Using Cellular Slime Molds in the High School Laboratory

P.B. Haskins

ARE THEY PLANTS OR ANIMALS? This question invariably accompanies the textbook description of the slime molds, which start their life cycle as one-celled animals, but end as typical plant-like fruiting bodies. Although the dubious nature of their classification is an interesting point, it is not the most important or even the most fascinating aspect of these social organisms. The Acrasiales, the cellular slime molds, resemble all other free-living amoebae at the beginning of their life cycle, but soon abandon their individuality to become part of a larger organism, and in so doing, sort themselves out into two different and distinct types of cells, each of which proceeds to carry out its proper function in the mature plant. Here, in microcosm, we have a lovely example of the riddle of development.

It should be pointed out that the cellular slime molds are not the slime molds with which most of us are familiar—the glistening mass of protoplasm, often bright yellow, found in the woods, slithering over decaying matter. These are the Myxomycetes and their multinucleate mass is a true plasmodium—that is, one in which the many nuclei are not separated by cell walls. The plasmodium is a result of the simple fusion of hundreds, sometimes thousands, of cells. The Acrasiales, on the other hand, demonstrate a pseudo-plasmodium, so called because the cell walls remain distinct at all times, and the final organism is a truly unified, multicellular individual. Despite their similarities, these two types of slime molds are not related phylogenetically, although they may have had a common one-celled ancestor.

The Acrasiales are ideal material for use in a high-school laboratory because they are easy to raise, interesting to observe, and can provide suitable material for a wide range of biology students, from the slowest to the most gifted. Lab work can consist merely in observing the three phases in the life of a slime mold: aggregation, migration and culmination. Or several laboratory periods can be devoted to their culture and include a variety of experiments, both simple and sophisticated. The study of these amoebae can tie in with such biological themes as the mechanism of development (as it pertains to the differentiation of cells); the evolution of simple organisms into more complex ones; orientation by means of physical stimuli such as light (phototaxis) and chemicals (chemotaxis); and all this can be demonstrated in a small creature whose life cycle takes only a few days.

Description

Of the Acrasiales, by far the most is known about *Dictyostelium discoideum*, and it is this species which is described here.

The cell that will become the future amoeba is encased in a spore covering, designed to protect it from long periods under unfavorable conditions. In a suitably warm and humid environment, the spore case breaks open and the amoeba emerges. It resembles a human white blood cell in size and appearance; moving by means of pseudopods, feeding by phagocytosis (engulfment), reproducing by binary fission. During this growth period the amoebae go their separate ways, feeding on bacteria, and dividing every 3 or 4 hours. While in this early stage, the amoebae appear to be identical to one another. They respond

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exactly the same to all lab tests and no morphological differences can be observed.

The amoebae will continue to multiply until their food source is exhausted. There is then a pause in their development, lasting from 4 to 8 hours. After this pause, aggregation begins. The amoebae come together, single cells at first, then groups of cells merging into streams and flowing together toward the center. When observed under the high power of a microscope, the amoebae can be seen to have assumed an elongated, somewhat rectangular shape.

The rate of movement toward the center is not one of continuous motion, but occurs in pulses or waves. The experiments of Dr. Bonner of Princeton University have shown that the cells are attracted by a chemical agent, which he named Acrasin. It is thought that acrasin is produced at first by a single cell, sometimes called a founder cell. This initiates the process of aggregation. As the first bit of acrasin attracts cells nearby, it also stimulates them to produce the chemical themselves. They in turn attract the amoebae in the next wider circle, and so on until each cell in the aggregation territory has been directed toward the center. (Several aggregation streams can be observed by the naked eye on one Petri dish; and it is interesting to note that under similar conditions, territory size remains almost constant, regardless of how many amoebae are present in that area.)

During aggregation as many as 100,000 amoebae will come together, and will heap up into a pile. The pile will turn on its side, becoming a cigar-shaped, migrating pseudoplasmodium. It is a colorless, glistening mass with very definite head and tail regions. The front end is more pointed and is frequently lifted upward. This slug, as it is sometimes called, glides along the surface of the agar, secreting a slime sheath which accounts for its shiny appearance. The slime secretion serves as the track along which the pseudoplasmodium moves, and the slime track can be seen in the wake of the migrating slug. The movement actually consists of the pseudopodial movements of the amoebae which make up the slug. It may migrate for long periods of time (24 hours would be considered long) or hardly at all.

During this migration stage we can see a differentiation in cells that, just a few hours before, seemed so much alike. In the front part of the pseudoplasmodium are found the larger cells: these will become the stalk cells of the mature fruiting body. In the hind end are smaller cells, which will become the spores. If some front end amoebae are stained with dye and placed in the hind end, they can be seen to move up through the slug to rejoin the other cells of their type. The same is true of posterior cells placed anteriorly. They will fall back as migration continues, until they reach their former position.

At the end of migration the slug comes to rest and begins to stand on end, front part up in the air. Thus begins the culmination stage of *D. discoideum*. During this phase the front end cells, which are now at the top of the pile push down through the mass of cells comprising the slug, and in so doing, the posterior portion is gradually pushed up. As the front cells reach downward they secrete a cellulose-type material, which gives them rigidity, and they form a stalk. As the stalk grows it lifts up the hind-end cells and they become the sorocarp, or spore-case, top of this fruiting body. The finished product is a thin graceful stalk composed of vacuolated cells holding into the air a delicate sorocarp containing the spores from which future generations of amoebae will arise.

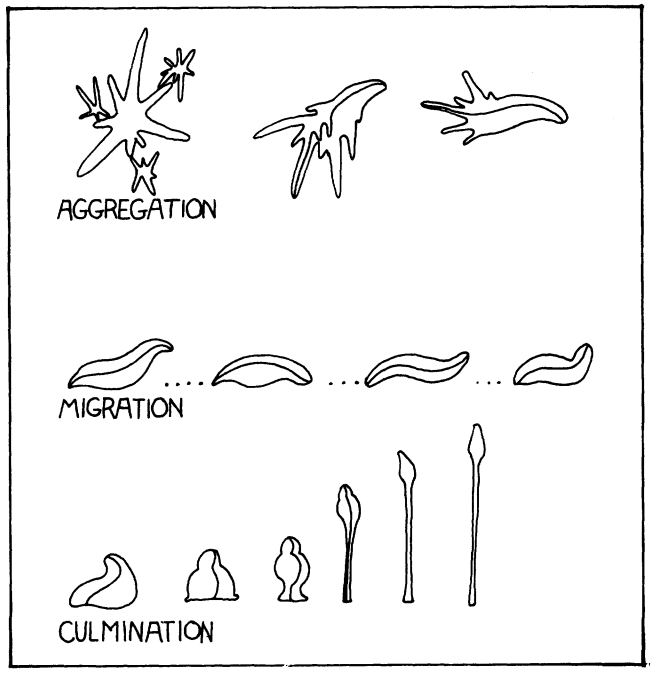

FIGURE 1. Stages in the development of a slime mold.

Laboratory Procedures

In preparing cultures of *D. discoideum* and its bacterial food supply, *E. coli*, it is very important that all equipment be sterile and that when inoculating an agar plate with bacteria or slime mold spores, the lid of the Petri dish be lifted only as much as is absolutely necessary, to avoid contamination. The exception is the class demonstration culture which, once prepared, should be available for examination and sterile precautions are not necessary. However, it must be discarded after use and spores from it should not be used for propagation purposes.

D. discoideum and *E. coli* and suitable agar for culturing them can be ordered initially from a commercial source. Macmillan Scientific Supply (also known as Turtox), 8200 South Hoyne Ave., Chicago, Ill. 60620 sells a complete kit of *D. discoideum*, *E. coli* and flask of medium containing 125 cc of 0.1% lactose agar plus instructions for using. Or any of these may be ordered separately. Carolina Biological Supply Co., Burlington, N.C. 27215 and Wards Natural Science Establishment, Rochester, N.Y. 14603 also carry cultures of *D. discoideum* and *E. coli* with complete instructions. Once obtained they are easy to keep on hand, even when not needed for long periods of time.

Should you wish to prepare your own agar solutions, the following directions may be helpful:

An effective nutrient agar is composed of:

lactose	1 (or 0.5) g
peptone	1 (or 0.5) g
agar	20 g
distilled water	1000 ml

Nutrient agar for raising *E. coli*

dextrose	10 g
peptone	10 g
agar	20 g
distilled water	1000 ml

Non-nutrient agar

agar	20 g
distilled water	1000 ml

Boil to dissolve agar; then add other ingredients. Sterilize in steam at 15 pounds pressure for 20 minutes. Pour into sterile Petri dishes. To prepare slanted test tubes, pour through a funnel into test tubes and rest at an angle until agar solidifies. Store Petri dishes and test tubes in refrigerator.

Before beginning your experiment, you will want to assemble the following materials:

Cultures of *D. discoideum* and *E. coli*
Nutrient and non-nutrient agar
Wire inoculating loop (made from thin wire, bent into loop at one end)
Petri dishes, with lids
Test tubes
Distilled water
Ingredients for nutrient agar: lactose, peptone, agar, dextrose

To prepare a good class demonstration culture, start with a sterilized Petri dish, with lid, containing non-nutrient agar. Using a wire loop that has been sterilized in the flame of a bunsen burner, spread a *very thin* layer of *E. coli* over the agar surface (bending the handle of the wire loop slightly will make this easier) and place only a few spores of the *D. discoideum* in the middle of the dish. To gather these spores merely touch the top of an aerial spore head with the wire loop, being very careful not to gather in any old bacteria, which are apt to mutate into varieties inedible by the slime molds. The culture should be kept at room temperature (21-25°C.).

After an interval of about 24 hours this agar plate will contain all stages of the development of *D. discoideum*. Zones of aggregation and migration should be seen, and also examples of the culmination stage, with the erect fruiting bodies.

To prepare cultures of *D. discoideum* for future use, use nutrient agar, and inoculate an agar plate with *E. coli* in the form of an X. Place a few spores of the slime mold in the center of the X. Again, it is important to collect only spores of *D. discoideum*, without any associated bacteria. The fewer spores collected, the better, as percentage of germination is decreased when dense spore concentrations are present. The amoebae on this plate will grow along the lines of the X and when they have reached culmination stage, can be placed in the refrigerator. If kept free of contamination this culture will be good for about 30 days. At that time it should be replated. If, however, you wish to keep some amoebae for longer periods of time, as over summer vacation, use the nutrient agar, slanted into a test tube. Place a thin layer of *E. coli* on the surface, then inoculate with spores of the slime mold and let culminate. Cover with a few drops of sterile mineral oil. (Since oil conducts heat poorly, it must be autoclaved for 45 minutes, instead of the usual 15.) Stopper tightly and store in the refrigerator. These amoebae will keep indefinitely. (But when using at a future time, they should be lightly rinsed with distilled water to remove the mineral oil.)

To keep a fresh supply of *E. coli* on hand for the slime mold, use nutrient agar, and inoculate with a small amount of the bacteria. The *E. coli* will multiply and the Petri dish can then be placed in the refrigerator. Whenever needed, the bacteria can be removed from this supply, and it should be replated when more are required. *E. coli* can also be stored using the method described above for the *D. discoideum*, that is, on the surface of nutrient agar which has been slanted into a test tube and tightly stoppered.

Suggestions for Experiments with D. discoideum

One of the simplest and most interesting experiments with this slime mold is the removal of the center of an aggregating mass. If this center is then placed to the side of the aggregating amoebae, they will change direction and head toward the new position of the center. The best tool for this is an eyelash sealed into the end of a glass rod. (First draw out the rod in the flame of a bunsen burner, and then insert eyelash.)

Numerous experiments can be devised to test the sensitivity of the migrating pseudoplasmodium to heat, light and other stimuli. For example, a very small light bulb can be placed to one side of the agar plate. The slug can detect a heat increase of only a fraction of a degree and will head toward it.

A slug can be bisected into two pieces to produce two fruiting bodies, each smaller than a normal one, but perfect in proportion and detail.

The percentage of germination of spores can be calculated under varying conditions of temperature, humidity, light (or darkness), and spore density.

Further experiments are suggested in the articles in the bibliography, especially Bonner and Stong.

(Concluded on p. 356)

TABLE 1. Natural Science Reading Test Scores, NEDT Test

Non-SAP Students (n-72)	
Class	*Average Score*
10th Grade, Spring 1974	34.40
9th Grade, Spring 1973	33.73
Change in Score	+0.67
SAP Students (n-74)	
10th Grade, Spring 1975	37.33
9th Grade, Spring 1974	32.87
Change in Score	+4.46

No detailed statistical analysis is needed to convince me that there is a significant increase in the average scores of the SAP students as compared with non-SAP students. I think it may be attributed to the SAP method.

In conclusion, I want to point out that I do not recommend that teachers follow exactly the procedures I used in the SAP. Indeed, I do not expect to follow them exactly myself in the coming school year. If I did not continually change and adapt to new circumstances, I would not really be following the ways of science. The important idea is that teachers should consider their own courses as part of the processes of science. They should analyze their own situation and adapt their teaching methods to it. Although I used the SAP in a school with modular scheduling, I see no reason why a similar system would not work in a school with traditional scheduling. Also, I would like to emphasize the two ideas about science teaching that were given at the beginning of this article. A science teacher should never forget that science teaching involves not only a formal structure but also the human beings who are being taught. I think that to be successful science teachers must always try to make these two ideas work together no matter how difficult the task may be.

Acknowledgments—I am indebted to the many inspiring teachers which I have had in the many schools I have attended. I am also indebted to the many informative articles in the *Science Teacher* and *American Biology Teacher*. Most of all, I am indebted to my students. They have taught me how to teach them.

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Slime Molds

. . .from p. 349

In the process of investigating the slime molds, we touch on many problems pertinent to biology today. Perhaps the most important of these is the study of development, and its mechanisms. Not only do the slime molds have a life cycle that is short and easy to follow, but it is much simpler to deal with only two types of cells: prespore and pre-stalk, than to sort through the many facets of, for example, a developing embryo. The embryo, besides, involves a more complex specialization of tissues as well as of cells. Another factor which complicates the study of higher organisms is the growth which accompanies cell differentiation. In the Acrasiales growth occurs only in the first, or feeding, stage. Thereafter, through aggregation and culmination, the amoeba must live on reserves stored early in its life. Growth and differentiation thus become two separate processes in time, as well as in fact.

Another matter of interest today is the mechanism of orientation in the slime molds. This not only includes the tendency of the migrating pseudoplasmodium to move toward heat, moisture, and away from light, but also the orientation of the amoebae to each other that is the basis of the aggregative process. The theory of the chemotaxic reaction to acrasin has been discussed. But we do not know what triggers the production of this chemical (aside from the starvation factor) by the founder cell, and how these solitary amoebae suddenly change their habits and stream together to become part of a larger organism. We wonder if this is how larger, more complex animals arose: by the clumping together of single cells. And we can compare this aggregation with experiments in which cartilage and muscle cells were separated and then mixed together, with the result that similar cells moved toward each other, the muscle cells in their proper relationship to the cartilage, that is, surrounding it. We have an example of such movement (called morphogenetic) in the position of prespore and pre-stalk cells, neatly arranged in their proper places in the migrating slug of *D. discoideum*.

It is possible that the research being done now on the cellular slime molds may point the way to a more complete understanding of such riddles as these.

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