodies from the Endoderm Cells of Hydra Viridis. Biol. Bul. Vol. 15.