Reports

Technique for the Study of the Behavior of Motile Microorganisms

Abstract. Rapid recording of the speed and the direction of the pathway of a swiftly moving microorganism is possible with the flying-spot microscope. The organism is televised, collisions between it and the scanning beam, which make a discrete pathway of "blips," being photographable on the viewing screen. The pathway of the miracidium of *Schistosoma mansoni* in water and in "host-factor" is recorded.

The study of the responses to external stimuli of rapidly moving microorganisms has been delayed by the technical difficulty in recording individual pathways with speed and accuracy. In the course of current studies on the behavior of the miracidium of *Schistosoma mansoni*, apparatus has been employed which makes possible the fast recording of a great number of tracks of single, rapidly moving microorganisms and hence the rapid gathering of statistical data on their behavior under different conditions.

The apparatus used was the flying-spot microscope (*1–4*), which essentially allows one to observe on a visual screen and at the same time to photograph a televised image of a fixed or moving microscopic object. This apparatus has been used for various purposes, including the counting of neurones in fixed tissues (*3*), but to our knowledge it has not been used as yet in the study of living organisms. In addition to the advantages described by its designers (*2*) the apparatus has a number of others which make it particularly useful in the study of the behavior of motile microorganisms. The greatest technical difficulty encountered in making any sort of a manual record with the aid of a camera lucida of the movements of a small organism which describes a devious pathway while moving swiftly (such as a miracidium or protozoan) results simply from the inability of the observer to follow such movements. It is, of course, possible to record such pathways with cinematography, but this requires much film and tedious hours of processing, tracing, and measuring, if one wishes data on changes in linear velocity or rate of change of direction occurring under different conditions. One could also make direct, short-duration time exposures of the organism under dark-field stroboscopic illumination.

However, the flying-spot system has the advantage that it minimizes the possible effects of directional illumination on the behavior of the organism. In addition, picture intensity is not dependent upon the level of illumination of the organism, as it would be if one were to make a direct photograph of the field under ordinary or dark-field stroboscopic illumination. Without using a dark-field optical system involving possible directional effects and without undue constant or intermittent illumination of the organism, one may nevertheless, by phase adjustment of the flying-spot system, obtain a brilliant track against a dark background on the viewing screen. This, of course, allows one to expose standard film to the image and obtain a track (Fig. 1), the length of which depends upon time of exposure. As can be seen, the track in the figure, a 2-sec exposure to the track of a *Schistosoma* miracidium moving in a droplet of water, is made up of discrete "blips." For this record the beam was scanning the microscope field at the rate of 12.5 times per second; hence in one second's travel there were 12.5 discrete images of the moving organism, producing as many blips. Calibration of the system with a stage micrometer by photographing the micrometer through the system gave an enlargement of 40 diameters, reduced in Figs. 1 and 2 to 16 diameters. Therefore 16 mm in the figures equal 1 mm of actual travel by the organism. In Fig. 1 there are approximately seven blips in 1 mm of actual travel by the miracidium. Since the organism is scanned 12.5 times per second, it follows that it is moving at a rate of 1.8 mm per second. Figure 1 also shows clearly the straightness of the normal unimpeded path of this miracidium in a water drop, most frequently involving smooth gliding motion more or less directly from miniscus to miniscus across the drop.

One can see from the above that, by setting up a number of preparations and making a large number of still photos at will while watching the screen, one may obtain data for statistical analysis with great rapidity and precision. Data on the effects of factors on linear velocity or rate of change of direction can be obtained from photographs such as the one in Fig. 2, a 2-sec exposure to the characteristic tracks of two miracidia in extract of whole ground host snail *Australorbis glabratus*. Here, as is subjectively obvious, the rate of change of direction has greatly increased and can be compared quantitatively with that of the straighter pathway in water alone by analysis of the angular changes occurring from blip to blip during one time interval of travel.

With the flying spot system, since the linear dimension of a single blip depends in part upon the velocity of the

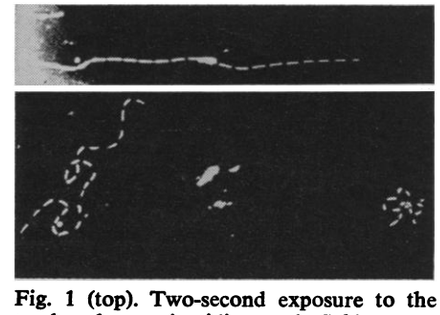

Fig. 1 (top). Two-second exposure to the track of a miracidium of *Schistosoma mansoni* in a water drop. Fig. 2 (bottom). Two-second exposure to the tracks of two *Schistosoma* miracidia in filtered extract of whole ground host snail, *Australorbis glabratus*. At the right, the "whirling dance" frequently exhibited on first encountering host extract. The "blips" have been reinforced for purposes of the cut.

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

organism making the blip, careful calibration may even make it possible to arrive at a rapid but fairly accurate estimate of changes in velocity by comparing the lengths of blips under different conditions. This, however, may have limitations, owing to the relative direction of the movement of the scanning spot and the organism.

The system is particularly well suited to the recording of pathways at low power (that is, with a 1-inch objective) in organisms with a wide range of size variation around the general order of magnitude of 100 μ. With little doubt, however, the apparatus could be used at higher magnifications and high velocities of travel. An advantage could be obtained when high velocities are encountered by the use of higher scanning rates and a long-persistence display tube which would enable one to photograph the "stored" track image.

A final advantage is that, since with the system illumination of the field is low and intermittent, heating is minimal. In our studies the miracidia were simply placed in an open drop on a well-slide without undue drying occurring during a long series of exposures. This makes easy the addition of materials to the drop, the effects of which one wishes to study.

The value of this apparatus as a tool for the analysis of linear and angular velocity under different conditions of many sorts of organisms (protozoa, trochophores, microplankters—indeed, motile cells of all sorts) can, we feel, be immediately grasped from the accompanying photographs (*5*).

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References and Notes

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5. We wish to express our appreciation to Rank Cintel Ltd. for making possible the use of the flying-spot particle resolver and to Donald Claugher for technical assistance.
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Response of the Neotenic Salamander Haideotriton wallacei to a Metamorphic Agent

Abstract. The neotenic, cave-dwelling plethodontid salamander *Haideotriton wallacei* undergoes only a few minor integumentary changes and loses only a single bone, the coronoid, when treated with sodium laevo thyroxin. Since neither the animal's thyroid nor the supplementary thyroxin is sufficient to effect substantial metamorphosis, genetic factors probably restrict the animal's ability to transform.

The systematic relationships of neotenic animals are best understood when the morphology of experimentally metamorphosed individuals can be compared with that of fully transformed types. The induction of metamorphosis also assists in ascertaining whether the neotenic features are due to environmental, physiological, or genetic factors.

The odd cavernicolous neotenic salamander, *Haideotriton wallacei,* was assigned by Archie Carr, who described it, to the lungless family Plethodontidae on the basis of its general conformation to the anatomy of larval plethodontids. I have shown in previous experiments (*1*) that the neotenic plethodontid salamander, *Eurycea tynerensis,* is clearly assignable to the genus *Eurycea* (it undergoes transformation upon administration of thyroxin) but that *Typhlomolge rathbuni,* another cavernicolous plethodontid, can undergo only a partial metamorphosis upon administration of thyroxin and therefore appears to have genetic resistance to the production of many of the skeletal and integumentary features of transformed plethodontids. It comes as no surprise, then, to find that *Haideotriton* is a second supposed plethodontid neotene that displays strong resistance to metamorphic agents.

For experimentation several subadult *Haideotriton* from Florida and Georgia were utilized (*2*). The animals were treated with various concentrations of sodium laevo thyroxin, a drug that normally causes almost complete transformation in neotenes such as *Eurycea tynerensis, E. neotenes,* and *E. nana,* and in various salamander larvae, within 3 weeks at laboratory temperatures and in the concentrations used for the experiment reported here (*3*).

The experimental animals were maintained in finger bowls containing 100 ml of the thyroxin solution made up with native water kept in a B.O.D. incubator in complete darkness at 19.5 ± 0.5°C (the temperature of the natural cave habitat). The thyroxin was first dissolved in a drop of 0.1*N* NH_4OH and then diluted to the desired concentrations. Three controls maintained under the same conditions exhibited no changes in the course of the experiment, and they showed no changes during the succeeding 2 months in which they were maintained.

The results of treatment with thyroxin are shown in Table 1. In no case did any treated animal give complete external evidence of loss of larval characteristics; gills atrophied but were never completely lost or resorbed. That the animals perhaps cannot withstand complete transformation is suggested by the failure of any to survive more than 25 days of treatment, whereas all the control animals were in excellent health for some time after the death of the last treated animal. The alterations that occurred were essentially only the most simple integumentary changes, such as loss or reduction of fins, gills, and labial folds. The only skeletal change observed was the loss of the coronoid (a bone which was not reported by Carr in his original description but which is definitely present in untreated animals). The only peculiarity in the response pattern is that a preparation from a large untreated adult male, on staining, shows ossification of the posterior basibranchium to form an os thyreoideum, whereas none of the treated animals give any indication of such ossification. This throws some doubt on the response observed in this species, for earlier stages of amphibian larvae tend to lack the potential to develop all adult structures when forced to metamorphose precociously (*4*).

All the treated animals were immature or female, but their response should be no different from that of males. What would happen if larger, older animals were treated with sodium *l*-thyroxin remains questionable at this time. Adult animals are exceedingly rare and difficult to capture, and further experimentation must await better collecting opportunities.

In addition to the responses shown in Table 1, the nonoccurrence of a number of other changes normally exhibited during metamorphosis was noted. None of the following occurred: development of nasolabial groove and nasolacrimal canal; development of eyelids; transformation to adult skin structure; development of a new integumentary pattern; atrophy of pterygoid; development of maxillary, prefrontal, nasal, and septomaxillary bones; devel-