Chemotactic Responses of *Halteria grandinella*

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Introduction

Chemotactic studies form a relatively recent field and have their origin in the work of Pfeffer and Englemann (2). Since that time considerable work has been done with a variety of microorganisms, including bacteria and protozoa.

Diehn (1), working with *Euglena gracilis*, concluded that the organism exhibited both positive phototaxic and inverse photophobic responses. He hypothesized that some single-celled organisms utilize homeostatic systems for orientation movements. Seravin and Orlovskaja (3) utilized six species of carnivorous protozoa in an investigation of the behavioral activity involved in feeding responses. Tamar (5) reported on the reactions of *Halteria grandinella* to a variety of substances, including KCl, $CaCl_2$, distilled water and acetic acid.

Previous work by Tamar (4, 5, 6, 7) supports the feasibility of using *Halteria grandinella* for chemotactic studies. It is a phylogenetically advanced ciliate protozoan possessing several different types of movement and a relatively short response time. The last property enables one to maximize testing periods.

The purpose of this experiment was to determine a series of chemotactic responses of *Halteria grandinella.*

Materials and Methods

The tests were run with different time periods for each substance, ranging from 15 to 120 minutes. However, in the case of the substances eliciting weak or strong positive responses, the reaction of the *Halteria* was maximal within 25 to 35 minutes. The optimal time for any *Halteria* response was determined from original tests. The temperature during all the tests never exceeded 23° C or dropped lower than 20° C. The pH of the test substance, whenever possible, was made equal to that of the culture medium. A specific medium was used to culture and test the *Halteria* (6).

Five major test methods were used in the course of the research. The first method involved the use of a petri dish cover or bottom. A smear of the test substance was dried in the dish and a lake of *Halteria*-filled culture fluid was then added. In the next method a lake was also employed, except that the lake was much smaller and extended over a slide smear. A third method again required a petri dish lake. However, in this case a capillary with a specific amount or concentration of the test substance was inserted into the lake. In addition to the petri dish-capillary method a slide-capillary method was also tried. Further, a slide with a coverslip held up by an empty capillary and one filled capillary was used. The final method, and the most effective, provided the best points of all the other methods. A slide with a tiny dried smear in one or two areas was covered with a lake. Two long pieces of thread were located at either end of the lake and a coverslip was placed on top of the lake and was held up by the thread.

The slide and petri dish tests were observed with a binocular dissecting microscope at a magnification of 10 to 70X. The light bulb serving as the light source was covered by 3 heat filters which effectively prevented any significant heat transmission. A phase contrast microscope was used mainly for identification of the *Halteria* and some of the test bacteria and mold.

Results

In the following tables the responses of the *Halteria* are grouped into five categories. These are: strongly positive, weakly positive, neutral, weakly negative and strongly negative. In a strongly positive response, the *Halteria* in a test are found almost exclusively over and around the test substance. A weakly positive response is a gathering of *Halteria* around and over the test substance, but *Halteria* are in other places of the test area also. A neutral response consists of an even distribution of the *Halteria* over the total test area. A weakly negative response is the even distributión of *Halteria* in most of the test area, but there are fewer over or around the test substance. Finally, a strongly negative response is the total avoidance by the *Halteria* of the substance placed in the test area.

The symbols that are used for these responses are: strongly positive = +, weakly positive = **θ**, neutral = 0, weakly negative = θ, and strongly negative = -. The symbols used for the methods are the following: lake-petri dish = 1, capillary-in-a-lake = 2, lake-on-a-slide =3, capillary-on-a-slide plus a coverslip = 4, smear-on-a-slide with a coverslip held up by thread = 5. An example of a recorded response may read θ3, +2, 01. This means 3 tests were weakly negative, 2 tests were strongly positive and 1 test was neutral.

This research was particularly directed at finding substances that elicit a positive response from *Halteria grandinella*. Many substances produced semi-positive or neutral responses. However, only three initiated a high percentage of strongly positive responses by the test organisms. These three substances are: a string soaked in skimmed milk, dried bacterial film and cultured water mold grown in a solution at a pH of 7.

Most of the hundreds of tests that were run produced a negative response from the *Halteria*. A neutral response by the *Halteria* was also common. Those substances out of over 75 that produced any kind of positive response are listed in the following tables.

All the substances listed in Table I were tested after they were brought to a pH of 7 and the culture medium was either at pHs between 6.8 and 7.2 or was also brought to pH 7.

Table II gives the results obtained with 7 amino acids that elicited a highest number of weakly positive responses when the medium was at pHs between 6.8 and 7.2 or was brought to pH 7 and the amino acids were at lesser (and their natural) pHs. These lesser pHs were the following: isoleucine 6.0, leucine 6, serine 5.7, proline 6.3, cystine 5.0, methionine 5.7 and tyrosine 5.6. When each of these amino acids was tested 10 times as above, with the exception that the pH of

TABLE I *A listing of the items tested at a pH near or at neutral.*

Item tested	Response	Method
albumen (raw)	**θ**10, +1	1
unwashed water mold	**θ**19, θ14, 02	1
bacteria (cocci?) from pond water (2 yellow colonies/10 ml of culture fluid)	**θ**9, 08, θ3	1
water mold and riboflavin	08, **θ**7, +4, θ1	2
cultured water mold	+5, **θ**3, 02	5
powdered skimmed milk	**θ**30	2
string soaked in skimmed milk	+7, 06, **θ**6, θ1	1

TABLE II *A listing of amino acids tested at lower than neutral pHs.*

Item tested		Response	Method
isoleucine	L(.125m)	**θ**24, 023, +1, -1, θ1	2
isoleucine	D(.125m)	**θ**9, 03, -3	5
leucine	D(.125m)	**θ**6, θ5, 03, -1	5
serine	L(.125m)	**θ**5, 04, +3, θ1	5
proline	L(.125m)	**θ**7, -5, 03	5
cystine	L(.125m)	**θ**9, -5, 02	5
methionine	L(.125m)	**θ**11, 04	5
leucine	DL(.125m)	**θ**6, θ5, 04	5
serine	L(.375m)	**θ**15, 05	5
cystine	L(.375m)	**θ**15, 03, θ2	5
tyrosine	L(.375m)	**θ**5, 04, θ1	5

the amino acids was brought to 7, predominantly neutral responses were obtained.

Table III lists all the substances that elicited a positive response from the *Halteria* but were at unknown pHs. The medium was always at a pH between 6.8 and 7.2 in the Table III tests.

TABLE III *A listing of the items tested that elicited a positive response, but at an unknown pH.*

Item tested	Response	Method
water plant stem (cut)	**θ**7, +2, 02	1
dried pond water	**θ**11, 07, θ1, -2	3
whole midge	**θ**5	1
whole raw egg	**θ**11, 08, +2, θ1	1
mealworms (cut)	**θ**12, 011, -2	5
fly larvae (cut)	**θ**14, 011	5
dried bacterial film	+8, 08, **θ**2, θ2	3

Discussion

Since skimmed milk powder is added to the culture medium it is possible that skimmed milk, bacteria and water mold may have to be present together to elicit a large proportion of positive responses from the *Halteria* with the experimental procedures employed in the present research. It may also be that bacteria attracted to the skimmed milk may in turn have attracted *Halteria* during the 25-35 minutes used in the skimmed milk tests.

The data showed that of all the 15 amino acids tested at their natural, lower than 7, pH, only the 7 given in Table II produced some kind of positive response and this positive response was very weak. The fact that each of these same 7 amino acids gave mainly neutral responses when it was brought to a pH of 7 indicates that the acidic nature of these amino acids may have been largely responsible for the attraction.

The other items that produced positive responses are logical choices for testing in view of the natural environment of the *Halteria*. Such items as a whole midge, mealworms and fly larvae, as well as pond water bacterial colonies and water mold, may be present in the pond water inhabited by *Halteria*. The preceding items plus egg, water plant stem and powdered skimmed milk all may have one factor in common. Each of them could offer the *Halteria* a concentration of food bacteria and perhaps also additional nutrients.

It should be remembered that skimmed milk, bacteria, and sometimes water mold were present in the culture medium, and this may have reduced the strength of the chemotactic responses.

Studies of the responses of *H. grandinella* to several amines and other nitrogenous substances are in progress.

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