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Sodium azide inhibition of germination, growth and development in Dictyostelium discoideum.

Nancy Taylor Morris

SODIUM AZIDE INHIBITION OF GERMINATION, GROWTH AND DEVELOPMENT IN *DICTYOSTELIUM DISCOIDEUM*

A Thesis

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In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

by

Nancy Taylor Morris

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

THESIS ACCEPTANCE

Accepted for the faculty of The Graduate College of the University of Nebraska at Omaha, in partial fulfillment of the requirements for the degree Master of Arts.

Graduate Committee

David M. Sutherland Biology
Name Department

William D. O'Dell Biology

James K. Wood Chemistry

Allen T. Wabon
Chairman

July 25 1975
Date

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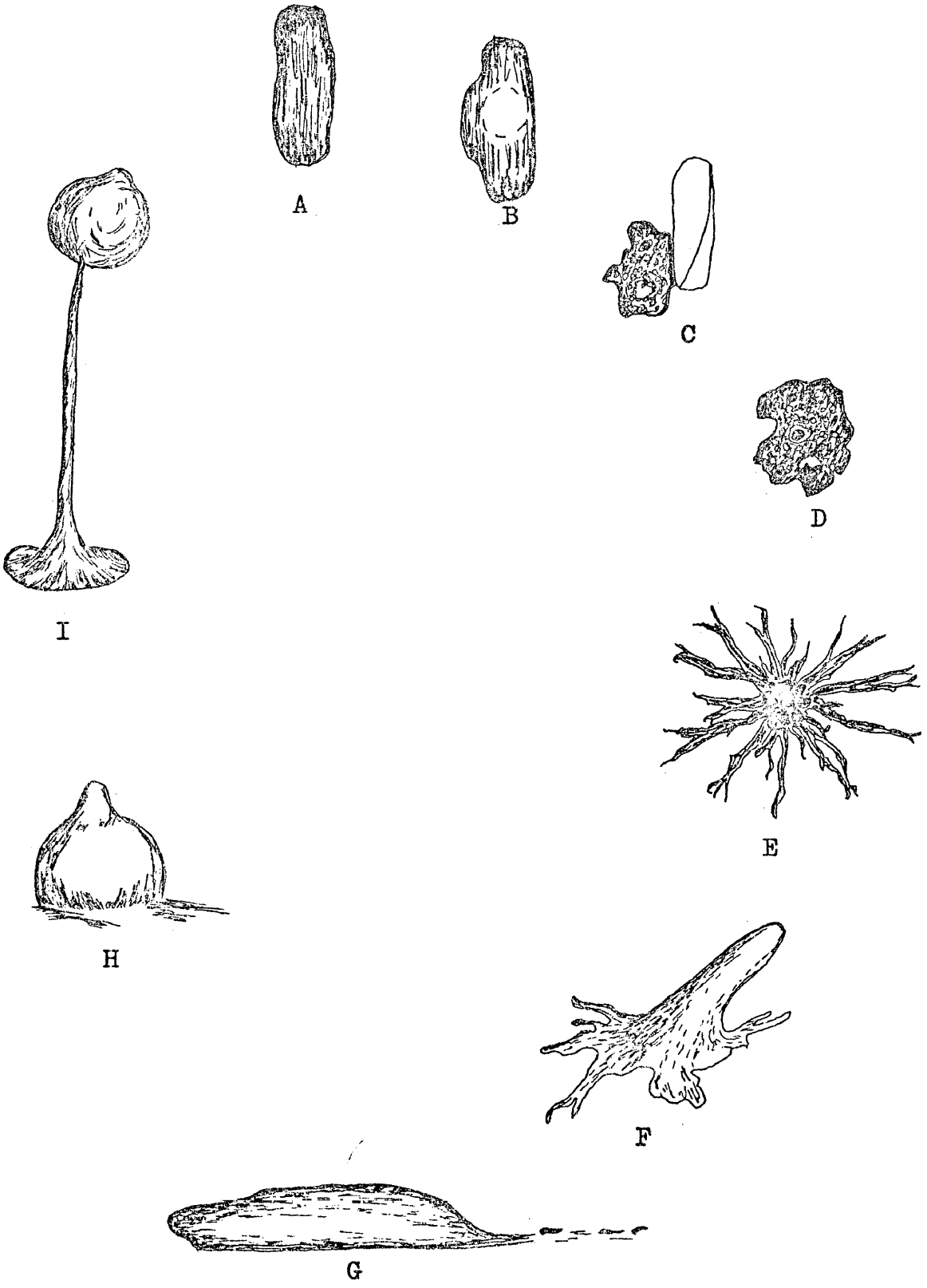
INTRODUCTION

In the life cycle (Fig. 1) of members of the Acrasiomycetes, the cellular slime molds, growth is separate from differentiation allowing independent study of these stages. The earliest stage in the life cycle is the spore. Each spore may germinate releasing a single amoeboid cell, the myxamoeba. Myxamoebae feed and grow independently until there is a depletion of the food supply. At this time growth virtually stops and the myxamoebae cease to move independently, streaming instead toward aggregation centers. By the end of aggregation, two morphogenetically different cell types appear, pre-spore cells and pre-stalk cells, which coexist in a structure resembling a common garden slug, called the pseudoplasmodium. The pseudoplasmodium migrates in a direction influenced by various physical conditions such as heat, light, pH and ionic content of the medium. Culmination follows migration and begins when the pseudoplasmodium assumes a vertical position on the substratum. The individual cells then complete differentiation with the pre-spore cells being raised up off the agar surface on a vertical, cellular stalk. The result is a mature, stalked structure, the sorocarp, with spores at the apex.

The purpose of this investigation was to determine the quantitative and qualitative effect of sodium azide

FIGURE 1 Life Cycle of Dictyostelium discoideum, representative member of the Acrasiomycetes.

A Dormant spore
B Swollen spore
C Myxamoeba emerging from spore case
D Vegetative myxamoeba
E Aggregation of myxamoebae
F Late aggregation
G Migrating pseudoplasmodium
H Early culmination
I Mature sorocarp


A
B
C
D
I
E
H
F
G

on the cellular slime mold Dityostelium discoideum. Spores, induced to germinate, were exposed to sodium azide and observed for normal swelling of the spores and emergence of myxamoebae. The effect of sodium azide on the vegetative multiplication of D. discoideum myxamoebae, the ability of pregrown myxamoebae to aggregate, to form pseudoplasmodia, and to continue normal morphogenetic development terminating in a mature sorocarp containing viable spores was investigated. The reversibility of sodium azide's effect on D. discoideum was tested. In addition, studies were made to determine if sodium azide affects oxygen uptake in vegetative myxamoebae.

LITERATURE REVIEW

Oxygen utilization in D. discoideum has been independently investigated by Gregg (1950) and Liddel and Wright (1961). Gregg found oxygen utilization, measured as μgO_2/hr/μgNitrogen with a respirometer, increased from the myxamoeba stage to the aggregation stage. The rate remained constant through aggregation into mid-culmination when a slow decrease began. This decrease continued until respiration completely ceased in the mature sorocarp. Liddel and Wright, using a Warburg apparatus, measured O_2 consumption as μgO_2/min/mg dry weight. They showed the oxygen consumption was highest in the myxamoeba stage, decreasing to a plateau from the migrating pseudoplasmodia stage through culmination and then decreasing again at the young sorocarp stage. The mature sorocarp continued to take up oxygen. These contradictory findings, particularly note worthy in the stages from myxamoeba to migrating pseudoplasmodium, have been considered by both authors. Gregg used total nitrogen as his reference in determining oxygen utilization. Liddel and Wright used dry weight as their basis. The ratio of total nitrogen/dry weight is greatest at the myxamoeba stage (Gregg and Bronsweig, 1956; Wright and Anderson, 1959) which would explain the lower rate found at this stage by Gregg (Liddel and Wright, 1961).

The free amino acid pool has been shown to decrease to 30% of its initial value during the transition from myxamoeba to migrating pseudoplasmodium thus a decrease in respiration could appear as an increase if based on total nitrogen (Wright and Anderson, 1960). Gregg (1964) considered the increased rate of movement (Samuel, 1961) and the increased oxygen tension requirements (Takeuchi and Tazawa, 1955) of myxamoebae composing the migrating pseudoplasmodium supportive evidence for his results since there would thus be increased energy requirements in the transition from myxamoeba to pseudoplasmodium. The conflicting evidence remains unresolved.

In the only study of oxygen concentration and spore germination of D. discoideum, Cotter and Raper (1967) showed that germination will not take place if the concentration of oxygen drops below 1mg/ml.

Mitochondria, the organelles associated with respiration in eucaryotic organisms, undergo distinct changes during the life cycle of D. discoideum. In the vegetative myxamoeba, the mitochondria have tubular rather than lamellar cristae and there are fewer inclusions than in the dumb-bell shaped mitochondria of the pseudoplasmodium. The appearance of mitochondrial inclusions reflect a degeneration of this organelle (George et al., 1972). These changes are correlated with the decrease in respiration from

myxamoeba to migrating pseudoplasmodium found by Liddel and Wright (1961). Further change in the morphology of the mitochondria is found in dormant spores where the mitochondria have a crenated appearance (Hohl and Hamamoto, 1969). During germination, mitochondria return to the vegetative form through a loss of density, reappearance of tubuli and the detachment of ribosomes from their periphery (Cotter et al., 1969).

A cytochrome-linked respiratory system is present in D. discoideum vegetative myxamoebae and pseudoplasmodia as evidenced by a color response to the Nadi reagent for cytochrome oxidase (Krivanek and Krivanek, 1958; Takeuchi, 1960). The activity of cytochrome oxidase decreases when the myxamoebae stop feeding, before they begin to aggregate, and it remains low through the pseudoplasmodial stage (Takeuchi, 1960).

2,4-dinitrophenol (DNP) is thought to be an uncoupler of oxidative phosphorylation allowing oxidation to occur without formation of high energy phosphate bonds (Lardy and Wellman, 1953). Hirschberg and Rusch (1950) and Gerish (1962) have found DNP inhibits aggregation in D. discoideum. Although these independent results show different inhibitory concentrations, the dependence of aggregation on oxidative phosphorylation is verified. Further, DNP has been shown to inhibit cells of D. discoideum in any stage of growth

or development by interfering with oxidative phosphorylations (Gerish, 1962).

Keilin (1936) first published information noting azide as an inhibitor of cellular respiration and oxidation of cytochromes. Azide acts to uncouple oxidative phosphorylation (Wilson and Chance, 1966). Sodium azide is known to inhibit respiration in some bacteria (Sevag, 1946; Lichstein and Soule, 1944; Bruemmer et al., 1957) while it stimulates respiration in others (Tissieres, 1951; Mitchell, 1962). Azide also inhibits bacterial catalase (Lichstein and Soule, 1944). Oxygen uptake in yeast cells is reduced by sodium azide (Keilin, 1936) which complexes with cytochromes a or a_3 (Ninnemann et al., 1970; Muijers et al., 1966). Endogenous respiration of fungal spores is stimulated with low concentrations of sodium azide while higher concentrations inhibit respiration (Mandels and Maquire, 1972).

Little data is available regarding the effect of sodium azide on D. discoideum. Hirschberg and Rusch (1950) found sodium azide at a concentration of 10^{-3}M partially inhibited the aggregation of D. discoideum myxamoebae, but they did not test the effects of sodium azide on other stages of the life cycle. Takeuchi (1960) showed that sodium azide will complex with cytochrome oxidase in pseudoplasmodia of D. discoideum. The process of germination can be stopped by high (7.5×10^{-4}M) concentrations of azide added after the spores are heat shocked (Cotter and Raper, 1968).

MATERIALS AND METHODS

Maintenance of Stock Cultures

Dictyostelium discoideum, strain NC4H, was maintained with routine transfers as follows: 0.5 ml samples of a mixed suspension of *Escherichia coli*, strain B/r, grown on glucose-salts medium (Adams, 1959) and spores from several sori were spread onto plates of glucose-salts medium. The composition of this medium is as follows (g/l): glucose, 4.0; NH_4Cl, 1.0; $MgSO_4$, 0.13; KH_2PO_4, 3.0; Na_2HPO_4, 6.0; and Bacto-agar, 15.

Germination Studies

Spores used in germination studies were removed from 2 to 7 day old sorocarps that had developed on glucose-salts medium, at 23 C. The spores were washed three times by centrifugation and suspended in 10 mM phosphate buffer, pH 6.4. In order to have synchronous germination, the suspension of spores was then heat shocked at 45 C for 30 minutes (Cotter and Raper, 1966). The heat shocked spores were pipetted into flasks of either phosphate buffer or sodium azide (Fisher Purified) and buffer to give from 10^5 to 10^6 spores per milliliter. The flasks were continuously shaken at 23 C on a gyratory water bath. At 30 minutes intervals, a 1 ml aliquot was removed from the flask, placed in a test tube, and stirred with a Vortex

Junior mixer to break up clumps of spores. A capillary tube fitted with an aspirator bulb was used to remove some of this suspension and transfer it to the chamber of an A-O Spencer hemacytometer for microscopic observation. The cells were examined and recorded as being dormant spores, swollen spores or emerged myxamoebae.

Reversibility of Germination Inhibition

In experiments to determine if germination inhibition was reversible, spores were washed, heat shocked and placed in flasks of phosphate buffer and sodium azide that were continuously shaken. The spores were washed free of sodium azide after 2 hours exposure and resuspended in buffer. Subsequently, the spores were examined at 0.5 hr intervals for germination. A spore was considered to have germinated when an emerged myxamoeba was seen.

Growth Studies

To obtain pregrown myxamoebae, spores of D. discoideum were added, with E. coli, as a food source, to flasks of liquid GYP medium (9/1); glucose, 1; yeast extract, 0.25; peptone, 2.0; KH_2PO_4, 4.2; and Na_2HPO_4, 2.7 (Weber and Raper, 1971). The flasks were incubated with constant shaking at 23 C. The emerged myxamoebae were observed for growth. When the cell number reached 10^4 to 10^5/ml, usually after 24 to 36 hr, sodium azide was added to the experimental

flasks. Cell number was recorded every 4 hr for approximately 24 hr by removing 1 ml samples from the flasks and agitating these samples in test tubes to break up clumps of myxamoebae prior to counting cell number with a hemacytometer. The growth rates of myxamoebae in various concentrations of sodium azide were compared with that of the control.

A Wild phase contrast microscope was used to determine cell number, and observe the refractility and pseudopod formation of the amoebae.

Reversibility of Growth Inhibition

In separate experiments, after the myxamoebae had been exposed to sodium azide for 4 hr, the approximate generation time, the myxamoebae were washed free of the sodium azide by three washings in phosphate buffer. The washed myxamoebae and fresh E. coli were resuspended in flasks of GYP medium and returned to the gyratory water bath at which time cell concentration was determined. Subsequent cell counts were made at 4 hr intervals, and the cells were microscopically examined for characteristics of sodium azide inhibition and to determine if they were active.

Developmental Studies

Myxamoebae were obtained from stock cultures as described above. Seventy-two hours after the flasks had

been inoculated with spores, the myxamoebae had reached the stationary growth phase. They were then harvested by centrifugation at 200 g and washed with phosphate buffer to free them of any remaining bacteria. A small amount of a dense suspension of these washed myxamoebae was pipetted onto Millipore filter discs in Millipore plates to give a concentration of approximately 10^4 myxamoebae/mm^2. The Millipore plates also contained an absorbent pad saturated with 2 ml of phosphate buffer. These plates were incubated at 23 C and observed for progress of development. At several stages of development the filter disc was removed and transferred to a plate prepared as above but with sodium azide included in the buffer solution. Development was followed for two days, unless culmination occurred earlier. The spores produced in the presence of sodium azide were heat shocked and spread on glucose-salts plates to determine viability.

Some filter discs were returned to plates containing only buffer after being exposed to sodium azide. The development in these plates was followed to determine if any effect observed would be reversed.

Anaerobic Experiments

Plates of glucose-salts medium were spread with a suspension of either heat shocked spores or myxamoebae and E. coli. The concentration of myxamoebae was determined

by averaging the number of cells present in 20 different fields of view at 100x. These areas were marked for later reference. The experimental and control plates were incubated at 23 C; experimental plates in GasPak anaerobic jars and control plates aerobically. After 2 days the plates were reexamined and the extent of germination and growth determined. Following these determinations, the experimental plates were left under aerobic conditions at 23 C for 4 days to determine if a return to aerobic conditions would reverse any effect of anaerobic incubation.

Detection of Oxygen Uptake

Myxamoebae were produced as described for growth studies. The myxamoebae were washed with buffer to remove the *E. coli*. The suspension of myxamoebae was adjusted to give a final concentration of $3x10^6$ myxamoebae per milliliter when added to a known amount of buffer or buffer and sodium azide. The buffer had been previously aerated by vigorous stirring with a teflon coated magnetic stir bar to produce an oxygen saturated solution. A Clark type oxygen probe (YSI series 5484) attached to a YSI model 54 oxygen meter, was inserted into the flask containing the myxamoeba suspension and it was sealed airtight with petroleum jelly. The oxygen meter was calibrated for ambient temperature and pressure prior to each experiment. Readings were taken approximately every seven minutes and the

temperature of the suspension was checked periodically. The liquid was constantly and unvaryingly mixed throughout the experiment to prevent oxygen depletion around the cathode. The mixing was accomplished by a stir bar contained within the flask which was raised off the surface of the power unit to avoid a temperature increase.

RESULTS

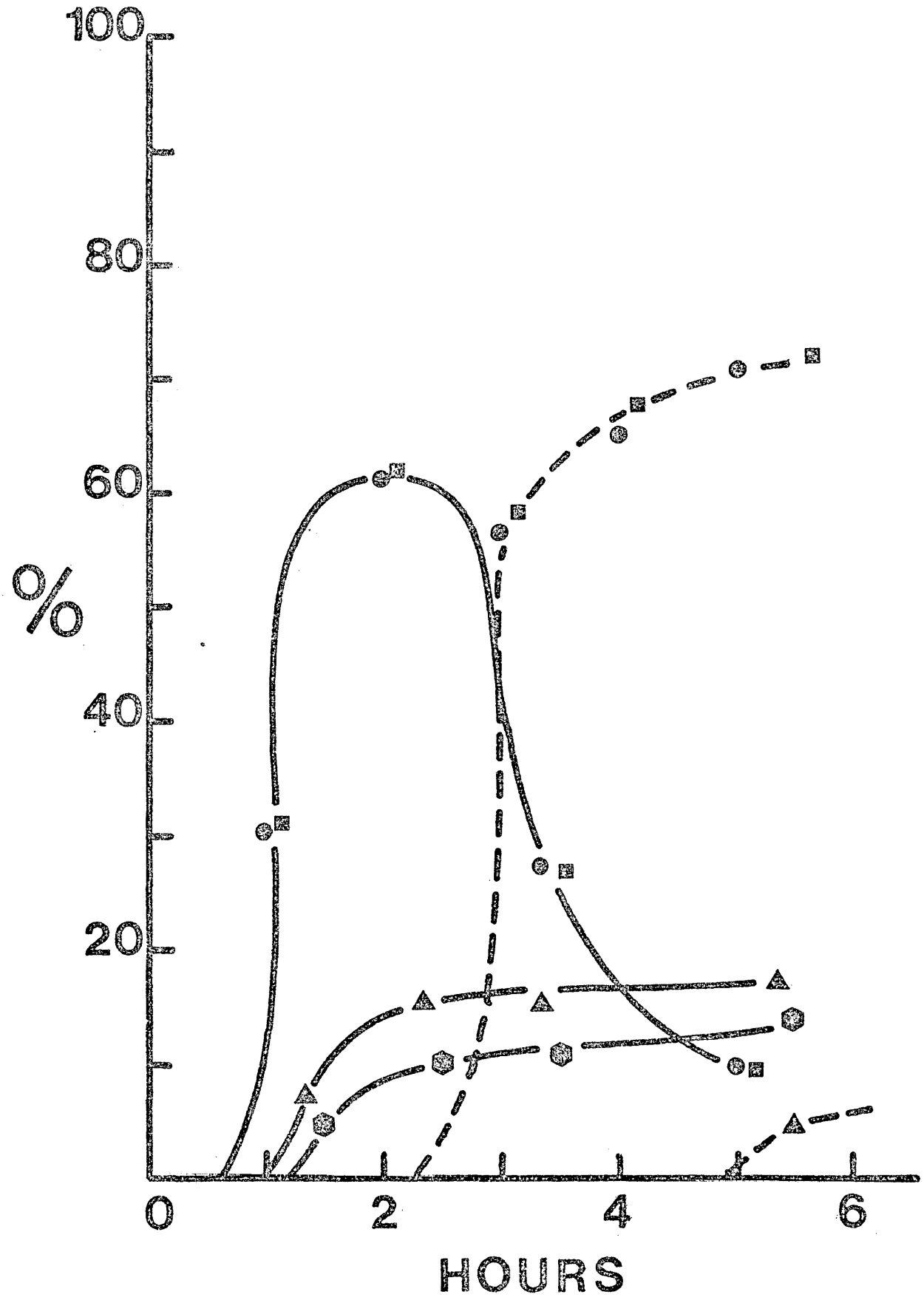
Germination and Sodium Azide

When heat shocked spores of D. discoideum were placed in a buffered azide solution at concentrations above 1.5×10^{-5}M, the percent germination was lower than in the control without azide. The percentage of spores that successfully completed germination decreased with an increase in sodium azide concentration from 1.5×10^{-5} to 2.5×10^{-4}M (Fig. 2). Emergence of myxamoebae was totally inhibited at an azide concentration of 3×10^{-4}M. Swelling did occur at 3×10^{-4}M azide but, in general, the number of swollen spores and the degree of swelling of spores decreased as the concentration of the inhibitor increased above 1.5×10^{-5}M.

The time sequence of events in germination was also altered when sodium azide was included in the buffer solution. The onset of germination, marked by the swelling of spores, was delayed when sodium azide was present (Fig. 2). The delay period lengthened with increased concentration of the inhibitor. In order to determine if the effect of sodium azide on spores could be reversed, spores were washed, heat shocked and exposed to sodium azide for 2.5 hr. After this length of time at least 70% of the control spores had entered the swollen state. The spores were then washed, to remove the sodium azide and returned to buffer. The

FIGURE 2. Germination kinetics of D. discoideum spores after heat shocking. Solid lines, percent swelling; broken lines, percent emergence.

- ● control without sodium azide
- ■ 1.5×10^{-5}M azide
- ▲ 1.5×10^{-4}M azide
- ◆ 3×10^{-4}M azide


100
80
60
40
20
%
0
2
4
6
HOURS

effect of the $3x10^{-4}$M solution of azide which had inhibited completion of germination was partially reversible. At this concentration of inhibitor the percentage of spores that completed germination after the removal of sodium azide was less than in the control. As the concentration of sodium azide was decreased from $3x10^{-4}$M to $1.5x10^{-5}$M the reversibility of its effect was more complete (Fig. 3). Spores were exposed for 2 hr to various concentrations of sodium azide, washed and then plated on buffered 1% peptone medium. Culmination occurred in all plates, but the higher the concentration of inhibitor, the longer it took for mature sorocarps to form. Also, the number of sorocarps varied; ie., more sorocarps were formed after exposure to lower concentrations of sodium azide.

Increase in Cell Number in Sodium Azide

The generation time for D. discoideum, strain NC4H, was approximately 4 hr under control conditions. Sodium azide concentrations of $1.5x10^{-6}$ to $1.5x10^{-5}$M had no observable effect on the growth of D. discoideum myxamoebae as determined by increase in cell number (Fig. 4). Concentrations of $7.5x10^{-5}$ to $2.5x10^{-4}$M azide reduced the rate of growth, with more inhibition occurring at higher concentrations. The formation of pseudopods and amount of movement was also limited at these concentrations. At $3x10^{-4}$M azide

FIGURE 3. Percent emergence of myxamoebae from heat shocked D. discoideum spores preexposed to sodium azide.

● control without sodium azide

▲ 1.5×10^{-4}M azide

◆ 3×10^{-4}M azide

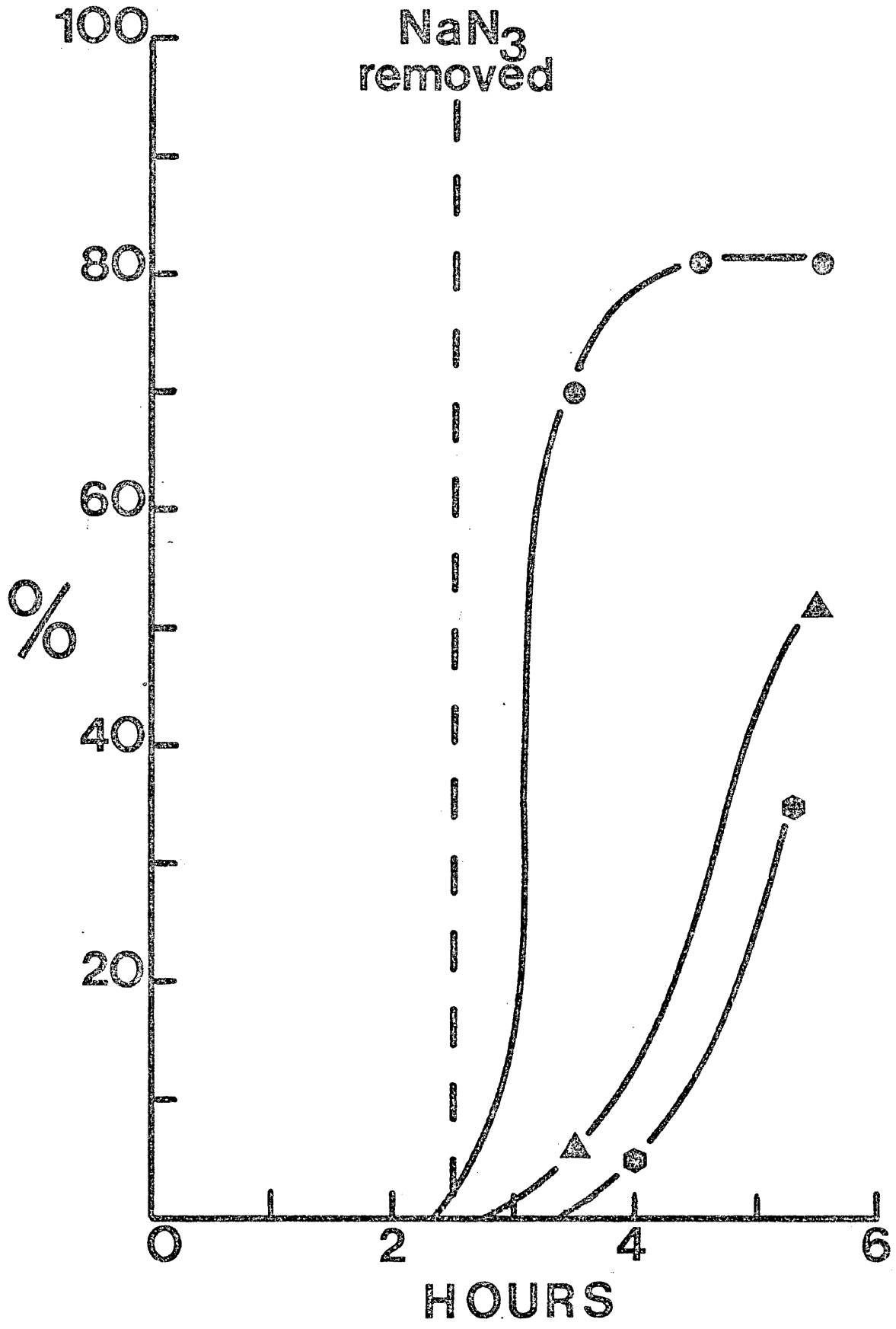

100
80
60
40
20
%
NaN_3 removed
0
2
4
6
HOURS

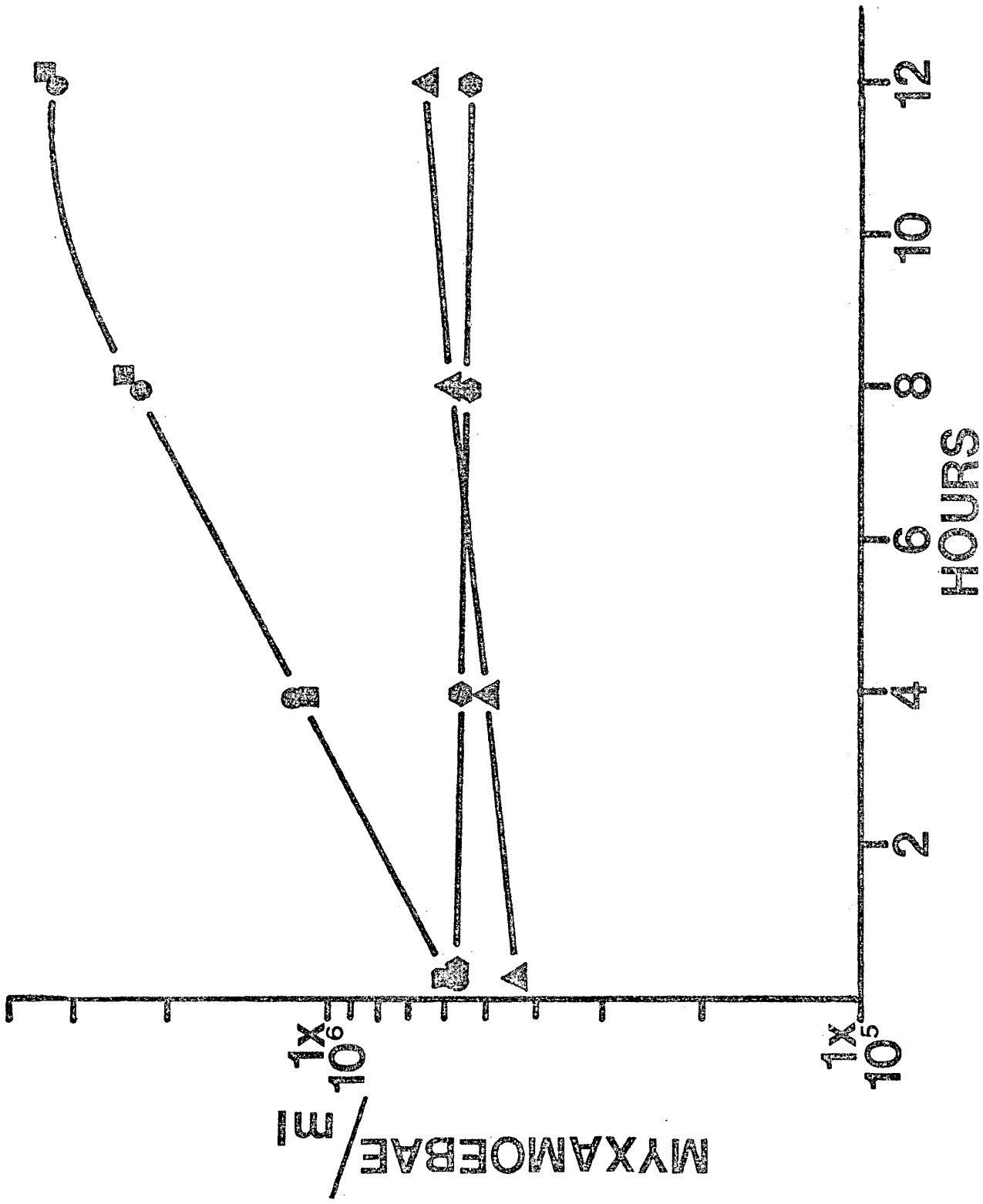
FIGURE 4. Growth of D. discoideum myxamoebae after addition of sodium azide.

● control without sodium azide

■ 1.5×10^{-5}M azide

▲ 1.5×10^{-4}M azide

◆ 3×10^{-4}M azide


MYXAMOEBAE/ml
1x10^6
1x10^5
2
4
6
8
10
12
HOURS

and above, growth was totally inhibited. Microscopic observation showed the myxamoebae had rounded, become less refractile and did not possess pseudopods after four hours exposure to $3x10^{-4}$M azide (Figs. 5 and 6).

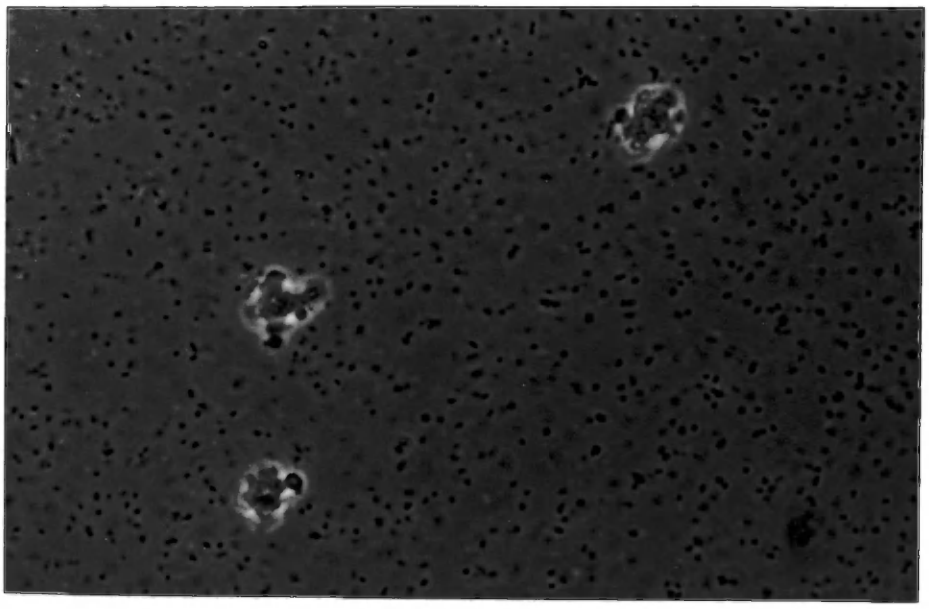
The growth rate of myxamoebae increased when myxamoebae, exposed to the inhibitor, were washed with buffer to remove the sodium azide and returned to the growth medium (Fig. 7). Normal logarithmic growth of the control cells did not resume until 4 hr after the cells were washed with buffer and returned to the growth medium. The treated cells also underwent a lag period after washing before they attained a growth rate comparable to that of the control cells.

Development Studies

When pregrown vegetative myxamoebae were placed on a Millipore filter disc and immediately exposed to sodium azide, culmination did not occur. Aggregation of myxamoebae did begin on plates of $1.5x10^{-4}$ and $2.5x10^{-4}$M azide producing mounds of cells visible at 10X magnification. Myxamoebae exposed to $3x10^{-4}$M azide did not begin aggregation and no observable mounds of cells occurred. Transfers made to $1.5x10^{-4}$ and $2.5x10^{-4}$M azide, after aggregation had begun, produced only 2 to 4 pseudoplasmodia-like structures as opposed to a minimum of 50 on control plates. These

FIGURE 5. Control myxamoebae in GYP medium without sodium azide.

FIGURE 6. Myxamoebae after 4 hr exposure to inhibiting concentrations of sodium azide.



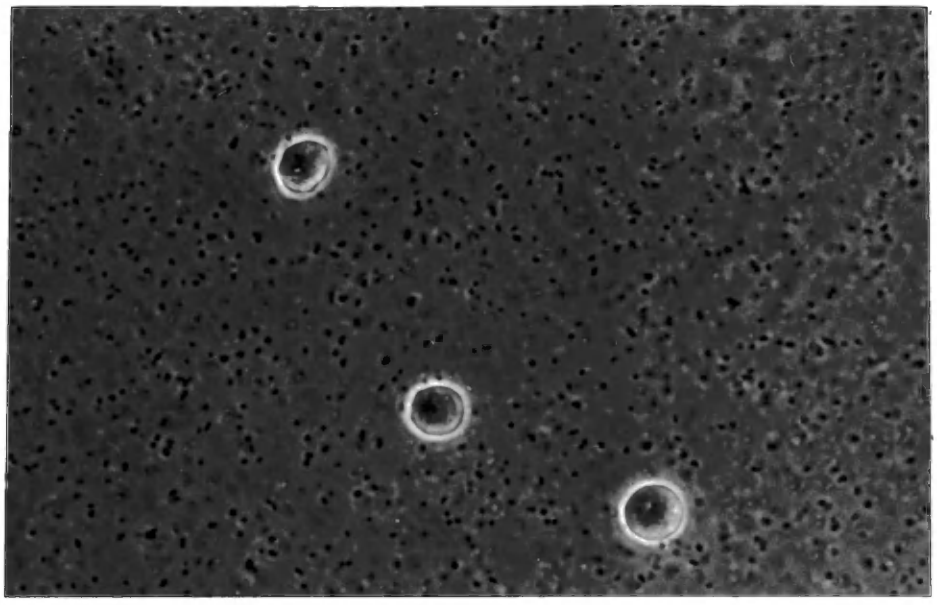
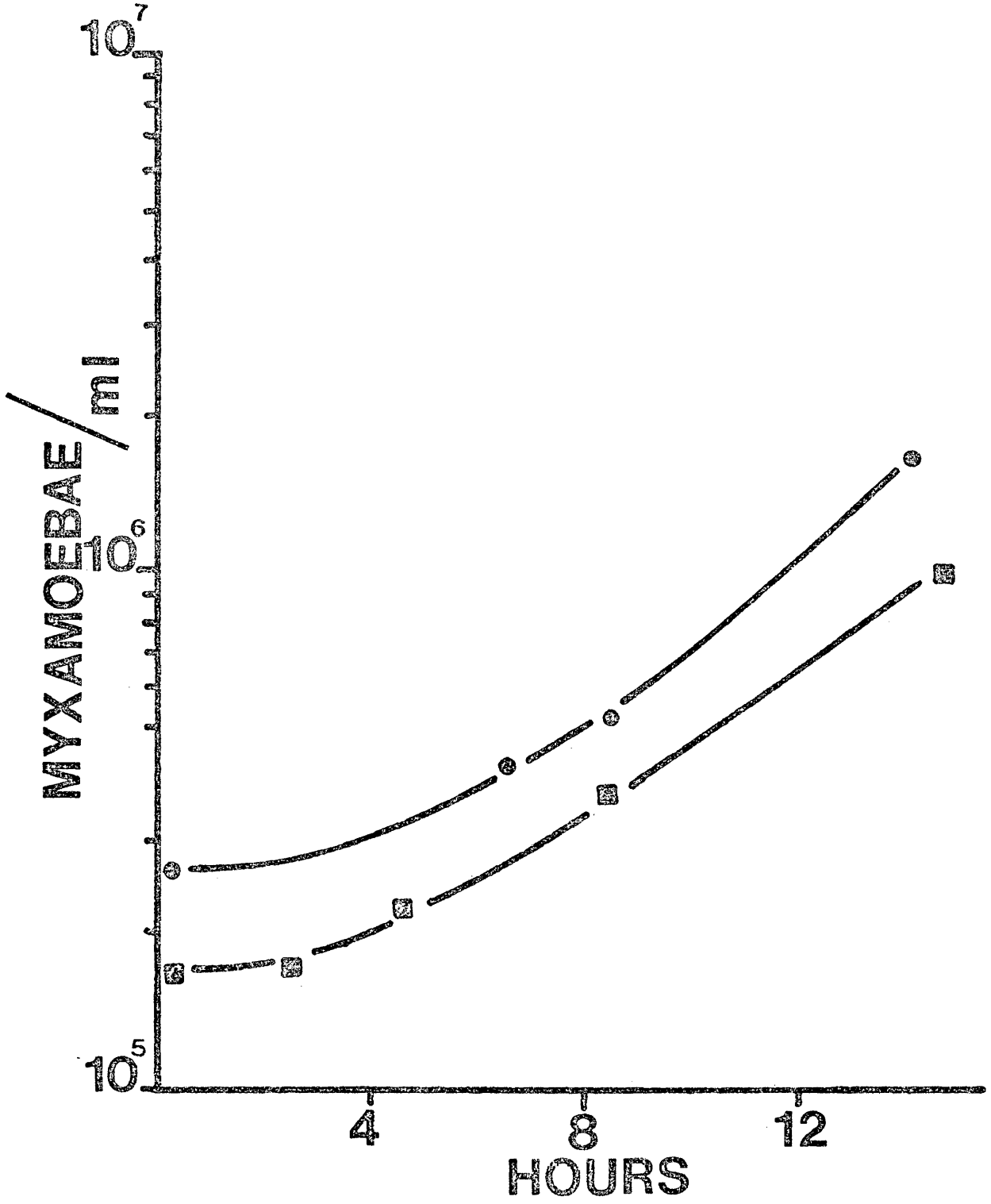


FIGURE 7. Growth of D. discoideum myxamoebae after the removal of an inhibitory concentration of sodium azide.

⊗ control without sodium azide

▩ 3×10^{-4}M azide


10^7
10^6
10^5
MYXAMOEBAE / ml
4
8
12
HOURS

structures were composed of vacuolate cells. Any further development of cells transferred in midaggregation was stopped by $3x10^{-4}$M azide.

Migrating pseudoplasmodia continued to develop and produce sorocarps, comparable to those in control plates, in the presence of $1.5x10^{-5}$M azide. Sorocarps were, on the average, smaller and slower to develop when formed in $7.5x10^{-5}$M azide. Further progress was stopped by sodium azide concentrations of $1.5x10^{-4}$, $2.5x10^{-4}$ and $3x10^{-4}$M.

Culmination was considered to have begun when the pseudoplasmodia oriented vertically. When transfers were made at the onset of culmination, sorocarps formed in the presence of all concentrations of inhibitor that were tested. However, these sorocarps had shorter stalks and smaller sori than the control sorocarps. Also, sodium azide affected the rate at which the sorocarps formed, slowing down the progress of culmination (Table I).

An attempt was made to determine if the inhibitory effect of sodium azide on development in D. discoideum might be reversed by returning the inhibited cells to a buffer solution without the inhibitor. Cells held at mid-culmination for 2.5 hr completed development and produced normal sized sorocarps when removed from the inhibitor. Migrating pseudoplasmodia, previously exposed to $3x10^{-4}$M azide for 4 hr, produced a few small sorocarps. Vegetative

Table I DEVELOPMENTAL STAGE REACHED FOLLOWING TRANSFER TO SODIUM AZIDE MEDIUM.

MOLARITY OF SODIUM AZIDE SOLUTION	STAGE WHEN TRANSFERED: VEGETATIVE MYXAMOEBAE	MID AGGREGATION	MIGRATING PSEUDOPLASMODIA	BEGINNING CULMINATION
0	SOROCARP	SOROCARP	SOROCARP	SOROCARP
1.5×10^{-5}	---	---	SOROCARP	---
7.5×10^{-5}	---	---	SOROCARP	---
1.5×10^{-4}	AGGREGATION	PSEUDOPLASMODIUM	PSEUDOPLASMODIUM	SOROCARP
2.5×10^{-4}	AGGREGATION	PSEUDOPLASMODIUM	PSEUDOPLASMODIUM	SOROCARP
3.0×10^{-4}	MYXAMOEBA	AGGREGATION	PSEUDOPLASMODIUM	SOROCARP

myxamoebae which were exposed to sodium azide for 13 hr did not begin development when removed from the inhibitor.

Anaerobic Effects on Germination and Growth

Heat shocked spores of D. discoideum did not germinate under anaerobic conditions on glucose-salts medium at 23 C. The spores showed no swelling after 48 hr. These same spores did however germinate, without further treatment, when returned to aerobic conditions.

The suspension of myxamoebae and E. coli was spread on plates containing growth medium. The density of myxamoebae on these plates was determined as number per 0.25 mm^2. There was not a noticeable increase in number of myxamoebae when the cells were incubated anaerobically. The myxamoebae present on the plates did not undergo any morphogenetic movement. When returned to aerobic conditions the myxamoebe aggregated and formed average size sorocarps within two days.

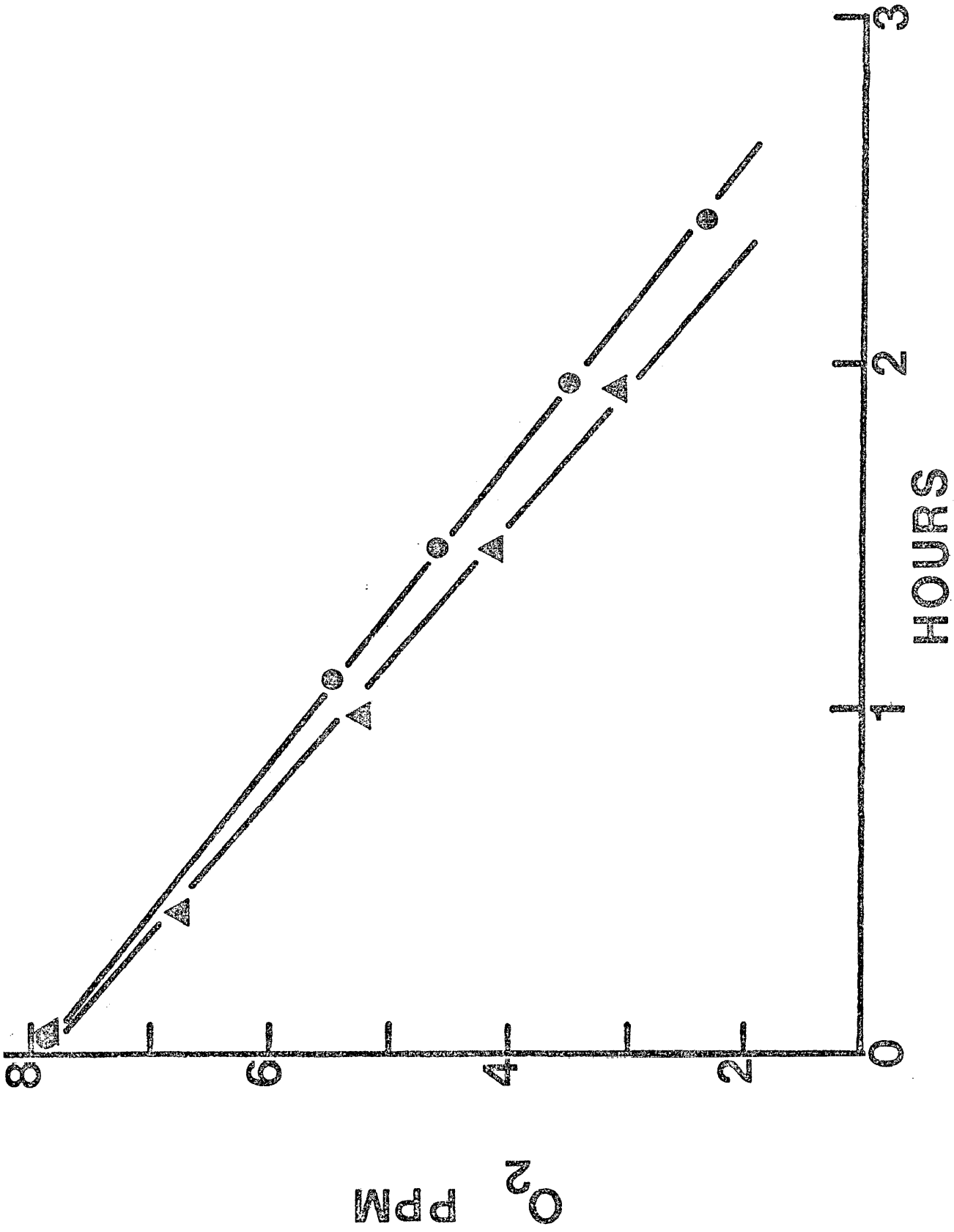
Oxygen Uptake in the Presence of Sodium Azide

Oxygen uptake by myxamoebae in a solution of buffer and sodium azide was comparable to the uptake by control cells (Fig. 8). The concentration of sodium azide which had proven inhibitory to cell growth was used in an attempt to demonstrate the possible mode of inhibition. These experiments showed that at $3x10^{-4}M$ azide, division of myxamoebae stops but oxygen uptake continues unaffected.

FIGURE 8. Oxygen uptake by D. discoideum myxamoebae suspended in buffer.

● control without sodium azide

▲ $3x10^{-4}$M azide


0
1
2
3
HOURS
0
2
4
6
8
O_2 PPM

DISCUSSION

It is evident from these investigations that sodium azide has an inhibitory effect on D. discoideum at nearly every stage in the life cycle. It is beyond the scope of this investigation to determine the mode of inhibition. However, my results show that sodium azide is not inhibiting the oxygen uptake of vegetative myxamoebae at the minimum inhibitory concentration for cell division.

Sodium azide does not stop the germination process at any one stage. At $3x10^{-4}M$ some spores will begin germination and swell, but emergence of myxamoebae is completely suppressed. At lower concentrations, emergence of myxamoebae will occur although the number of spores completing germination is less than in the control. At a concentration of $1.5x10^{-5}M$ sodium azide did not inhibit the swelling of spores or the emergence of myxamoebae. Cotter and Raper (1968) found that $7.5x10^{-4}M$ azide inhibits swelling in heat shocked spores of D. discoideum. More recently, Cotter (personal communication) has shown that the swelling of dimethyl sulfoxide induced spores can be prevented by $2x10^{-3}M$ azide. The method of inducing germination used in my investigations was heat shock. The mechanisms involved in the two ways of activation are not the same and only produce a similar overall result. Cotter may be observing

a different type of inhibition in the dimethyl sulfoxide induced spores.

If spores are exposed to $3x10^{-4}$M azide, immediately after heat shocking, they begin the germination process, some proceeding to the swollen state. When the sodium azide is removed from the heat shocked spore suspension some of the swollen spores complete germination within 1 hr as compared to a control time of 2.5 hr after heat shocking. These results indicate that sodium azide does not inhibit the initial stage(s) of spore germination. Whatever the effect sodium azide has on the spores, they are able to respond to heat activation. There may be a change in permeability to sodium azide after the initial stage(s) of spore germination. Similar results have recently been obtained by Charest and Cotter (personal communication).

The heat shocked spores that had been exposed to $3x10^{-4}$M azide and then washed free of the inhibitor did not show as great a percentage of germination as the control spores. The myxamoebae which emerged from reversibly exposed spores were capable of vegetative growth and morphogenetic development into mature sorocarps. At high concentrations of inhibitor there were fewer viable cells, therefore fewer sorocarps, and these required more time to form.

After four hours exposure to $3x10^{-4}M$ azide, all vegetative myxamoebae appeared characteristically inhibited (Fig. 6). The inhibition was reversible in nearly all myxamoebae after exposure for four hours. Reversible inhibition is known in other organisms (Lichstein and Soule, 1944). Washing the myxamoebae two times with buffer delayed the attainment of a logarithmic growth rate, even with the control cells. Normal growth of myxamoebae requires aeration of the suspension and this could not be accomplished while centrifuging during the process of washing out the inhibitor and thus may in part explain this lag period. It is common to observe a lag in growth rate when bacteria are washed in a similar manner. Compounds are present which must exist at a minimum concentration in order to promote growth, and these can be washed out.

The development of D. discoideum was also inhibited by sodium azide. Aggregation was not completed inhibited until a concentration of $3x10^{-4}M$ was reached. This evidence contradicts the findings of Hirschberg and Rusch (1950) who listed $10^{-3}M$ azide as the lowest concentration which would inhibit aggregation of D. discoideum myxamoebae. This is the same concentration that inhibited the movement of vegetative myxamoebae which explains why aggregation could not occur, assuming that inhibition by sodium azide is the same for feeding and non-feeding myxamoebae.

Concentrations of $1.5x10^{-4}$ and $2.5x10^{-4}$M azide permitted development up to initial aggregation, but no further. Therefore, it would be expected since development had stopped at these concentrations, that cells which had already aggregated would not progress beyond that stage. Pseudoplasmodia-like structures did appear on these plates but there were only 2 to 4 per plate and these were small structures composed of vacuolate cells which had been raised vertically off the substratum.

Sorocarps did form when pseudoplasmodia were placed in a concentration of $7.5x10^{-5}$M azide and below but not at concentrations above that. It is likely that some cells were inhibited even at the lower concentrations because the sorocarps were smaller than those in control plates and were slower to develop, an indication fewer cells were participating.

Once the cell mass was up off the substratum and not in direct contact with the inhibitor, development continued, even in $3x10^{-4}$M azide. The size of sorocarps and the rate at which they were produced was different from the control for those formed in plates containing the inhibitor. Some cells were necessarily placed in direct contact with the inhibitor and may have been affected. Thus there may have been a redifferentiation of pre-spore cells to stalk cells which would take time and reduce the number of cells comprising the mature sorocarp.

Sodium azide's effect(s) on D. discoideum cells undergoing development could be reversed by removing the cells to buffer without the inhibitor. This reversibility was possible at the aggregation and pseudoplasmodia stages. More information is needed before any further statement can be made on this matter.

The heat shocked spores placed in anaerobic jars during this investigation did not show any swelling after 48 hr, although when heat shocked spores were exposed to $3x10^{-4}$M azide noticeable swelling did occur. Oxygen is required for the germination of heat shocked spores of D. discoideum (Cotter and Raper, 1967). In the presence of $3x10^{-4}$M azide, activated spores can utilize oxygen and will proceed to the swollen state.

There is some question whether division of vegetative myxamoebae occurs under anaerobic conditions (Liddel and Wright, 1961). The results of this investigation show that cell division does not occur in vegetative myxamoebae kept on a solid growth medium with a food supply when oxygen is not present. The myxamoebae incubated anaerobically for 48 hr were rounded similar to azide treated cells, and aggregation had not occurred.

When these investigations were begun I thought that sodium azide would act as a repiratory inhibitor of D. discoideum. It is known that azide inhibits oxidative

phosphorylation in other organisms. The concentration of sodium azide which completely inhibited emergence of myxamoebae, cell division and stages of development did not decrease the oxygen uptake of myxamoebae. The myxamoebae were exposed to the inhibitor and oxygen concentration was monitored for 3 hr. Within the first three hours after addition of sodium azide, oxygen consumption occurred at approximately the same rate in the presence or absence of the inhibitor (Fig. 8). Charest and Cotter (personal communication) have found oxygen uptake of spores to be inhibited by $2x10^{-3}M$ azide. Concentrations in that range were not used in these investigations. At the high concentrations of sodium azide used by those authors the prevention of oxygen uptake may not be the primary result of the treatment. As has been mentioned earlier in the text, sodium azide may have two modes of action; e.g., at low concentrations it stimulates endogenous respiration and at higher concentrations it inhibits respiration (Mandels and Maguire, 1972). This biomodal action is common to other inhibitors such as DNP (Mathre, 1969). The critical point here is that at $3x10^{-4}M$ azide cell division is prevented although oxygen uptake is not inhibited and one might expect that the respiration of spores would be similarily unaffected.

Other respiratory inhibitors should be further tested on D. discoideum. The effect of $10^{-3}M$ azide on oxygen

uptake of myxamoebae needs to be determined. Knowledge of the permeability of spores to sodium azide would help establish the reason for the swelling of spores exposed to sodium azide.

SUMMARY

The effect of sodium azide on the germination, growth and development of Dictyostelium discoideum, a cellular slime mold, was determined. Heat shocked spores were exposed to sodium azide in phosphate buffer. The spores reached the swollen stage in a $3x10^{-4}M$ solution of sodium azide but there was no emergence of myxamoebae.

Pregrown myxamoebae were exposed to sodium azide in glucose-salts medium containing Escherichia coli. The amount of growth inhibition increased with an increase in concentration of sodium azide. Myxamoebae, developing on Millipore filter discs, were transferred at various stages to plates containing sodium azide. Morphogenetic development was influenced by the presence of sodium azide. The degree of inhibition depended on the stage of development that had been reached prior to exposure. The minimum inhibitory concentration of sodium azide was $3x10^{-4}M$ for germination and growth with incomplete inhibition occurring at lower concentrations. The inhibitory effect of sodium azide could be reversed at all stages of the life cycle by washing the cells with phosphate buffer. The time for recovery from sodium azide treatment after washing was longer when higher concentrations of the inhibitor were used.

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