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THERMOSENSING AND PHOTOSENSING IN
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CHOO BONG HONG

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THERMOSENSING AND PHOTOSENSING IN *DICTYOSTELIUM DISCOIDEUM* AMOEBAE

By

CHOO BONG HONG

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ABSTRACT

THERMOSENSING AND PHOTOSENSING
IN DICTYOSTELIUM DISCOIDEUM AMOEBAE

by

Choo Bong Hong

Amoebae of D. discoideum, the parental strain HL 50, showed only positive thermotaxis after 3 hr on temperature gradients(0.22°C/cm) throughout the temperature range checked. This response was somewhat stronger at lower temperatures than higher temperatures. Negative thermotaxis developed with exposure time on the thermal gradients. After 16 hr on the gradients, the amoebae clearly showed negative and positive thermotactic responses with the transition temperature between negative and positive at about 19°C.

The thermotactic responses of mutants derived from HL 50 by means of N-methyl 1-N'-nitro-N-nitrosoguanidine also have been investigated. The thermotactic responses of mutants, HO 596 and HO 1445, also changed with time on the thermal gradients(0.22°C/cm). Amoebae of these mutants showed a negative as well as a positive response after 3 hr on the thermal gradients. HO 428 is a mutant which lacks a

negative thermotactic response, and its response does not change with time on the gradient(0.22°C/cm). This mutant shows a stronger positive response at lower temperatures and a weaker response above the growth temperature of the amoebae.

Temperature response curves of amoebae after 16 hr on the gradient were similar to those of the slugs from both the parental and mutant strains. Based on this fact, it is proposed that amoebal thermotaxis is the basis for slug thermotaxis.

Both axenically and nonaxenically grown D. discoideum amoebae show positive and negative phototactic responses dependent upon the light intensity. Low temperature(77°K) absorption spectra of the nonaxenic strains lack the peak at 638 nm previously observed in the axenically grown amoebae and associated with protoporphyrin. This fact argues against protoporphyrin as the primary photoreceptor pigment for amoebal phototaxis. An alternative function for protoporphyrin in amoebal phototaxis may be as a screening pigment permitting the amoebae to detect the direction of unilateral radiation.

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LIST OF ABBREVIATIONS

ATPase	Adenosine triphosphatase
cAMP	Adenosine 3',5'-cyclic phosphoric acid
CAPS	Cyclohexylaminopropanesulfonic acid
cGMP	Guanosine 3',5'-cyclic phosphoric acid
CTAB	Cetyl trimethyl ammonium bromide
Cyt. c	Cytochrome c
nE	nano Einstein
EDTA	Ethylenediamine tetraacetate
MES	2-(N-Morpholino)ethanesulfonic acid
MOPS	3-(N-Morpholino)propanesulfonic acid
NADH	Nicotinamide adenine dinucleotide (reduced form)
P_{630}	Photoreceptor pigment which has an absorption peak at 630 nm
P_{638}	Photoreceptor pigment which has an absorption peak at 638 nm
SDS	Sodium dodecyl sulfate
STF	Slug turning factor
Tris	Tris(hydroxymethly)aminomethane
Zero C.P.	Zero-crossing point

INTRODUCTION

In contrast with those processes, such as photosynthesis in plants and ingestion of food in animals, which provide energy to an organism, the ability of an organism to sense its environment has been heavily studied only in recent years. The major difference in these important phenomena is that the energy providing processes usually depend on a single form of energy, i.e., in photosynthesis, light is the sole energy source, whereas, in sensing, an organism may perceive many environmental stimuli. Through its sensory processes an organism is able to detect and respond to subtle changes in its environment, allowing it to efficiently find those conditions which are optimal for its survival.

Environmental stimuli which organisms sense are light, sound, chemicals, mechanical force, temperature, and gravity. Of these, sound alone is not perceived by higher and lower plants.

In plants, environmental sensing usually causes a common result, i.e., change in shape of the organism or movement of the organism. Phytochrome-controlled photomorphogenesis in plants and shedding leaves from a tree in preparation for winter are good examples showing a change

of shape of the organism as a result of sensing its environment. Chemotaxis of bacteria and phototropism of plants are two representative examples of the ability of an organism to sense its environment and, as a result, change its location.

These sensory processes are extremely efficient, and as a consequence, require high sensitivity and stability of the detecting mechanism. Thus, it would come naturally to use a unicellular, even prokaryotic organism, such as bacteria, as a model system for dealing with the sensing mechanism. Multicellular organisms usually add a great deal of complexity to studying these sensory processes.

Dictyostelium discoideum has been found in nature as a soil amoeba in forest detritus throughout the world. The cells feed on the bacteria of decaying matter and continue vegetative growth by binary fission. However, when the environment is depleted of food, D. discoideum follows a characteristic developmental cycle which is initiated by aggregation of amoebae, and this life cycle has been readily reproduced in the laboratory. Thus, this organism had a characteristic feature which usually cannot be found in others, i.e., unicellular and multicellular stages can be separated and controlled conveniently, and probably it is the main reason that D. discoideum has become a model eukaryotic system in which to study a variety of aspects concerning development, morphology, biochemistry, genetics and so on. In addition to this characteristic life cycle

D. discoideum has diverse responses to its environment which have been relatively easy to detect. These various sensing abilities together with a well characterized development, conveniences in doing genetics and biochemistry, and ease of culture have made D. discoideum an amenable system in which to study sensory transduction in a eukaryotic organism.

Among the important environmental factors for life, chemicals, temperature and light have been convenient handles and the most studied areas in search of the sensory system of D. discoideum. This dissertation will present results concerning thermal responses and photoresponses of D. discoideum amoebae in an attempt to contribute toward an understanding of the mechanism of sensory transduction in D. discoideum. It is hoped that such an understanding will contribute toward an understanding of sensory transduction in general. The dissertation is divided into two parts. The first part will be concerning thermal responses and the second part will be about photoresponses.

Chapter one includes a literature review and general materials and methods which have been used throughout the experiments. The literature review will start by covering the life cycle of this organism because its well-characterized development has contributed greatly to the works of sensory transduction. In Chapter two, thermotaxis of D. discoideum amoebae will be reported, and in Chapter three, several thermotaxis mutants will be compared and possible mechanisms in amoebal thermotaxis will be discussed.

Chapter four describes the photobehaviour of D. discoideum amoebae and includes an action spectrum of amoebal phototaxis. In Chapter five, possible photoreceptor(s) in D. discoideum amoebal phototaxis and their intracellular localization will be described. Phototactic behaviours of those thermotactic mutants used in Chapter 3 have been examined based on the indications from slugs which show possible connections between thermo- and photo-transduction pathways in D. discoideum, and will be used to discuss a possible photosensory transduction pathway in D. discoideum amoebae in Chapter six.

In Chapter seven, possible connections between amoebal and slug photo- and thermo-sensings will be presented and discussed.

CHAPTER 1

LITERATURE REVIEW AND GENERAL MATERIALS AND METHODS

1.1. Literature Review

D. discoideum is a primitive eukaryotic organism with a well-defined morphogenesis and a rich variety of responses to environmental stimuli. These facts, in addition to the ease of culture, contribute to the popularity of D. discoideum as a model system from which we hope many results may be extrapolated both to higher plants and animals.

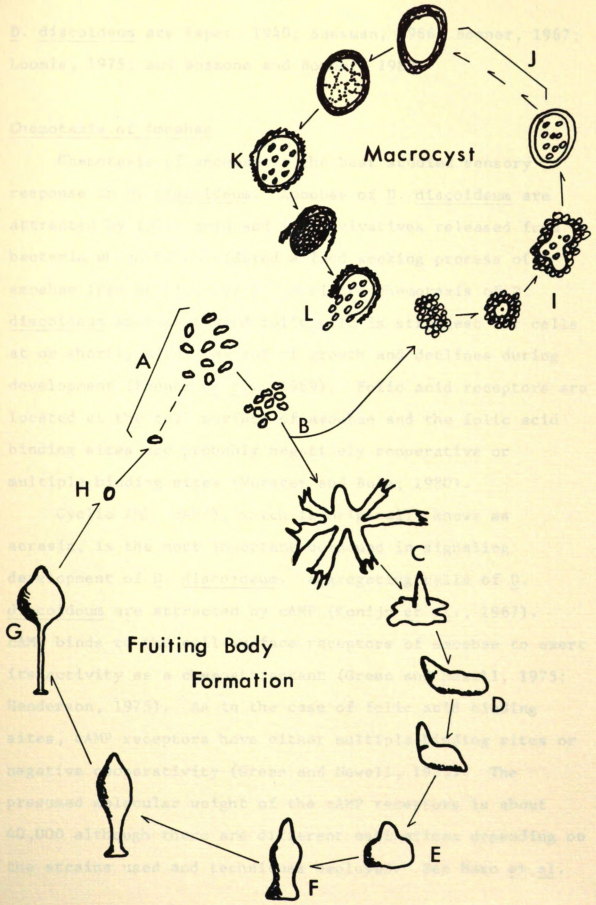
The literature review will cover the life cycle of this organism first and then will cover the fields of sensory transduction of this organism. The literature in the field of sensory transduction which will be reviewed are: 1. Chemotaxis of amoebae, 2. Thermotaxis of amoebae, 3. Phototaxis of amoebae, 4. Slug chemotaxis, 5. Slug thermotaxis, 6. Slug phototaxis.

Life Cycle

Amoebae growing in association with bacteria as a food source start development when the bacterial food is exhausted. These amoebae can follow two different morphogenetic sequences depending on the conditions at that time (Figure 1). These conditions would allow for the changing

cell surface properties of amoebae which can determine the destiny of these cells. Macrocyst-forming amoebae respond differently to treatment with various lectins and sugars than amoebae developing into spores. When the starved amoebae in the dark face an excess of moisture or absence of phosphate, two amoebae may fuse and form a macrocyst which can eventually liberate amoebae. Macrocyst formation occurs before approximately 12 hr following the onset of starvation, and once this critical time point has passed, amoebae are irreversibly developed into another alternative step of the life cycle which will generate spores. Instead of forming a macrocyst, the amoebae may form a pseudoplasmodium and a fruiting body which contains many spores. After an interphase period, the amoebae aggregate into mounds containing about 10^3 to 10^5 cells by responding chemotactically to cyclic AMP (cAMP). A tip of the mound extends upward and then falls over resulting in a finger-like structure called a pseudoplasmodium, glex, or slug. Following a period of migration, the tip of the slug stops migrating causing the slug to round up and form a structure which looks like a "Mexican hat". The prestalk cells which are found in the center of the aggregate develop into vacuolated stalk cells. The stalk cells extend lengthwise pushing the maturing spore mass upward. The spores, then, can be dispersed by wind, rain, or insects, and germinate under suitable conditions to produce amoebae which can reinitiate the developmental cycle. For reviews concerning the life cycle of

Figure 1. Life cycle of D. discoideum. The center of the figure depicts the commonly studied aspects of the slime mold life cycle: vegetative growth of haploid amoebae and fruiting body formation. Fruiting body formation occurs when starved amoebae are placed on an air-water interface in the presence of visible light, and it culminates with the differentiation of approximately 80% of the cells into spores. Such spores, under appropriate conditions, germinate and liberate amoebae, which can then grow vegetatively. The upper right-handed portion of the figure depicts the formation and germination of macrocysts. This cycle is favored by starved amoebae in the dark, the presence of excess moisture, or the absence of phosphate. Two amoebae fuse to form a diploid giant cell. It has been implied from genetic experiments that meiotic divisions yield four haploid nuclei. Amoebae liberated in macrocyst germination can commence vegetative growth or aggregation. A) vegetative growth, B) interphase, C) aggregation, D) pseudoplasmodium, E) mexican hat stage, F) culmination, G) fruiting body, H) spore, I) formation of giant cell, J) meiosis, K) appearance of amoebae within cyst, and L) liberation of amoebae. From Jacobson and Lodish (1975).


J
K
Macrocyst
L
I
A
B
H
C
G
Fruiting Body
Formation
D
E
F

D. discoideum see Raper, 1940; Sussman, 1966; Bonner, 1967; Loomis, 1975; and Bozzone and Bonner, 1982.

Chemotaxis of Amoebae

Chemotaxis of amoebae is the best studied sensory response in D. discoideum. Amoebae of D. discoideum are attracted by folic acid and its derivatives released from bacteria which is considered a food seeking process of amoebae (Pan et al., 1975). Positive chemotaxis of D. discoideum amoebae toward folic acid is strongest for cells at or shortly after the end of growth and declines during development (Bonner et al., 1969). Folic acid receptors are located at the cell surface of amoebae and the folic acid binding sites are probably negatively cooperative or multiple binding sites (Wurster and Butz, 1980).

Cyclic AMP (cAMP), which is originally known as acrasin, is the most important compound in signaling development of D. discoideum. Aggregating cells of D. discoideum are attracted by cAMP (Konijn et al., 1967). cAMP binds to the cell-surface receptors of amoebae to exert its activity as a chemoattractant (Green and Newell, 1975; Henderson, 1975). As in the case of folic acid binding sites, cAMP receptors have either multiple binding sites or negative cooperativity (Green and Newell, 1975). The presumed molecular weight of the cAMP receptors is about 40,000 although there are different estimations depending on the strains used and techniques employed. See Mato et al.

(1978) and Juliani and Klein (1981) for recent reviews about cAMP receptors.

Following depletion of the food source, amoebae become aggregation-competent cells. Amoebae start to move into the center of aggregation and produce cAMP probably to relay signal by responding cAMP stimuli (Tomchik and Devreotes, 1981).

Chemotactic response of the amoebae to cAMP is a fast process and can be observed 5 sec after stimulation by cAMP. D. discoideum amoebae show several short-term biochemical changes in response to cAMP stimuli which can be considered potential mediators of the fast chemotactic responses of amoebae to cAMP. An increased influx of calcium and the intracellular cGMP concentration, decreases in the phosphorylation of myosin heavy chains and of extracellular pH, complex changes in the methylation of phospholipids, and the activation of adenylate cyclase have been the short-term biochemical changes in response to cAMP stimuli and have been of special interest in dealing with the chemotactic response of amoebae to cAMP (Gerisch, 1982). Among these effects, a decrease of extracellular pH is probably due to increased CO_2 production which indicates a transient increase of the respiration rate in stimulated cells (Gerisch et al., 1977). The activation of adenylate cyclase causes cAMP-stimulated cAMP production and thus results in the relay of cAMP signals. The remaining four short-term biochemical changes are believed to be involved in the

chemotactic response of D. discoideum amoebae (Gerisch, 1982). Addition of cAMP to chemotactically active cells causes the intracellular cGMP concentration to increase about tenfold; the intracellular cGMP concentration starts to increase in 5 sec and reaches its peak after about 10 sec, then declines sharply thereafter (Mato et al., 1977). There is no change in cAMP concentration during this period (Wurster et al., 1977). Thus, apparently cGMP and not cAMP is involved in the processing of chemotactic signals. cGMP's probable mode of action is by binding to soluble intracellular proteins, though not much is known about these proteins (see Mato et al., 1979 for a recent review). There had been also an indication that cGMP mediates a fast, transient increase in phosphatidylcholine in the amoebal membranes which would result in a change of the local intracellular Ca^{++} concentration, i.e., influx of Ca^{++} (Alemany et al., 1980), and D. discoideum contains a calmodulin which mediates the regulatory effects of Ca^{++} (Bazari and Clarke, 1981; Clarke et al., 1980). Thus, a change of intracellular Ca^{++} concentration inhibits myosin heavy chain kinase via calmodulin (Maruta et al., 1980). This inhibition of myosin heavy chain kinase results in reduced phosphorylation of myosin, and this change causes an increase in the actin-activated Mg^{++}-ATPase activity of myosin (Kuczmarski and Spudich, 1980). Thus, a tentative scheme of signal processing from cAMP receptors at the cell surface to the contractile system involves the

opening of calcium channels, inactivation of myosin heavy chain kinase in a calmodulin—mediated reaction, decrease of steady-state phosphorylation of myosin, increase of the actin-activated Mg^{++}-ATPase activity, and polymerization of myosin. Futrelle et al. (1980) showed that cAMP stimulation causes local extension of pseudopods at the area close to the source of cAMP and a fast general contraction of the cell as well. It is possible that dephosphorylation of myosin heavy chains is related to the contraction, and changes in phospholipid are related to the local extension of pseudopods which will sum up apparent amoebal movement (Gerisch, 1982).

Thermotaxis of Amoebae

Thermotaxis of D. discoideum amoebae has not been reported previously (Bonner et al., 1950; Häder and Poff, 1979a). In Chapters 2 and 3 of this dissertation, results will be presented which demonstrate the capacity of amoebae for thermotaxis.

Phototaxis of Amoebae

Häder and Poff (1979 a,b) recently demonstrated positive and negative phototaxis of D. discoideum strain Ax 2 amoebae by several methods. Light-induced accumulations of amoebae in "light-traps" are dependent on wavelength, fluence rate and density of the cells. Similarly photodispersal of amoebae from light traps is also dependent

upon wavelength, fluence-rate and density of the cells. At relatively low light intensity, amoebae of D. discoideum gather into the light traps, and at higher light intensity they have a tendency to move out from the light traps. The action spectra of photoaccumulation and dispersal of amoebae are very similar (Häder and Poff, 1979 a,b). Both spectra show a primary maximum at 405 to 410nm and a broad band in the long-wavelength blue and green region of the spectrum, with a secondary maximum at about 638nm. In photoaccumulation, another maximum at 580nm and minima at 560nm and 600 to 620nm are apparent which are somewhat obscure in the action spectrum of photodispersal. Microvideographic analysis of movement of individual amoebae exposed to the unilateral light stimuli support the conclusion that the photoaccumulation of amoebae in the light traps and dispersal from the light traps are the result of oriented movements of individual amoebae, positively and negatively, phototaxing in response to the scattered light beam from amoebae inside the light traps (Burkart and Häder, 1980). Possible photolysis and heating effects in these responses have been excluded (Häder and Poff, 1979 a,b).

Häder and Poff (1980) have also reported the effect of ionophores and a protonophore on photoresponses of D. discoideum amoebae, and have shown that, although amoebae may use the same photoreceptor for photoaccumulation and photodispersal, the downstream transfer pathway of these two photoresponses appears to be separate. Häder and Poff have

concluded that slug and amoebal phototaxis in D. discoideum are different processes based on the differences in action spectrum, effects of ionophores and protonophore and response by a phototactic mutant (Häder et al., 1980).

Slug Chemotaxis

Fisher et al. (1981) reported that a low molecular weight metabolite from developing cells of D. discoideum can induce negative chemotaxis of D. discoideum slugs, i.e., slugs turn away from the site of higher concentration of this metabolite. The bioassay suggested that production of this low molecular weight metabolite, which they called "slug turning factor" or "STF", from developing cells can be increased under the light. A high concentration of STF interferes with phototaxis and thermotaxis of the slug. They also proposed that phototaxis of D. discoideum slug is the result of negative chemotaxis of this organism: light is focused onto the distal side of slug, by a "lens effect" and generates a lateral STF gradient in the slug (Fisher et al., 1981).

Fontana (1982) tested STF effects in slug thermotaxis and phototaxis with other strains of D. discoideum and detected no effect of STF on thermotaxis and phototaxis. It is necessary to reconsider the function of STF as a common chemical which functions in thermotaxis and phototaxis of D. discoideum slug.

Slug Thermotaxis

Since the early reports of thermotaxis of D. discoideum pseudoplasmodia (Raper, 1940) and its extreme sensitivity to temperatures (Bonner et al., 1950), little effort was put into understanding thermotaxis of the slug for almost three decades. Recently, Poff and Skokut (1977) re-examined thermotaxis of D. discoideum slugs and confirmed its extreme sensitivity. Slugs of D. discoideum can detect a temperature gradient as small as 0.0004°C across a slug by turning toward the warmer side of the gradients when the gradient midpoint temperatures were in the range of 22° to 28°C. They also showed that thermotactic response curve could be shifted toward lower temperatures when the amoebae were grown at 20°C instead of 23.5°C. Based mainly on the results of thermal adaptation and the relatively narrow thermal response range, Poff and Skokut proposed that membrane lipids are involved in thermo-sensory transduction in D. discoideum slug.

Later, Whitaker and Poff (1980) found another thermal response of D. discoideum slugs (negative thermotaxis) by lowering the temperature gradients more than one to three degrees below the growth and development temperature. The transition point--the temperature where the transition from negative to positive thermotaxis occurs--and degree of response were gradient-strength dependent, and adaptation of the thermal response to a new growth and development temperature was apparent. From these results Whitaker and

Poff proposed three sensors in D. discoideum slug thermotaxis. One sensor mediates positive thermotaxis, the second sensor mediates negative thermotaxis and the third is responsible for thermal adaptation in slug thermotaxis.

In contrast, Fisher and Williams (1982) proposed one sensor which can reverse the sign of the response dependent upon signal strength. Schneider et al. (1982) isolated thermotactic mutants of D. discoideum which can be classified into three groups; a mutant which only shows positive thermotaxis, mutants with weakened positive thermotaxis and normal negative thermotaxis, and a mutant which exhibits weakened positive and negative thermotaxis. The mutants classified into the first two groups clearly support the multiple biothermometer model proposed by Whitaker and Poff (1980).

Whitaker (1979) examined the change in fatty acid composition of amoebae, maintained in the slug stage at different growth temperatures and found an increase of the level of unsaturation with a decrease in the number of cyclopropane fatty acids. These results seem to support the hypothesis that a membrane-bound desaturase functions as a sensor which controls adaptation in thermotaxis, but Fontana (1982) showed no change in bulk lipid fluidity in response to the different growth and development temperature which suggests the proposed sensor to be untenable. Further investigations are necessary to clarify this problem.

Slug Phototaxis

Phototaxis of D. discoideum slugs was also discovered by Raper (1940) and confirmed by Bonner et al. (1950) who examined the effects of a wide-range of wavelengths on slug phototaxis. More thorough investigation of the effects of different wavelengths of light on slug movement gave an action spectrum of D. discoideum slug phototaxis which shows a maximum at about 430nm, a secondary maximum at about 555nm and weak responses at all other visible light regions (Francis, 1964).

An action spectrum of D. discoideum slug phototaxis was re-measured by using a technique similar to the so called null-response, i.e., a uniform population of pseudoplasmodium is placed between a pair of lights of different wavelengths held at right angles to each other, and the preferred wavelength was determined by measuring the angle of migration relative to the light sources. Poff et al. (1973) measured the angle of migration of slug away from the bisect of two orthogonal light sources with adjacent wavelengths, and used to build an action spectrum. The resultant action spectrum was similar to that of Francis (1964). It showed that D. discoideum slugs are most responsive to blue light at about 430nm and to green light in the range of 550-590nm. Poff et al. (1973) also reported a light-induced absorbance change--a rapid increase in absorption at 411nm could be induced by illuminating cells with other wavelengths of light. This light-induced

absorbance change could be followed during the purification of the possible photoreceptor pigment, and was solubilized and purified to greater than 90% homogeneity (Poff et al., 1974). This purified photoreceptor pigment--phototaxin--also shows the light-induced absorbance change. It appears to be located in the mitochondria and was identified as a heme protein with a molecular weight of about 240,000. It can undergo a reversible photooxidation when irradiated with the proper wavelength of light and this photooxidation shows up an increase in the absorption difference at 411nm and 428nm. The action spectrum of photooxidation and the absorption spectrum of phototaxin are similar to the action spectrum of slug phototaxis. Based on this similarity, phototaxin has been proposed as the primary photoreceptor pigment for phototaxis of the slug (Poff et al., 1974). Thus, photooxidation of this pigment, possibly in conjunction with some mitochondrial function, is proposed to be involved in controlling the oriented movement of slug.

Poff and Loomis (1973) re-examined the possible "lens effect" in slug phototaxis, originally proposed for the phototropism of Phycomyces and applied to D. discoideum slug phototaxis by Francis (1964). Partly, based on the observation that there is an increase in the rate of migration by light stimulation, Poff and Loomis (1973) explained the orientation of slug by "lens effect". Light focused onto distal cells of the pseudoplasmodia by the lens effect preferentially increases the photooxidation of the

phototaxin located in the cells of distal side. This increases the migration rate of that region resulting in subsequent turning of the pseudoplasmodium toward the light. This phenomenon has been questioned in a recent report by Smith et al. (1982) who have found no effect of light on the speed of slug movement.

Bidirectional phototaxis of D. discoideum slugs was recently reported by Fisher and Williams (1981) and Fontana (1982). The slug moves or migrates at some discrete angle with respect to the incident light rather than moving directly toward the light. From their phototactic mutants, Fisher and Williams proposed that bidirectionality in slug phototaxis is the result of the balance between positive and negative slug phototaxis with respect to the preferred angle in slug phototactic movement. Available phototactic mutants may facilitate the efforts in search of an explanation for this interesting phototactic behavior of slugs.

1.2 General Materials and Methods

Culture of D. discoideum Amoebae

D. discoideum amoebae, strain Ax 2, have been grown axenically in rotary shake culture in HL 5 liquid medium as described in Häder and Poff (1979a), and strain HL 50 and mutants derived from HL 50 have been grown on SM solid medium in association with Klebsiella aerogenes as described by Sussman (1966). All cultures were kept in darkness and at 23.5 ± 0.3°C, unless otherwise mentioned in each chapter.

Harvest of D. discoideum Amoebae

Amoebae of the axenic strain Ax 2 were harvested by centrifugation and washed twice with potassium phosphate buffer (15 mM, pH 6.1). Non-axenic strains grown in association with bacteria were harvested when they were in the "exponential-phase" of growth by washing the agar plate with phosphate buffer, then pelleting and washing the cells with phosphate buffer until the supernatant was clear. The centrifugations for the harvest and wash were performed for 1 or 2 min at about 500 x g. Unless otherwise mentioned in each chapter.

Thermal Gradients and Temperature Measurements

The thermal gradients used are basically the same as those described by Poff and Skokut (1977). An aluminum slab, approximately 10 cm wide and 3 cm thick, with two lengthwise drilled conduits was surrounded by styrofoam except for the connecting water circulation. The conduits were connected to the circulating water baths (Model 2095, Forma Scientific, Marietta, Ohio; RTE-8, Neslab, Portsmouth, New Hampshire; Lauda NDB 8/17, Brinkmann Instruments, Inc., Westbury, New York). Temperature gradients were established by changing the temperature of the water baths, and the temperature was measured with a mercury-in-glass thermometer accurate to less than 0.1°C which is traceable to National Bureau of Standards. The temperature difference between the two water baths was maintained at 4°C, for a

gradient of 0.22°C/cm, which can be produced with this set-up. See Fontana (1982) for the conversion of the difference of temperature between water baths to the actual gradient strength. The temperature gradients were adjusted at least one hour before use to permit temperature equilibration across the aluminum block. These thermal gradients along with the water baths were kept in a dark and temperature-controlled room, unless otherwise mentioned in each chapter.

Measurement of Light Intensity and Absorbancy

The light intensity was measured with a Kettering model 65 radiometer and/or an International Light Spectroradiometer (Newburyport, Mass) which can be calibrated with a standard lamp traceable to National Bureau of Standards.

Absorption spectra were measured using a single-beam spectrophotometer on line with a small computer as described in Häder and Poff (1979b). The spectrophotometer uses a monochromater from a Cary 14-R spectrophotometer, a 200-W tungsten-halogen lamp as the measuring beam source, a sample compartment designed for a vertical light path, and EMI 9659 QA phototube directly beneath the sample. A Hewlett-Packard 2108 MX computer is connected to the spectrophotometer via a 12-bit analog-to-digital converter and a logarithmic amplifier. A cylindrical cuvette and Dewar flask with optical windows on the bottom were used for absorbance

measurements at 77°K. For routine absorption measurements a Cary or Gilford spectrophotometer was used.

Light Source and Control of Light Intensity and Wavelength

A slide cube projector (Bell and Howell, CP 40) or a Unitron lamp was used as a light source. Light intensity was controlled by means of a trimatron dimmer (Leviton) or neutral density filters (Balzer or Inconnel). For an IR cut off filter, a 35mm pathlength aqueous 5% (w/v) $CuSO_4.5H_2O$ solution was used, and monochromatic light was obtained using interference filters (Baird Atomic) with half band widths of 9-11nm.

All the phototactic experiments were performed in a light- and temperature-controlled room, unless otherwise mentioned in each chapter.

CHAPTER 2

THERMOBEHAVIOUR OF *DICTYOSTELIUM DISCOIDEUM* AMOEBAE

Introduction

Amoebae of the cellular slime mold, *D. discoideum*, live and grow in the mulch on the forest floor where they feed on bacteria. Following the depletion of food, the amoebae aggregate, forming a multicellular pseudoplasmodium which eventually develops into a sorocarp consisting of a stalk bearing a sorus containing spores. Under the proper conditions, the spores may germinate releasing amoebae, thus repeating the developmental cycle (Raper, 1940). Much of the work with this organism has centered upon its development because of the fact that one can easily separate growth and cell division from development.

Dictyostelium is also being increasingly recognized as an ideal system for the study of sensory transduction in a eukaryotic organism, probably due to its simplicity and ease of culture along with the variety of its responses toward environmental stimuli. Sensory responses thus far described are chemotaxis by the amoebae to cAMP (Robertson *et al.*, 1972), folate (Pan *et al.*, 1975), and an unidentified repellent(s) (Bonner, 1977), both positive and negative phototaxis by the amoebae (Häder and Poff, 1979 a,b),

chemotaxis by the pseudoplasmodia to an endogenous "slug turning factor" (Fisher et al., 1981), phototaxis by the pseudoplasmodia (Bonner et al., 1950; Francis, 1964; Poff et al., 1973; Poff and Butler, 1974), and positive and negative thermotaxis by the pseudoplasmodia (Bonner et al., 1950; Poff and Skokut, 1977; Whitaker and Poff, 1980). In addition to the descriptive work, progress is also being made toward an understanding both of the primary steps and of the transduction sequence for each of these responses (see Chapter 1).

Thermotaxis by the pseudoplasmodia has been known for many years (Bonner, 1950). The characteristics of this response include: a very high sensitivity (response to less than 0.0004°C across an individual pseudoplasmodium), a relatively narrow temperature range across which the response is given; negative thermotaxis at temperatures several degrees below the growth temperature of the amoebae and development temperature of the pseudoplasmodia, and positive thermotaxis at higher temperatures; and adaptation--a dependence of the direction of thermotaxis on the recent thermal history of the organism.

This work was undertaken to determine whether or not individual amoebae of D. discoideum also exhibit thermotaxis.

Materials and Methods

Amoebae of D. discoideum, strain HL 50, were grown in association with Klebsiella aerogenes (Sussman, 1966) for about 22 hr at 23.5 ± 0.3°C. At this time, the bacteria had been cleared from about 80% of the agar plate.

The amoebae were freed of bacteria by washing three or four times with phosphate buffer at a final concentration of 1×10^7 amoebae/ml. Aliquots of 1 ml were mixed with 0.3 ml of a suspension of washed charcoal in phosphate buffer (2% w/v). The charcoal was added to mark the position at which the suspension was deposited on 2% water agar which was about 1 mm thick and covered a 1 x 25 x 75 mm glass microscope slide. A fine line of amoebae (Figure 2A) was formed on the agar with the aid of a micromanipulator. The micromanipulator was constructed of two pieces of fine platinum wire, 0.1 mm in diameter, which were attached at their ends to two metal bars such that the wires were appressed together. The bars were attached to a rack and pinion gear so that the wires could be raised and lowered (see the appendix A for the diagram of the micromanipulator). After spotting, the slides were placed on a temperature gradient, 0.22°C/cm, formed as described in Chapter 1. The amoebae were exposed on the gradients for 3, 6, 9, 12, 16, or 20 hr.

After the exposure to the thermal gradient, the number of amoebae which moved in each direction away from the original line was counted. This was done by examining, on

each slide, five randomly chosen microscope fields of about 3 x 5 mm. The response (Δ%) for each replicate was calculated in the following manner. Δ% = 100 (# of cells moving up gradient - # of cells moving down gradient)/total # of cells which moved. Thus, Δ% is a measure of the directed movement on a thermal gradient. For thermal response curves, relative Δ% was determined for thermal gradients with varying midpoint temperatures.

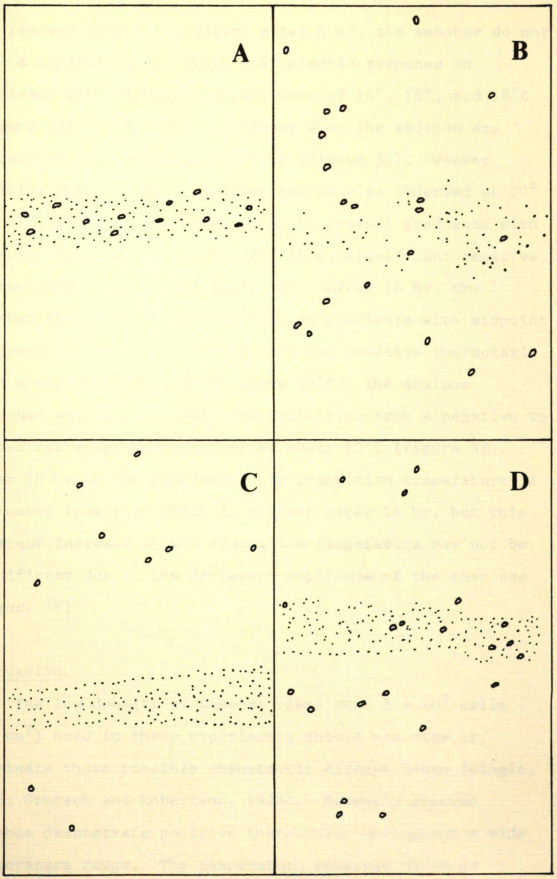
The vertical error bars in the figures represent 95% confidence intervals calculated as described in Steel and Torrie (1980).

Results

Under these experimental conditions, amoebae of D. discoideum strain HL 50 showed amoeboid movement for at least 20 hr without any sign of aggregation. In the absence of a thermal gradient, amoebae moved randomly on the agar surface. Depending on the midpoint temperature of the thermal gradient used, there were conditions which resulted in random movement, more cells on the warmer side, or more cells on the cooler side (Figures 2B, 2C, 2D). These results show that amoebae are capable of positive and negative thermotaxis.

Amoebae of strain HL 50, grown at 23.5°C, were then exposed to thermal gradients for six different time intervals. After 3 hr, positive thermotaxis is seen throughout the entire temperature range (Figure 3A). If the amoebae

Figure 2. Thermotactic responses of D. discoideum amoebae. Tracings from photomicrographs showing: A) original spotting, B) random response, C) positive thermotaxis, and D) negative thermotaxis. The larger dots represent amoebae and smaller dots are charcoal which aid in locating the original spotting line.


A
B
C
D

are removed from the gradient after 6 hr, the amoebae do not show a significant positive thermotactic response on gradients with midpoint temperatures of 14°, 16°, and 18°C (Figure 3B). This trend continues when the amoebae are exposed on the gradients for 9 hr (Figure 3C). Weaker positive thermotactic responses can be also observed at 20° and 21°C after 9 hr. After 12 hr on thermal gradients with midpoint temperatures of 14° and 16°C, significant negative thermotaxis is evident (Figure 3D). After 16 hr, the amoebae show negative thermotaxis on gradients with midpoint temperatures of 14°, 16°, and 18°C and positive thermotaxis with a midpoint temperature above 20°C. The maximum response was at 23°C, with the transition from a negative to a positive response occurring at about 19°C (Figure 3E). After 20 hr on the gradients, the transition temperature is increased from that which is evident after 16 hr, but this apparent increase in the transition temperature may not be significant due to the decreased amplitude of the response (Figure 3F).

Discussion

The low density of amoebae (less than 3×10^3 cells per cm^2) used in these experiments should minimize or, eliminate those possible chemotactic effects known (Gingle, 1976; Grutsch and Robertson, 1978). Recently starved amoebae demonstrate positive thermotaxis throughout a wide temperature range. The temperature response curve of

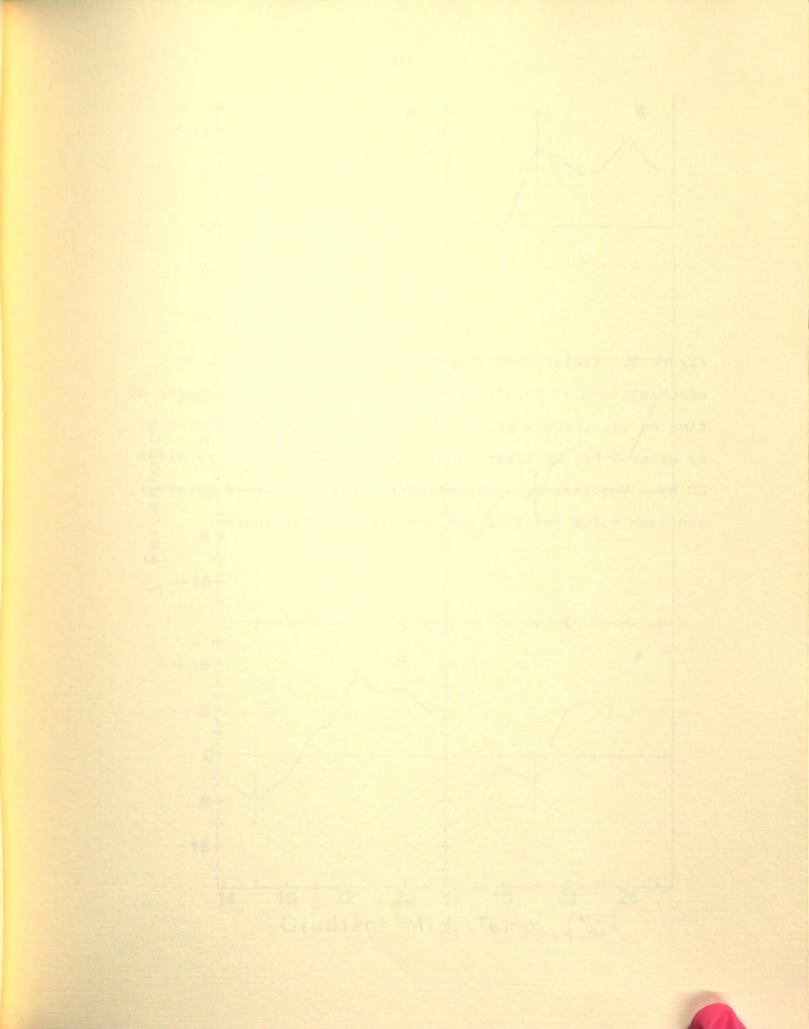
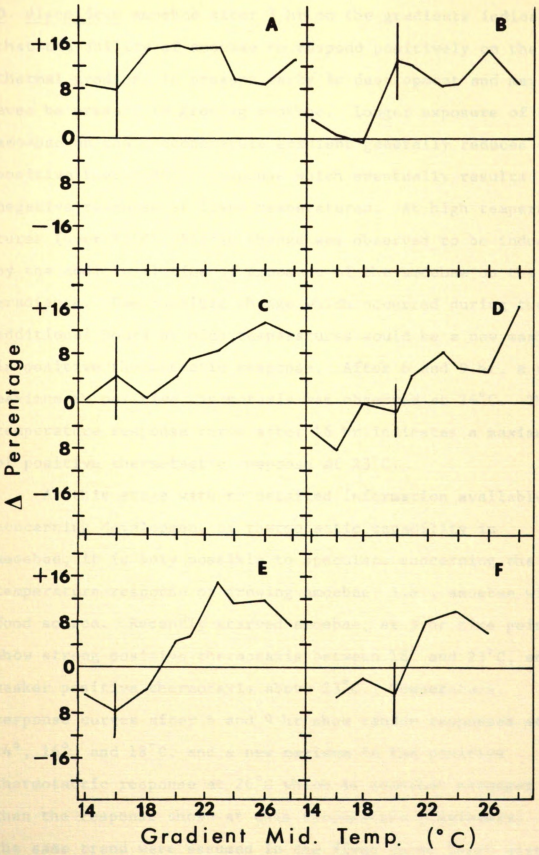


Figure 3. Temperature response curves for strain HL 50 amoebae grown at 23.5°C and exposed for different lengths of time on the thermal gradient. A) after 3 hr, B) after 6 hr, C) after 9 hr, D) after 12 hr, E) after 16 hr, and F) after 20 hr. Vertical bars represent the 95% confidence interval, and each datum point is the average of 8 replicates.


A
B
C
D
E
F
+16
8
0
8
−16
Δ Percentage
14
18
22
26
Gradient Mid. Temp. (°C)

D. discoideum amoebae after 3 hr on the gradients indicates that the ability of amoebae to respond positively on the thermal gradient is present early in development and may even be present in growing amoebae. Longer exposure of the amoebae on the temperature gradient generally reduces positive thermotactic response which eventually results in a negative response at lower temperatures. At high temperatures (over 21°C), little change was observed to be induced by the additional time of exposure of the amoebae on the gradients. One possible change which occurred during the additional hours at high temperatures would be a new maximum in positive thermotactic response. After 6 and 9 hr, a new maximum in positive thermotaxis was observed at 26°C. The temperature response curve after 16 hr indicates a maximum of positive thermotactic response at 23°C.

At this stage with no detailed information available concerning development of thermotactic capability in amoebae, it is only possible to speculate concerning the temperature response of growing amoebae, i.e., amoebae with food source. Recently starved amoebae, at 3 hr time point, show strong positive thermotaxis between 18° and 23°C, and weaker positive thermotaxis above 23°C. Temperature response curves after 6 and 9 hr show random responses at 14°, 16°, and 18°C, and a new maximum in the positive thermotactic response at 26°C which is somewhat stronger than the response shown at this temperature previously. If the same trend were assumed in the first three hours since

the food depletion, a tentative temperature response curve of D. discoideum amoebae in the vegetative stage would probably show stronger positive thermotactic responses than the temperature response curve at 3 hr time point at the gradients with lower midpoint temperatures, but slightly weaker positive thermotactic response at the gradients with high midpoint temperatures, i.e., around 26°C.

After 12 hr on the gradient, D. discoideum amoebae start to show a temperature response curve with both positive and negative thermotaxis. An indication of the developing negative thermotaxis of amoebae can be seen on the temperature response curve at the 6 hr time point which shows random responses at 14°, 16°, and 18°C midpoint temperature gradients. A possible explanation of these random responses is the movement of amoebae toward the warmer side of the gradients at around 3 hr or earlier timepoint followed by the change of preferred direction, i.e., negative thermotaxis. It indicates that the capacity of amoebae to respond negatively develops early in the development process (before 6 hr or possibly even 3 hr) and subsequent time points show that it increases as development proceeds. This increase in the ability to respond negatively is also reflected in the upward shift in the transition point as development proceeds. The stages of development which occur after starvation but prior to cell-cell contact are: 1) acquired chemotactic sensitivity to cAMP; 2) acquired capability to relay cAMP signal;

3) initiation of autonomous cAMP signalling; and 4) increased cohesiveness which is the result of the formation of contact sites and large amounts of carbohydrate-binding proteins (McMahon et al., 1977). Because the time course of development depends on cell density, the total number of cells, the homogeneity of the initial population and culture conditions (Gingle and Robertson, 1976), it is not possible without further information to temporally place the development of the capacity for negative thermotaxis in the development framework.

Two possible models (temporal sensing and spatial sensing) to account for a cell's ability to perceive and to migrate along a stimulus gradient have been proposed especially for bacterial chemotaxis (McNab and Koshland, 1972; Koshland, 1976) although later investigations clearly established temporal sensing in bacteria (Koshland, 1979). These models also have been considered to explain chemotactic sensing of D. discoideum amoebae (Gerish et al., 1977), but at this time it is not clear which mechanism would count for amoebal sensory movement. It will be absolutely necessary to find out if amoebal thermotactic sensing is temporal or spatial, or both, before we can understand thermotaxis of D. discoideum amoebae.

Two sensors, one for positive thermotaxis and the other for negative thermotaxis, have been proposed to account for the thermotaxis of D. discoideum slug, a cumulative organism of 10^3 to 10^5 amoebae (Whitaker and Poff, 1979; Schneider

et al., 1982). The results presented here show that negative thermotactic response and positive thermotactic response can be separated by following a developmental time course. This unique induction of negative thermotaxis upon food depletion and/or exposing the amoebae on the thermal gradient should be helpful in investigating the sensors responsible for these responses.

Summary

Thermotaxis of D. discoideum amoebae has been described. Amoebae of D. discoideum, strain HL 50, show only positive thermotaxis throughout the temperature gradients checked shortly after food depletion. Negative thermotactic responses become apparent at the thermal gradients with low midpoint temperatures after 12 hr on the gradients. After 16 hr on the thermal gradients, amoebae of D. discoideum show a distinct negative thermotaxis at the thermal gradients of 14°, 16°, and 18°C midpoint temperatures and positive thermotaxis at the other temperature gradients.

Development of negative thermotaxis of amoebae shortly after exposure on the gradient (6 hr or earlier) was observed supporting the conjecture that negative thermotaxis and positive thermotaxis are at least partially independent.

CHAPTER 3

THERMOTACTIC MUTANTS OF *DICTYOSTELIUM DISCOIDEUM* AMOEBAE

Introduction

Amoebae of *D. discoideum* feed on bacteria in the mulch of the forest floor. Once the food is depleted, they follow a developmental cycle which includes formation of a pseudoplasmodium and a fruiting body (Raper, 1940). During this life cycle, *D. discoideum* has two motile stages, the amoebae and the slug, which are able to respond to environmental stimuli by showing oriented movements.

Amoebae of *D. discoideum* are positively chemotactic to folic acid (Pan *et al.*, 1975) and cAMP (Robertson *et al.*, 1972), negatively chemotactic to an unidentified "repellent(s)" (Bonner, 1977), and positively or negatively phototactic (Häder and Poff, 1979 a,b). Pseudoplasmodia of *D. discoideum* are negatively chemotactic to "STF" (Fisher *et al.*, 1981), phototactic (Poff *et al.*, 1973), and positively and negatively thermotactic (Whitaker and Poff, 1980).

In Chapter 2, thermotaxis of *D. discoideum* amoebae was presented. Amoebae of strain HL 50, grown at 23.5°C, showed only positive thermotaxis throughout the thermal gradients checked shortly after food depletion. The temperature

response curve of amoebae at this time point (3 hr) showed a strong positive thermotactic response between 18° and 23°C, while above 23°C, there was a drop in the strength of positive thermotaxis. Random responses could be observed on the thermal gradients with low midpoint temperatures at 6 hr and 9 hr time points, and became negative beyond 9 hr. Negative thermotaxis on the thermal gradients with low midpoint temperature became apparent after 12 hr on the gradients, and grew stronger as exposure time on the gradients increased. Based on these data, it has been proposed that the vegetative amoebae of D. discoideum are capable of a positive thermotaxis, and the negative thermotactic response at low temperature is probably induced by food depletion and/or exposure of the amoebae on the thermal gradients.

In this work, thermotactic responses of mutants derived from HL 50 were checked at three different time intervals on the thermal gradients. The results were then used to postulate the way vegetative amoebae respond on the thermal gradients.

Thermotaxis mutants used in these works were originally selected based on the different slug thermotactic responses. HO 428, HO 596 and HO 813 have been isolated by Schneider et al. (1982), while HO 1445 was isolated as mentioned in the appendix.

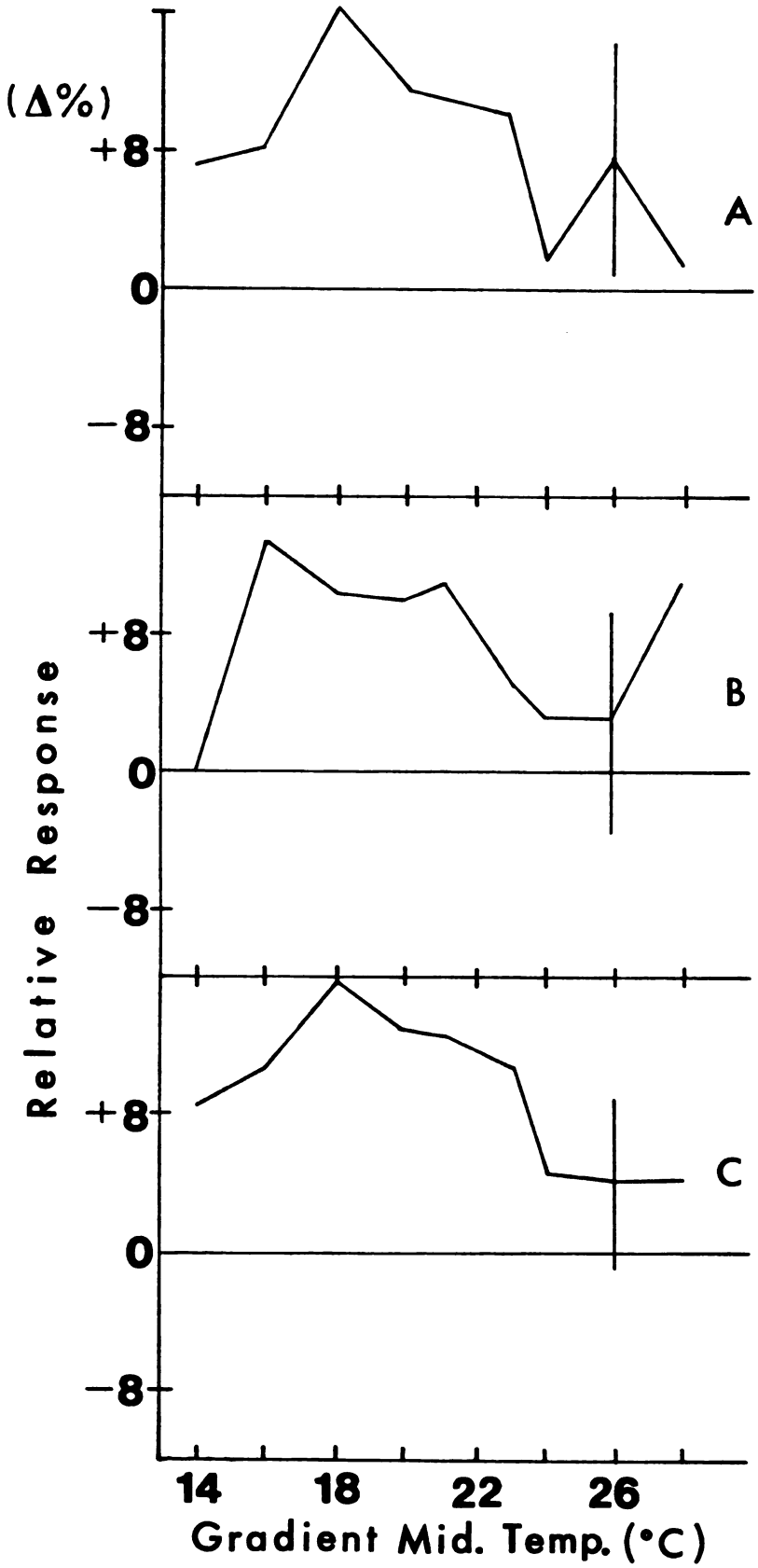
Materials and Methods

The characterization of thermotaxis of D. discoideum, strains HO 428, HO 596, HO 813 and HO 1445, was conducted as follows: amoebae were grown in association with K. aerogenes (Sussman, 1966) for about 20 - 24 hr at 23.5 ± 0.3°C in the dark. At this time, the bacteria had been cleared from about 80% of the agar plate. The amoebae were freed of bacteria by washing with buffer, aliquotes of the washed amoebae were mixed in suspension with washed charcoal, and the mixture of amoebae and charcoal was linearly spotted on the agar covering a glass microscope slide by means of the micromanipulator. The slides were then placed on a temperature gradient, 0.22°C/cm, and left there for 3, 6, and 16 hr. After the exposure on the thermal gradient, the response was measured, Δ% was calculated, and the thermal response curves were plotted. Vertical error bars represent 95% confidence intervals. See Chapters 1 and 2 for detailed methods.

Results

Amoebae of D. discoideum, strain HO 428, grown at 23.5°C showed positive thermotaxis throughout the entire temperature range after 3 hr on the gradients. Stronger positive responses were observed at the lower temperatures (Figure 4A). These positive responses continued at 6 hr (Figure 4B) and 16 hr (Figure 4C) with no significant differences.

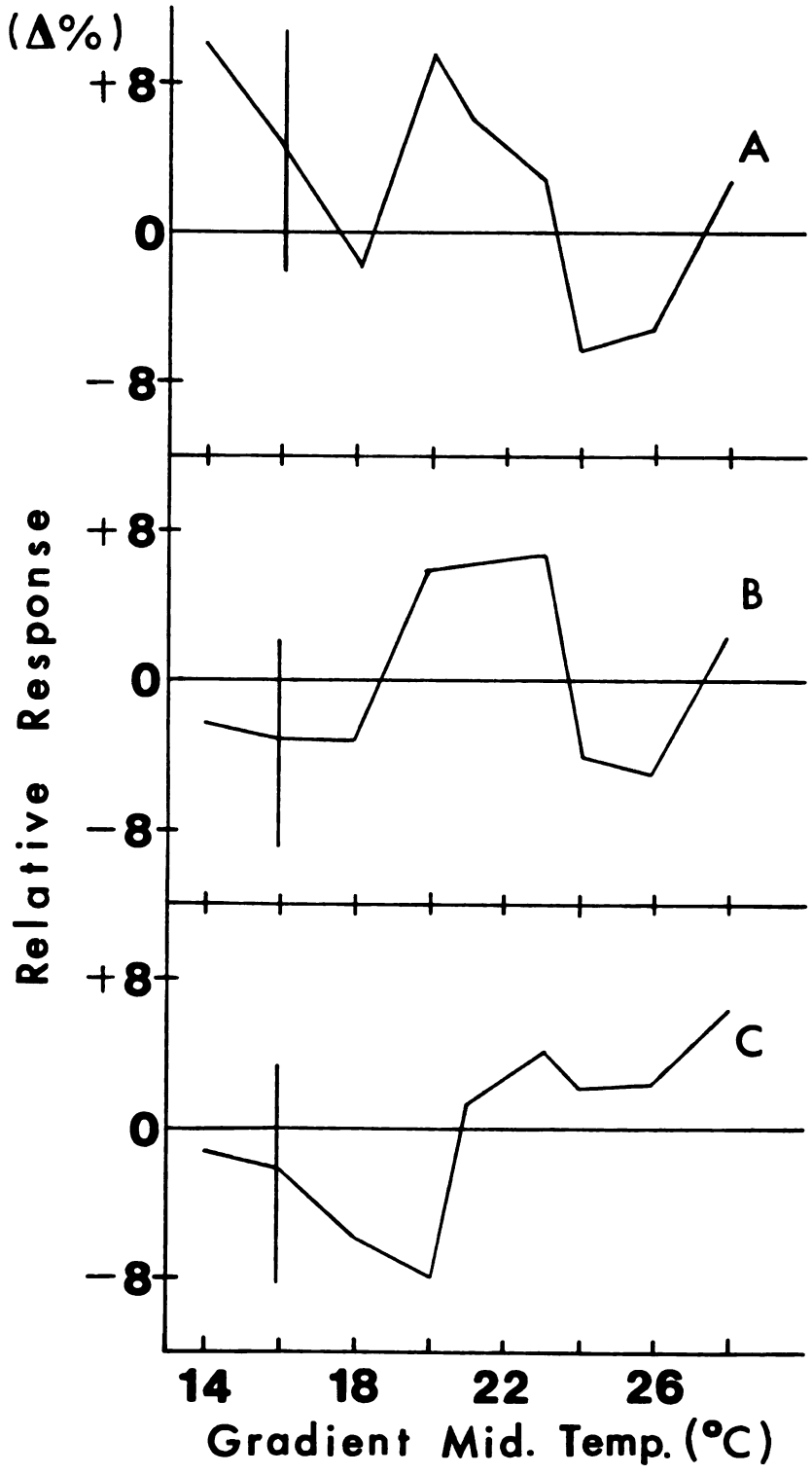
Figure 4. Temperature response curves for strain HO 428 amoebae grown at 23.5°C and exposed for different lengths of time on the thermal gradient. A) after 3 hr, B) after 6 hr, and C) after 16 hr. Vertical bars represent the 95% confidence interval, and each datum point represents the average of 8 replicates.


(Δ%)
+8
0
−8
A
+8
0
−8
B
+8
0
−8
C
Relative Response
14
18
22
26
Gradient Mid. Temp. (°C)

Amoebae of strain HO 596 showed complicated thermotactic responses after 3 hr on the gradients. Positive thermotactic responses were observable on the thermal gradients with the midpoint temperatures lower than 23°C except at 18°C, and negative thermotactic responses were also seen at 24° and 26°C (Figure 5A). Positive thermotactic responses at low temperatures, 14° and 16°C, were quickly diminished after 6 hr, but little change was observable from other midpoint temperatures (Figure 5B). This change of response (a shift in the thermal response curve toward the negative direction at lower temperatures) was more apparent at the 16 hr time point. After 16 hr, the temperature response curve showed negative thermotactic responses at lower temperatures and weak positive thermotaxis above 21°C with a transition point from negative to positive thermotaxis at about 20.8°C (Figure 5C). Noticeable changes which occurred during this time course were more apparent negative thermotactic responses at low temperatures and absence of the previously existing negative thermotactic responses at 24° and 26°C.

D. discoideum amoebae, strain HO 813, showed no negative thermotactic responses over the entire temperature range after 3 hr on the gradients. Although the strength of the positive response is much weaker than the parental strain HL 50 and mutant HO 428, HO 813 amoebae exhibited positive thermotactic responses on most temperature gradients (Figure 6A). After 6 hr, negative thermotaxis is

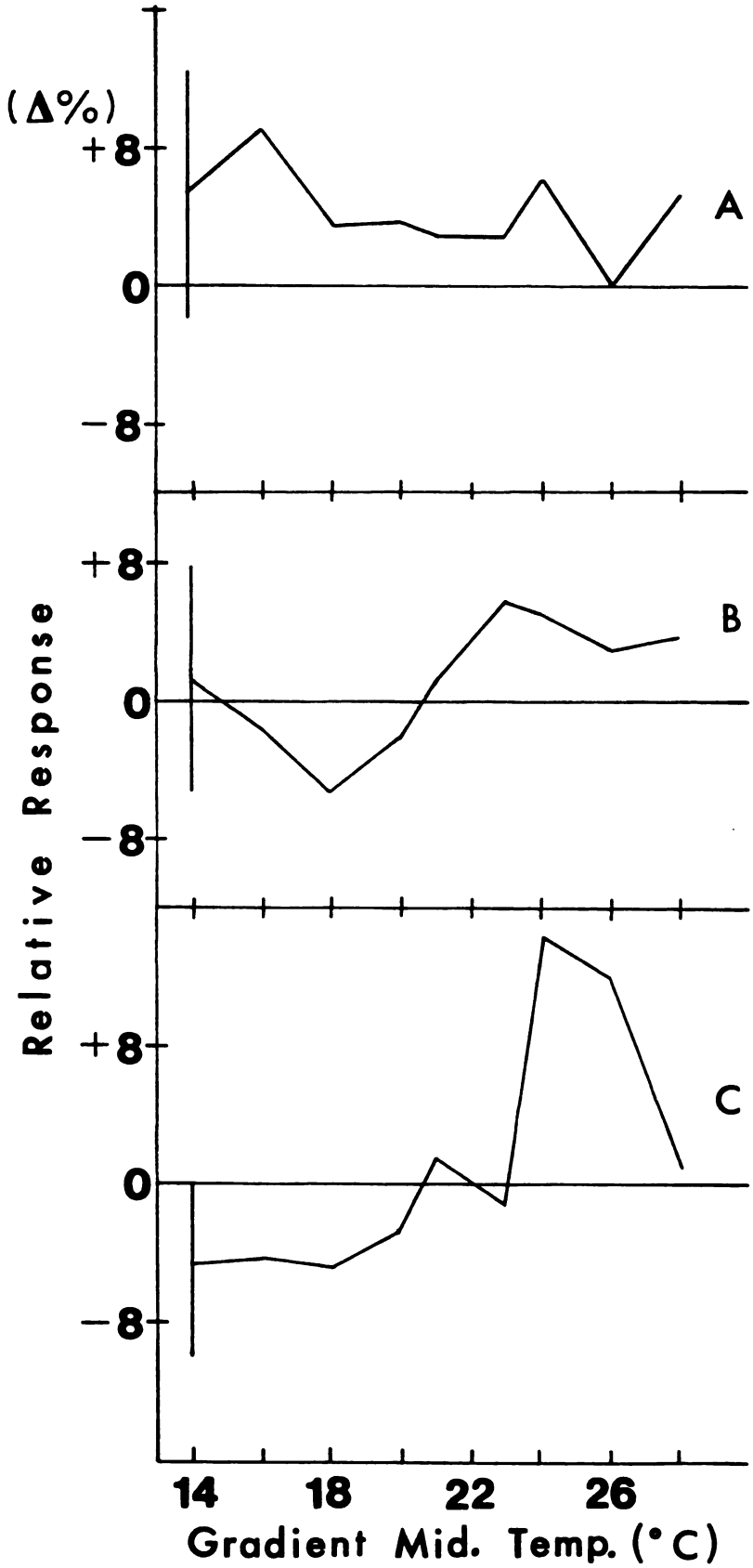
Figure 5. Temperature response curves for strain HO 596 amoebae grown at 23.5°C and exposed for different lengths of time on the thermal gradient. A) after 3 hr, B) after 6 hr, and C) after 16 hr. Vertical bars represent the 95% confidence interval, and each datum point represents the average of 8 replicates.


(Δ%)
+8
0
−8
A
+8
0
−8
B
+8
0
−8
C
Relative Response
14
18
22
26
Gradient Mid. Temp. (°C)

apparent at 18°C, but not much change was observed in the strength of positive thermotactic response on the gradients with higher midpoint temperatures, and the transition point from negative to positive thermotaxis was at about 20.8°C (Figure 6B). During the time interval between 6 and 16 hr, negative thermotaxis was observed at a broader temperature range, positive thermotaxis was strengthened at temperature gradients with higher midpoint temperatures and there appeared to be a small shift in the transition point. After 16 hr, negative thermotactic responses were seen at the gradients below 21°C while positive thermotaxis was observable at the gradients above 23°C (Figure 6C).

After 3 hr on the gradients, HO 1445 amoebae showed complicated temperature responses similar to those of HO 596 amoebae. Positive thermotaxis at 14°, 20°, 21°, 23° and 28°C and a weak negative thermotactic response were observed at 26°C (Figure 7A). During the next 3 hr, the temperature response curve was shifted downward on most gradients with low midpoint temperatures. Negative thermotactic responses were observable below 21°C after 6 hr (Figure 7B). Between 6 hr and 16 hr time interval, strong positive thermotactic responses were given by amoebae at the higher midpoint temperatures. The maximum negative response was seen at 18°C, and the maximum positive response was at 26°C. The transition point from negative to positive thermotactic responses was at about 20.6°C (Figure 7C).

Figure 6. Temperature response curves for strain HU 813 amoebae grown at 23.5°C and exposed for different lengths of time on the thermal gradient. A) after 3 hr, B) after 6 hr, and C) after 16 hr. Vertical bars represent the 95% confidence interval, and each datum point represents the average of 8 replicates.


(Δ%)
+8
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+8
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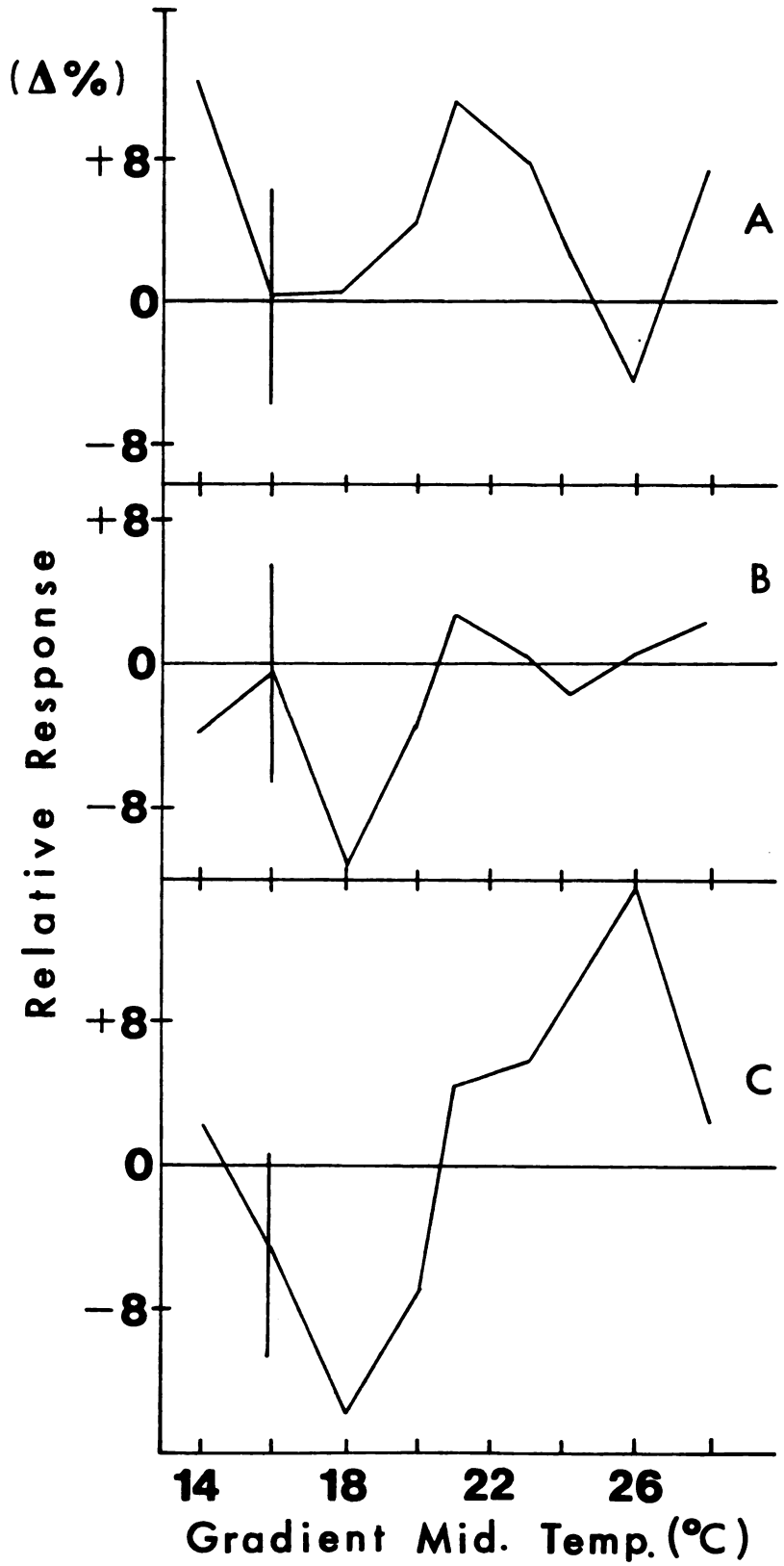
−8
C
Relative Response
14
18
22
26
Gradient Mid. Temp. (°C)

Figure 7. Temperature response curves for strain HO 1445 amoebae grown at 23.5°C and exposed for different lengths of time on the thermal gradient. A) after 3 hr, B) after 6 hr, and C) after 16 hr. Vertical bars represent the 95% confidence interval, and each datum point represents the average of 8 replicates.


(Δ%)
+8
0
−8
A
B
C
Relative Response
14
18
22
26
Gradient Mid. Temp. (°C)

Discussion

The low density of amoebae (less than 3 x 10^3 cells/cm^2) used in these experiments should be low enough to eliminate any possible chemotactic effects (Gingle, 1976; Grutsch and Robertson, 1978) in amoebal movement of these mutants.

Early in development, D. discoideum amoebae, mutant strain HO 428, show strong positive thermotactic responses at temperatures lower than the growth temperature of the amoebae, while above the growth temperature, the thermotactic responses are very weakened. Extending the exposure on the temperature gradients to 16 hr did not induce noticeable differences from the temperature response curve obtained after 3 hr. Thus, in the mutant strain, HO 428, development does not affect the thermotactic response in amoebae. Based on this, one can postulate a temperature response curve of the growing amoebae of the mutant strain, HO 428, which is quite similar to the ones appearing in Figure 4.

In another mutant strain, HO 596, recently starved amoebae seemed to have both thermotactic responses, i.e., positive and negative. After 3 hr on the gradients, HO 596 amoebae were positively and negatively thermotactic. As the exposure time on the gradients increased, negative thermotaxis was induced at lower temperatures and diminished at higher temperatures. As development proceeds negative thermotactic responses at higher temperatures, 24°C and

26°C, diminished; this change probably supports the existence of negative thermotaxis in the vegetative amoebae at higher temperatures. This postulated negative thermotaxis of the vegetative amoebae at higher temperatures probably is at least as strong as the responses seen from the temperature response curves at 3 and 6 hr time points. This suggests that growing amoebae, strain HO 596, would have a negative thermotactic transduction path as well as a positive path. Mutant HO 1445 amoebae showed a temperature response and change of the response along development similar to that of HO 596. Negative thermotaxis increases in strength on the gradients with lower midpoint temperatures, and positive thermotaxis becomes more apparent at higher temperatures as development proceeds. Thus, the indication from these two mutants, HO 596 and HO 1445, is that vegetative amoebae of D. discoideum have the capacity for both positive and negative thermotaxis. The temperature ranges of the positive and negative thermotaxis expected from the results of these two mutants would be below the growth temperature and above the growth temperature of the amoebae, respectively, and this feature may suggest a mechanism for seeking an optimal environment in growing D. discoideum amoebae, i.e., vegetative amoebae gather at their growth temperature.

The proposed temperature response curve of the growing amoebae of the parental strain, HL 50, presented in the previous chapter is similar to the temperature response curve

of HO 428 amoebae. Both have strong positive thermotactic responses below the growth temperature of the amoebae and a weaker response above that temperature. The results with HO 596 and HO 1445 strongly suggest negative thermotaxis in the vegetative amoebae of D. discoideum. Based on the temperature response curves from these mutants one can suggest the following points:

1) HO 428 is a mutant with a defect in negative thermotactic transduction path in amoebae as it shows only positive thermotaxis.

2) HO 596 and HO 1445 are mutants which have mutations in the development of positive thermotaxis at higher temperatures, 24° and 26°C, such that they have delayed expression of positive thermotaxis at these temperatures, i.e., the observed negative thermotaxis at higher temperatures in these mutants is due to a delayed development of the positive thermotactic response at these temperatures. This suggests that growing HL 50 amoebae also have a capacity of negative thermotaxis at higher temperatures, but the first three hours on the thermal gradient is long enough to induce a positive thermotaxis at these temperatures.

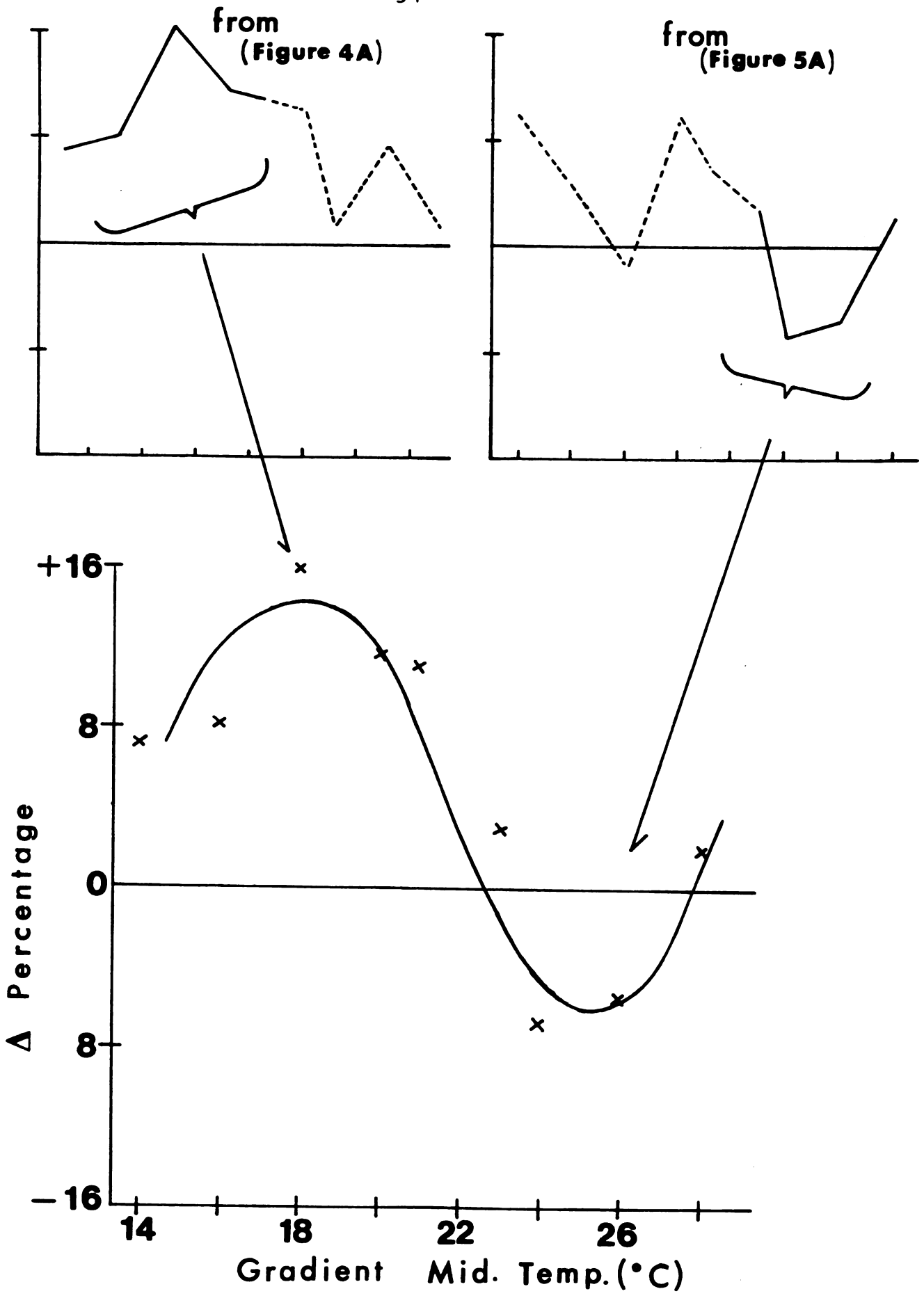
3) Positive thermotactic responses at 16° and 18°C in growing HO 596 and HO 1445 amoebae are rapidly diminished compared to other strains after food depletion.

Based on these results and assumptions, a tentative temperature response curve of vegetative HL 50 amoebae may be proposed. Its main features would be a positive

thermotactic response at the temperature gradients lower than the growth temperature of amoebae with the maximum at about 18°C and a negative thermotactic response above the growth temperature (Figure 8). Probably the strength of negative thermotaxis would be weaker than that of positive thermotaxis because of its narrower and weaker responses as can be seen in Figures 5A and 7A.

In Caenorhabditis elegans, a nematode which shows thermotaxis (Hedgecock and Russell, 1975), and the slug of D. discoideum (Whitaker and Poff, 1980), two sensors, one for positive thermotaxis and the other for negative thermotaxis, have been proposed to accommodate their thermotactic responses (actually these two organisms show an opposite way of responding on the thermal gradients; thermotaxis of C. elegans can be called an optimum seeking process, and thermotaxis of D. discoideum slug has been called as an "optimum leaving" mechanism) (Schneider et al., 1982). In these two-thermosensor models, the apparent response will be determined by the input from the two sensors. Based on the model proposed here for vegetative amoebae of D. discoideum, growing amoebae have the ability to respond positively or negatively on the thermal gradients dependent on the temperature. The shift of sign of response from positive to negative would occur at about the growth temperature of the amoebae. This sign reversal could be a result of two competing sensors as suggested in C. elegans and D. discoideum slug, or could be a result of a difference in stimulus

Figure 8. Proposed temperature response curve of D. discoideum amoebae at the vegetative growth stage. This model is primarily based on the positive thermotaxis of amoebae, strain HO 428, at lower temperatures (Figure 4A) and negative thermotaxis of amoebae, strain HO 596, at higher temperatures (Figure 5A).


from
(Figure 4A)
from
(Figure 5A)
+16
8
0
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16
Δ Percentage
14
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22
26
Gradient Mid. Temp.(°C)

strength which suggests only one sensor, examples of which can be found in photoresponses (Forward, 1976; Häder and Poff, 1979b). More evidence is necessary to decide which is the case for vegetative amoebal thermosensing.

Amoebae of mutant strain HO 813 showed only weak positive thermotactic responses at most temperatures after 3 hr. After 6 hr, HO 813 amoebae showed a negative thermotactic response at around 18°C, but there was no significant change at higher temperatures. Weak responses at the 3 hr time point at lower temperatures could be a result of a transition from stronger positive thermotaxis at 0 hr time point, i.e., growing amoebae, to negative thermotaxis at 6 hr time point, or, it is also possible that recently starved HO 813 amoebae have a mutation at a signal amplification path which results in only weak responses.

In contrast with the sensing of other environmental parameters, thermosensing usually demands consideration of the whole organism in signal reception, because all of the molecules can gain or lose heat. This nonspecificity in receiving signals makes it a great deal more difficult to identify the thermoreceptor. Amoebae of HO 428 show no significant change in their thermoresponses as they develop. This stable thermotactic expression of HO 428 may be an excellent control system when dealing with thermoreceptors. The increase of negative thermotaxis in other strains can be compared with the steady positive temperature response

of HO 428 at the level of the whole organism or at a molecular level.

A major difficulty in interpreting these results has been in determining the significance of the responses. It would be positively helpful in studying the thermal response of this organism to have a more sensitive device for detecting the response or to find conditions which would induce stronger responses.

Summary

Temperature responses of amoebae of thermotactic mutants have been investigated. Amoebae of the mutant strain HO 428 showed positive thermotaxis which is strong at lower temperatures and drops sharply above the growth temperature of amoebae. The temperature response of HO 428 amoebae was not affected by the length of exposure of amoebae on the gradients. HO 596 amoebae seemed to have both positive and negative thermotactic responses shortly after food depletion. Longer exposure of these amoebae on the thermal gradients induced a stronger negative response at lower temperatures and an apparent positive response at higher temperatures. A similar change could be observed in HO 1445 amoebae. Based on the steady positive thermotactic response by HO 428 amoebae and the mode of change in temperature response at higher temperatures, 24°C and 26°C, by HO 596 amoebae, a model for the temperature response of vegetative D. discoideum amoebae, strain HL 50, has been proposed. The

main features of the model are: a positive response at the thermal gradients with midpoint temperatures lower than the growth temperature of amoebae and a negative response above it.

CHAPTER 4

PHOTOBEHAVIOR OF *DICTYOSTELIUM DISCOIDEUM* AMOEBAE

Introduction

Amoebae of *D. discoideum* feed on bacteria in the mulch of the forest floor. Once the food is depleted, they follow a developmental cycle in which the pseudoplasmodium and the fruiting body are formed. During this life cycle, *D. discoideum* has two motile stages, the amoebae and the slugs, which are able to respond to environmental stimuli showing oriented movements.

Amoebae of *D. discoideum* are chemotactic to folic acid (Pan *et al.*, 1975), and unidentified repellent(s) (Bonner, 1977), and cAMP (Robertson *et al.*, 1972), and are thermotactic (Chapters 2 and 3) and phototactic (Häder and Poff, 1979 a,b). Pseudoplasmodia of *D. discoideum* are also chemotactic to "STF" (Fisher *et al.*, 1981), thermotactic (Whitaker and Poff, 1980), and phototactic (Poff *et al.*, 1973).

Häder and Poff (1979 a,b) used "light traps" to investigate the photoresponses of *D. discoideum* amoebae, and observed accumulation of amoebae in the light trap and

dispersal of amoebae from the light trap. These responses depend on cell density in the trap, the light intensity and wavelength used. This photoaccumulation, which occurs at relatively low doses of light, was the result of directed movement by the amoebae toward the light scattered from amoebae inside the trap. Photodispersal of amoebae at relatively high light intensities was the result of negative phototaxis by amoebae responding to the light scattered from the amoebae inside the trap. Microvideoscopical analysis again confirmed these results (Häder and Poff, 1979 a,b).

Because these photoresponses of amoebae are based on the light scattered from other amoebae, it is necessary to use very high intensities to induce oriented movement of amoebae. Possible undesirable side effects, such as a heat gradient between the inside and outside of the light trap and photodamage of the cells, could have resulted from the use of high light intensities. To avoid these problems, a device has been built which can use more directed light stimulation (at lower light intensities). Using this device, phototactic movements of amoebae have been confirmed, and an action spectrum of amoebal phototaxis measured.

Materials and Methods

Amoebae of an axenic strain of D. discoideum (Ax 2) were grown in darkness in a rotary shake culture at 23.5 $\pm$ 0.3°C in 400ml Erlenmeyer flasks as previously described

(Häder and Poff, 1979a). When the cells had grown to a concentration of about 6 x 10^6 cells/ml, they were harvested by centrifugation and resuspended in the buffer at 5 x 10^6 cells/ml. A 1 ml portion of the suspension was evenly distributed on the surface of 1% (w/v) non-nutrient water agar contained in a 90mm diameter polystyrene petri dish. The cells were subjected to the light treatment within 1 hr of the transfer to the non-nutrient agar in order to avoid cell aggregation which begins about 7-8 hr after this transfer (Häder and Poff, 1979c). Phototactic orientation of the amoebae was measured in an apparatus based on a device previously used for measuring phototactic orientation in flagellates (Feinleib and Curry, 1967; Nultsch and Throm, 1975). (See appendix for a detailed description of this apparatus for measuring phototaxis.) In this apparatus, PIN diodes in combination with a 660nm measuring light beam were used to monitor the density of a population of amoebae while the amoebae were irradiated with a lateral actinic light. The 660nm measuring beam was obtained using a Unitron lamp in combination with an interference filter and was directed at the sample from above.

This beam was partially absorbed by the cells before striking the two PIN diodes in the bottom of the petri dish holder. The PIN diodes, which were matched in their electrical characteristics, were connected in a Wheatstone bridge with two resistors, one of which was a potentiometer

for zero adjustment. The Wheatstone bridge was powered by 2.0V DC from a variable power supply. The fluence of the measuring beam was adjusted to 400 mW/m^2 at the surface of the PIN diodes. Actinic light was screened from the PIN diodes by a Corning filter No. 2030. Preliminary experiments showed that the measuring beam had no effect on the orientation or movement of the cells.

A collimated, actinic light beam from a slide cube projector (Bell and Howell, CP 40) was directed at the suspension from an angle of about 15° above the horizontal plane. Monochromatic light was produced using interference filters (Baird Atomic) with a half band width of 9-11nm and an average transmission of about 45%. In this case, the energy of the incident irradiation was controlled by varying the lamp voltage by means of a trimatron dimmer (Leviton). White light was produced by a Unitron lamp and the light intensity was controlled by inserting neutral density filters (Balzer) in order to guarantee a uniform color temperature of the incident light.

Oriented movement toward the actinic light source caused an increase in the number of cells over the PIN diode closest to the light source and thus reduced the amount of light received by this diode. One possible reason for this change would be that more cells are squeezing into the space over the PIN diode. If the cells dispersed from over the PIN diode farthest from the light source, it would cause an increase in the light received by that diode. The signal

from the Wheatstone bridge corresponded to the change in light striking the two diodes, and was measured with a high impedance digital voltmeter and recorded on an Esterline Angus model E1101E strip chart recorder after being filtered with a long-pass filter having a time constant of about 200ms. The entire apparatus except the actinic light source was enclosed in a dark box which was placed on styrofoam to reduce mechanical vibrations and was maintained in a dark room with temperatures controlled at 23 $\pm$ 1°C.

All light intensities were measured with a Kettering model 65 radiometer.

Results

When the amoebae are placed in the apparatus described above, they move randomly over the agar surface while being exposed only to the 660nm measuring beam. This movement is recorded as an inclining or declining line, which levels off as the cells become uniformly distributed (Figure 9A). In most experiments an equilibrium was reached after about 1 hr and was stable over several hours. Therefore, a dark period of 1 hr was allowed in all experiments before the onset of the light treatment.

With unilateral actinic white light at intensities of 40 and 90 mW/m^2, the absorbance increased over the PIN diode next to the light source, indicating a movement of the amoebae toward this source. At higher light intensities (160 and 200 mW/m^2) there is an increase in absorbance

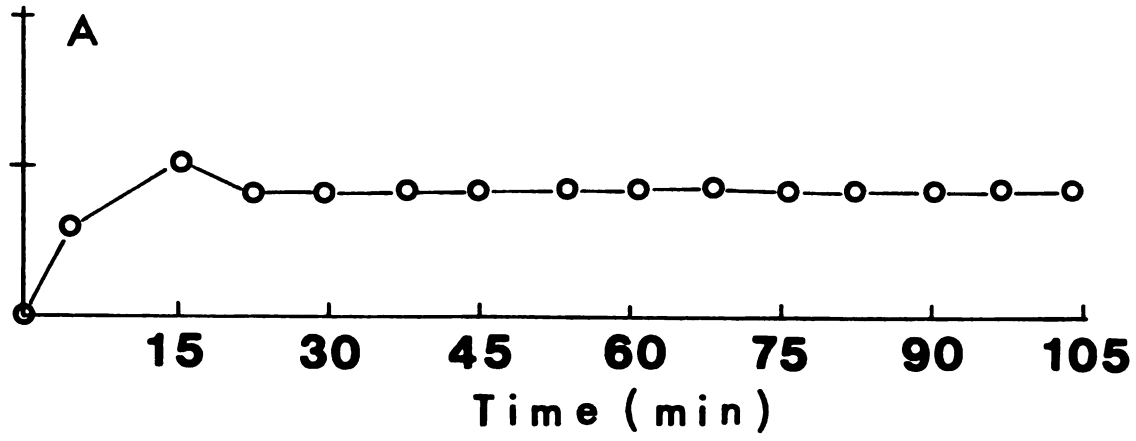
over the PIN diode furthest from the light source, indicating a movement of the amoebae away from the light (Figure 9B). The average slope (over the first hour) of such curves is the rate of response by the amoebae to the actinic light. This rate has been measured as a fluence-response curve (Figure 10). It is evident that light intensities up to about 100 mW/m^2 induce positive phototaxis while higher intensities induce negative phototaxis.

Fluence-response curves for monochromatic irradiation have been produced using the same procedure. Figure 11 shows three representative curves (for 402, 420 and 560nm). It is interesting to note that, as in the case of the white light fluence-response curve, there was a decrease in the negative response at higher quantum flux densities. An action spectrum (Figure 12) based on the zero-crossing between positive and negative phototaxis shows a major action maximum at about 405nm and secondary maxima at about 450, 520, 580 and 638nm. At wavelengths below 380nm, no oriented movements were observed at any fluence rate up to 500 mW/m^2.

Discussion

Since an accumulation of cells in a light field can be caused by a number of different mechanisms, a detailed analysis of the behavior of the cells is necessary to determine which of the possible photoresponse mechanisms is responsible for the observed population effect. Preliminary

Figure 9. Recorded movement of D. discoideum amoebae in darkness (A). Response of D. discoideum amoebae to white light. △ ,40 mW/m^2; ■ ,90 mW/m^2; ▲ ,160 mW/m^2; o ,200 mW/m^2 (B). Relative response is the change in electrical signal from the two PIN diodes and is proportional to the difference in number of amoebae over the two diodes.


A
15
30
45
60
75
90
105
Time (min)

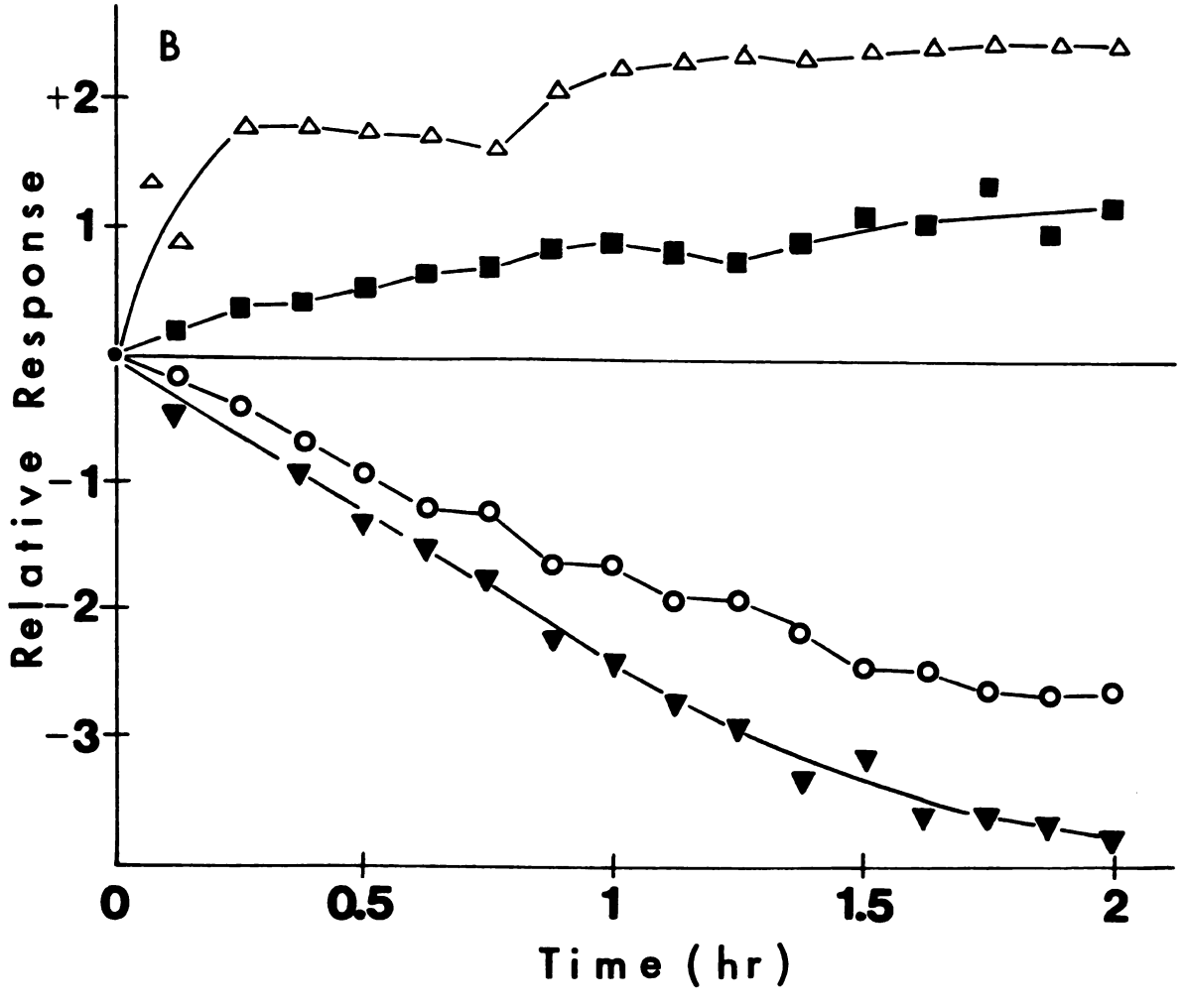

B
+2
1
-1
-2
-3
Relative Response
0
0.5
1
1.5
2
Time (hr)

Figure 10. Fluence-response curve for photomovement in white light. Phototaxis was measured as the rate of movement of a population of amoebae in response to each intensity of light. Each datum point represents a separate experiment.

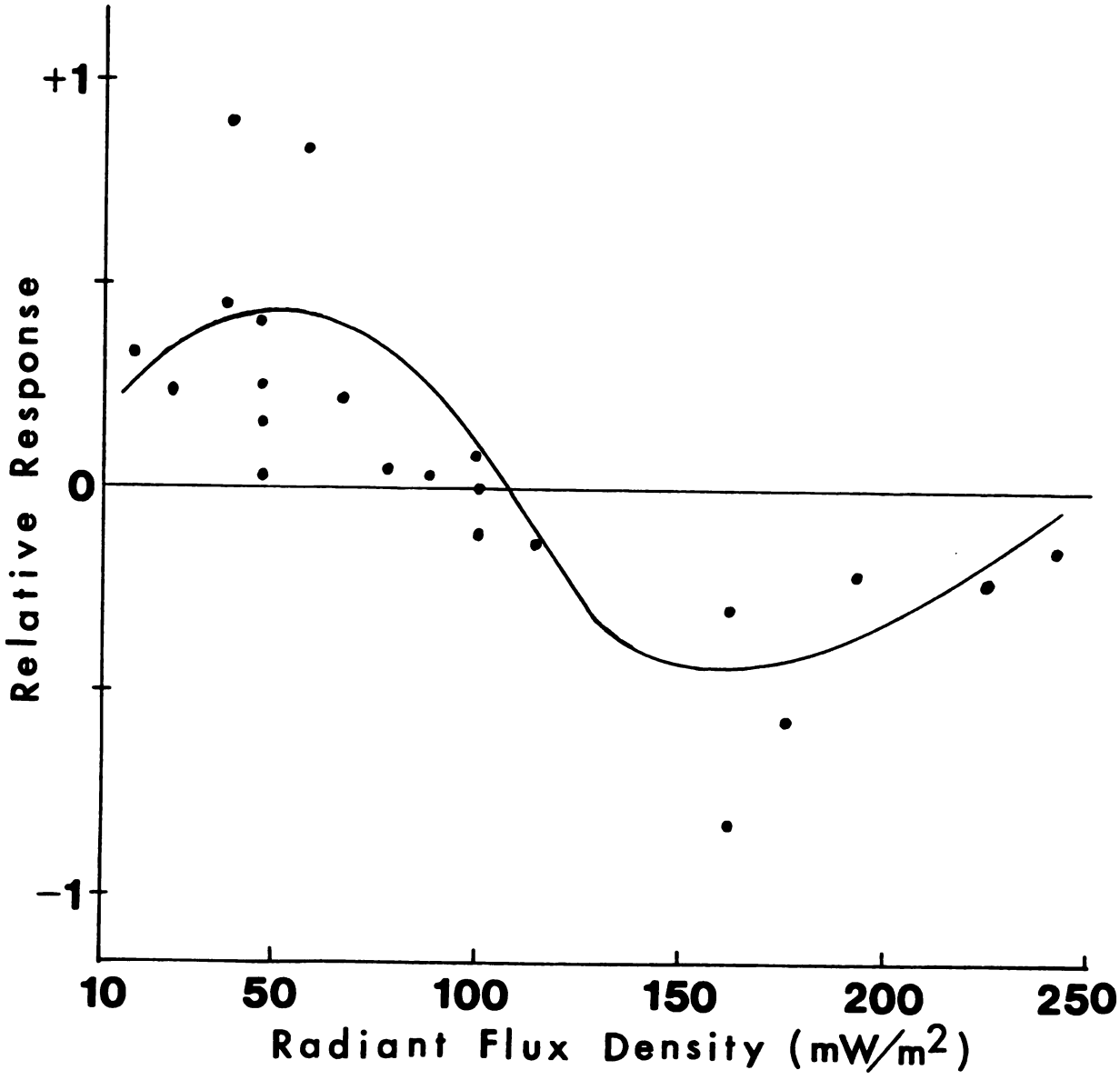

+1
0
−1
Relative Response
10
50
100
150
200
250
Radiant Flux Density (mW/m2)

Figure 11. Fluence-response curves for phototactic orientation in monochromatic light. ▲, 402nm; □, 420nm; ■, 560nm. Phototaxis was measured as the rate of movement of a population of amoebae in response to each intensity of light. From Hong et al. (1981).

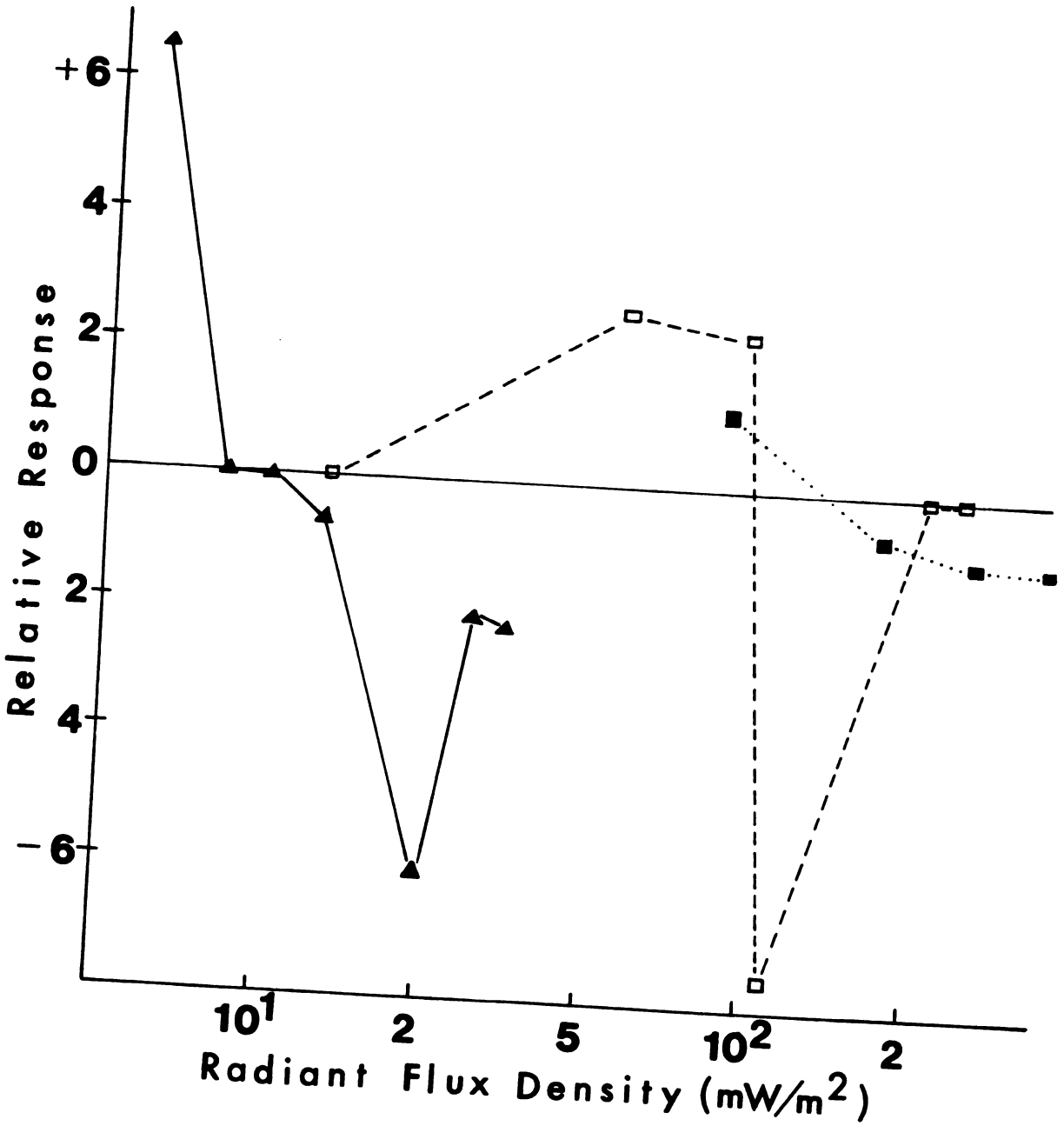
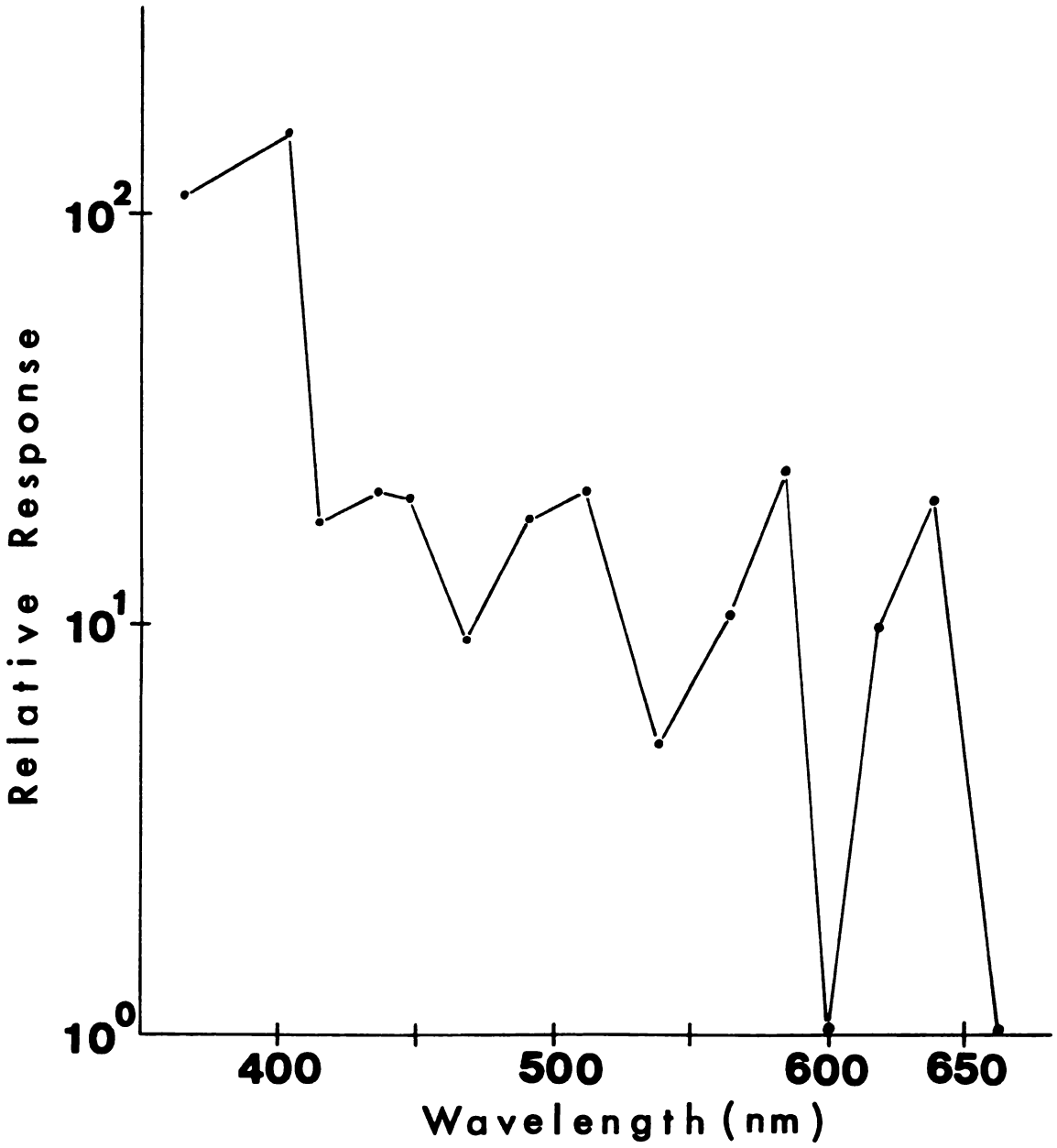

+6
4
2
0
2
4
−6
Relative Response
10¹
2
5
10²
2
Radiant Flux Density (mW/m²)

Figure 12. Action spectrum for phototaxis by amoebae based on the "zero-crossing" points in fluence-response curves for monochromatic light. Relative response is the reciprocal at each wavelength of the quantum flux density in nE, for the "zero-crossing" point. From Hong et al. (1981).


Relative Response
10^2
10^1
10^0
400
500
600
650
Wavelength (nm)

microvideographic observation has shown that amoebae move toward light at low intensities and away from light at high intensities, whether the actinic light is produced from a lateral light source or due to light scattered from cells or particles in an illuminated field. Thus, it has been previously concluded that the observed responses of D. discoideum amoebae in light fields result from positive or negative phototaxis of the individual amoebae (Häder and Poff, 1979 a,b).

The mass movement of a population of cells toward or away from unilateral light can be due only to an oriented movement (phototaxis), although the speed of movement of the cell can be influenced by a photokinetic effect (Schuchart, 1975). The fluence-response curves indicate that amoebae are positively phototactic at low light intensities and negatively phototactic at higher light intensities. This is consistent with the observation that amoebae accumulate in light fields of lower light intensities (Häder and Poff, 1979 a,b).

In addition, these results permit an estimation of the fraction of light scattered by the amoebae. This can be done by comparing the intensity at the "zero crossing" between positive and negative phototaxis and the intensity for the "zero crossing" between accumulation and dispersal from light fields. The "zero crossing" point between accumulation and dispersal in a white light trap is between 10^4 and 10^5 mW/m^2 (Häder and Poff, 1979a); the "zero

crossing" point between positive and negative phototaxis in response to unilateral light is at about 100 mW/m^2. Thus, about 0.1 to 1.0% of the white light incident on the amoebae in a light field appears to be scattered laterally out of the trap and responded to phototactically by the amoebae outside the trap.

The action spectrum presented here for phototactic orientation resembles the action spectra for accumulation in or dispersal from light traps by D. discoideum amoebae (Häder and Poff, 1979 a,b). Moreover, these spectra are similar to the action spectrum for the photoinhibition of aggregation (Häder and Poff, 1979c). The photoreceptor pigment for amoebal phototaxis is unknown at present, although the spectrum resembles the absorption spectrum of a porphyrin.

Summary

An apparatus had been developed to measure phototactic movement in a population of amoebae of D. discoideum. Fluence-response curves in white light show a positive phototaxis to light below 100 mW/m^2. Higher intensities cause a negative phototaxis. An action spectrum, based on the "zero-crossing" points in fluence-response curves for monochromatic light, shows a major peak at about 405nm and secondary maxima at about 450, 520, 580 and 638nm. This action spectrum resembles the action spectra for

accumulations of amoebae in and dispersal from light traps and that of inhibition of aggregation by light.

CHAPTER 5

PHOTORECEPTOR(S) IN DICTYOSTELIUM DISCOIDEUM AMOEBAL PHOTOTAXIS

Introduction

Amoebae of D. discoideum feed on bacteria in the mulch of the forest floor. Once the food is depleted, they follow a developmental cycle in which the pseudoplasmodium and the fruiting body are formed. During this life cycle, D. discoideum has two motile stages, the amoebae and the slug, both of which are able to respond to environmental stimuli by showing oriented movements.

Amoebae of D. discoideum are chemotactic to folic acid, unidentified "repellent(s)", and cAMP, and are thermotactic and phototactic. The pseudoplasmodium of D. discoideum is also chemotactic to "STF", thermotactic, and phototactic.

Häder and Poff (1979 a,b) observed photoaccumulation of amoebae inside the "light trap" and photodispersal of amoebae from the trap. Action spectra for those two responses, photoaccumulation and photodispersal, showed a primary maximum at 405 to 410nm and a broad band in the long-wavelength blue and green region of the spectrum, with a secondary maximum at about 638nm.

In Chapter 4, directed lateral light stimulation has been used to orient amoebae of D. discoideum. The results clearly suggested positive and negative phototactic responses of amoebae, strain Ax 2. The action spectrum of phototaxis of amoebae based on the "zero-crossing point" of the fluence response curves with monochromatic lights looks similar to the action spectra of photoaccumulation and photodispersal of amoebae. The similarity between those action spectra supports the previous interpretation that photoaccumulation and photodispersal of amoebae result from positive and negative phototaxis of amoebae, respectively.

Absorption spectra at 77°K of Ax 2 amoebae grown axenically in HL 5 liquid medium also show similar peaks as these action spectra. Such as absorption spectrum also has a primary maximum at 405 to 410nm, a broad band in the long-wavelength blue and green region, and a secondary maximum at about 638nm.

Among those peaks, the peak at 638nm in the absorption spectrum and action spectra is rather unique in biological examples--not many known in vivo compounds have a peak at this wavelength. Therefore, this peak at 638nm had been used to assay for the photoreceptor pigment regulating phototaxis in Ax 2 amoebae. (This photoreceptor pigment is designated as "P_{638}".)

This chapter will include two sections. In the first section, observed effects on P_{638} will be described

and protoporphyrin will be discussed as P_{638}. In the second section, P_{638} will be localized inside the cell.

5.1. Identification of P_{638}

Materials and Methods

D. discoideum amoebae, axenic strain Ax 2, were cultured axenically in HL 5 liquid medium in rotary shake culture at 23.5 ± 0.3°C in darkness, harvested at the appropriate culture age, washed with phosphate buffer, and resuspended in the same buffer as described in Chapter 1.

The titer of the culture was measured using a Hemacytometer (American Optical) after the proper dilution.

A very small aliquot (less than 2ul) of the resuspended amoebae suspension at the concentration of 3×10^7 cells/ml was put on 1% water agar (w/v) covering a 1 x 25 x 75 mm glass microscope slide and linearly spotted by means of the micromanipulator as described in Chapter 2. The thickness of 1% water agar was about 2 mm, and a marker, consisting of small blue cellophane, was inserted in water agar before it solidified. This marker was used to locate the original spot line.

The microscope slide was then placed on the transparent plastic slab, 9 x 90 x 2 cm, in a large, 40 x 100 x 25 cm, covered box constructed of transparent plastic. To keep enough moisture in the box, the box was filled with about 1.5 cm of distilled water. The transparent plastic slab was grooved lengthwise at the proximal side, such that neutral

density filter(s) could be placed in front of each microscope slide. The box was covered and wrapped totally with wet black cloth except for the front face through which light was admitted.

After 7 hr of exposure to the light, the microscope slides were taken out and the results were checked. The degree and direction of response were determined by comparing micrographs of the original spot and of that following the resultant. Values of $\Delta\%$ were calculated and used to plot fluence-response curves as described in Chapter 2.

Photomicrographs were taken by means of a Zeiss microscope equipped with a 35 mm camera.

All the preparations and checking the slides were performed at room temperature under dim white light, and the microscope slides with the sample were kept in the dark box with enough moisture. All the phototactic experiments were performed in a dark room at $23.5 \pm 0.3^{\circ}C$.

A slide cube projector (Bell and Howell, CP 40) was used as the light source and was directed at the microscope slides from an angle of about 10° to 15° above the horizontal plane. A 35 mm path length aqueous 5% (w/v) $CuSO_4$ solution was used as an IR-absorbing filter throughout the experiment.

Light intensity was controlled by placing neutral density filters (Balzer or Inconnel) in front of the sample and/or the light source. Light intensity was measured with

a Kettering model 65 radiometer and/or an International Light Spectroradiometer (Newburyport, Mass).

Low temperature absorption spectra were measured with a single-beam spectrophotometer on line with a small computer as described in Chapter 1.

The following buffers were used: phosphate buffers at pH 2.3, 6.5 and 10.2, phosphoric acid at pH 2.12, formic acid at pH 3.75, acetic acid at pH 4.75, MES at pH 6.15, MOPS at pH 7.20, Tris at pH 8.30, boric acid at pH 9.24 and CAPS at pH 10.40. The strength of all buffers was 0.2 M.

Chemicals used: Biochemicals were the products of Sigma, Aldrich, Calbiochem, or Fisher.

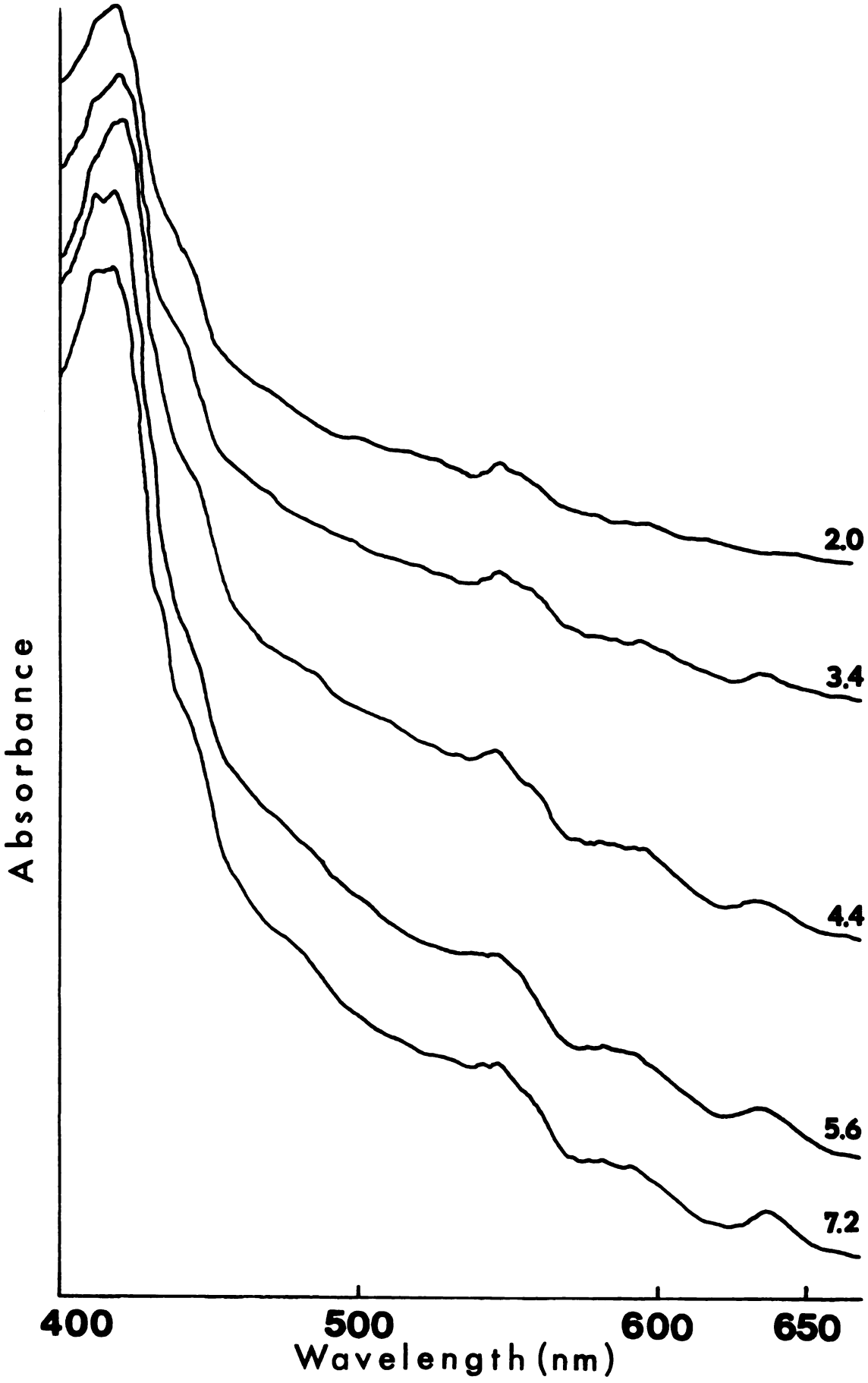
Results

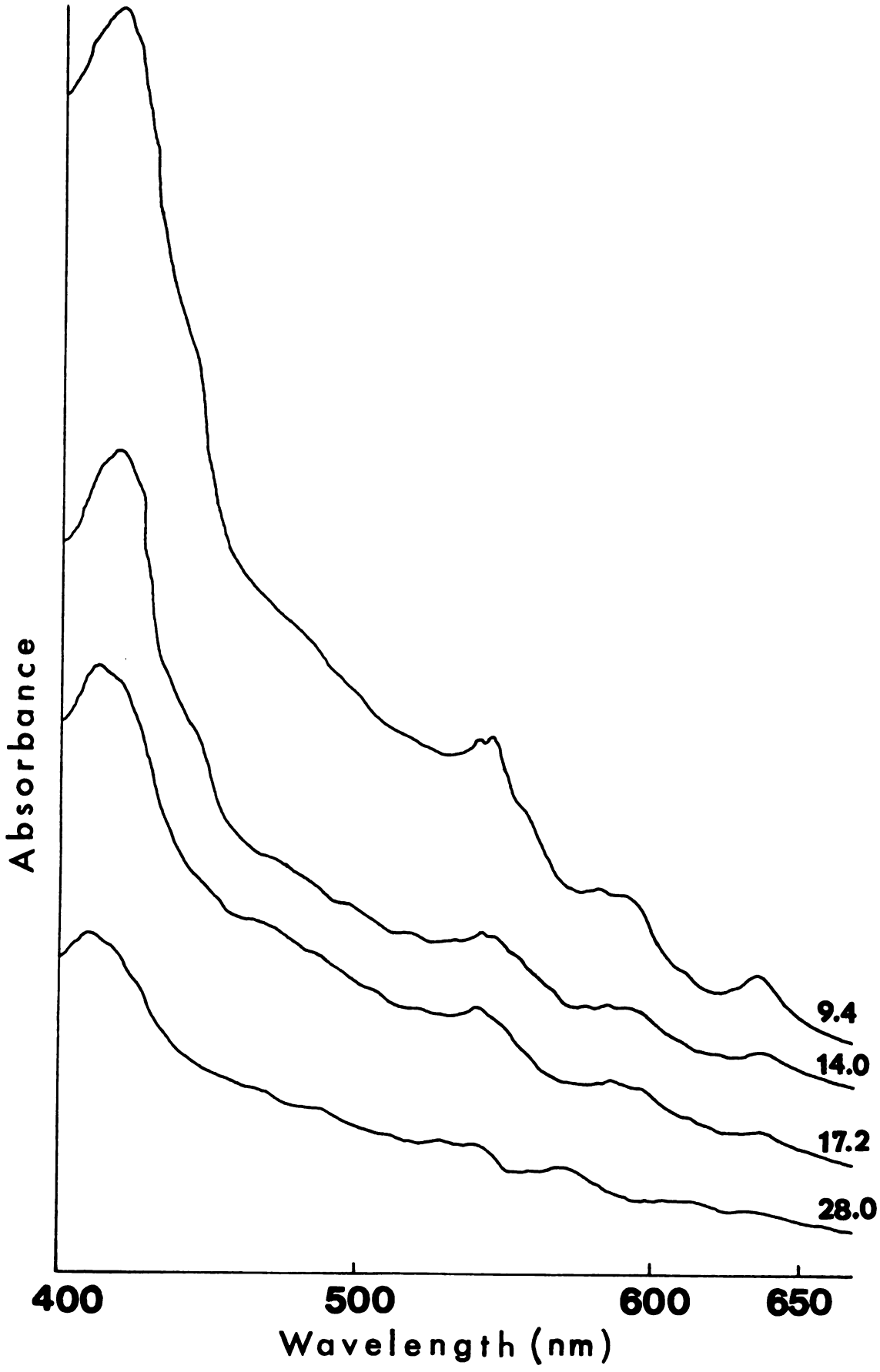
Amoebae of D. discoideum, strain Ax 2, irradiated with uni-lateral light for 7 hr moved randomly (Figure 13B), toward the light (Figure 13C), or away from the light (Figure 13D).

Amoebae of D. discoideum, axenic strain Ax 2, grown in the liquid medium, HL 5, increase their content of P_{638} with increasing cell titer in the culture, while in the "log-phase" of growth. As the cells enter the stationary phase, the amoebae secrete P_{638} into the liquid medium which results in a decrease of internal P_{638} concentration (Figure 14). Figure 15 clearly shows the change of internal P_{638} concentration at different cell ages.

Figure 13. Tracings from photomicrographs showing original spot (A), random movement (B), positive phototaxis (C), and negative phototaxis (D) of amoebae. Small dots are amoebae, and lines crossing or curving the middle of each picture are from markers. Dotted lines represent the position of original spots which have been traced from the photomicrographs of original spots.

Figure 14. Low temperature, 77°K, absorption spectra of Ax 2 amoebae grown in HL 5 liquid medium at nine different titres of culture. The titer of culture in 10^6 cells/ml is indicated on the right side of correspondent absorption spectrum.


Absorbance
2.0
3.4
4.4
5.6
7.2
400
500
600
650
Wavelength (nm)


Absorbance
9.4
14.0
17.2
28.0
400
500
600
650
Wavelength (nm)

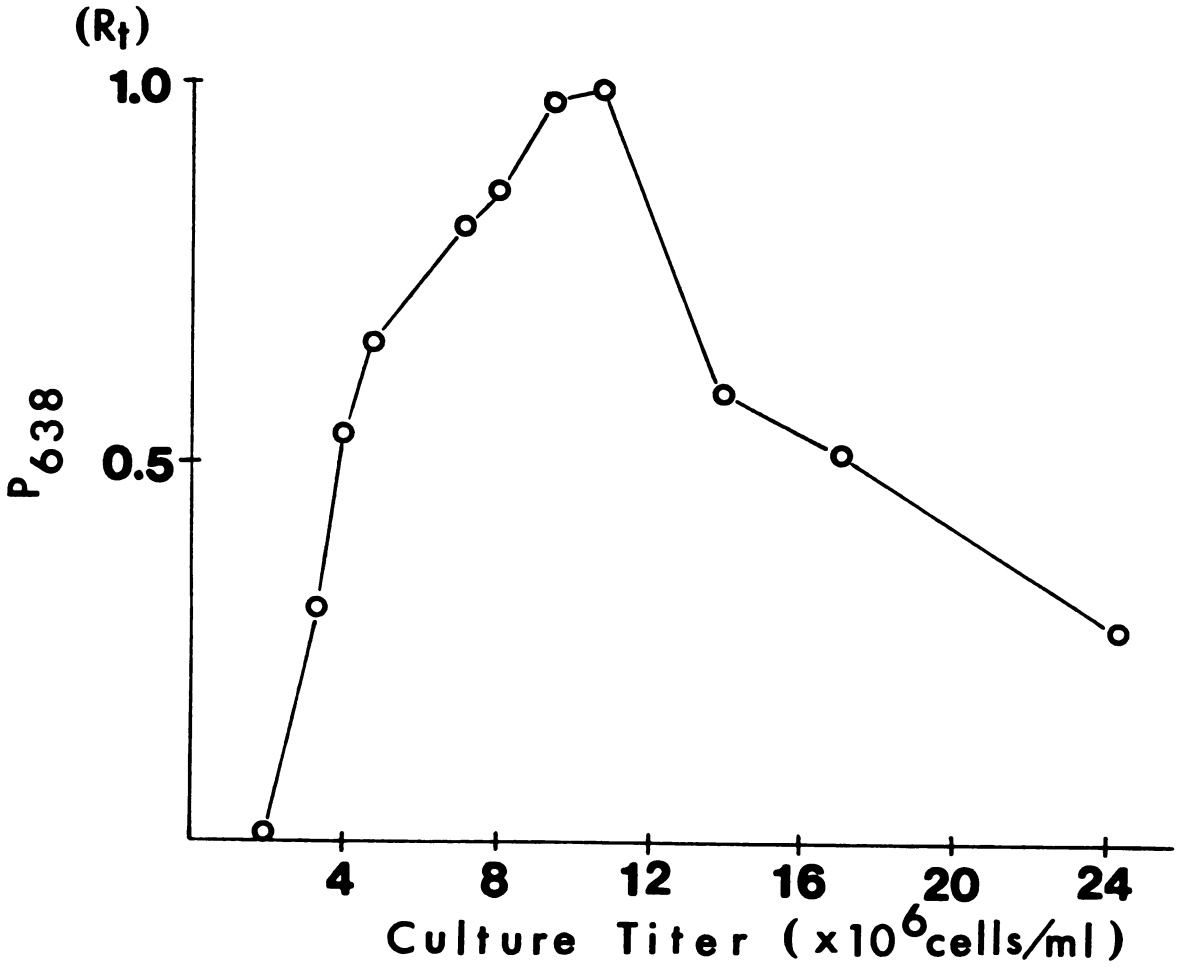
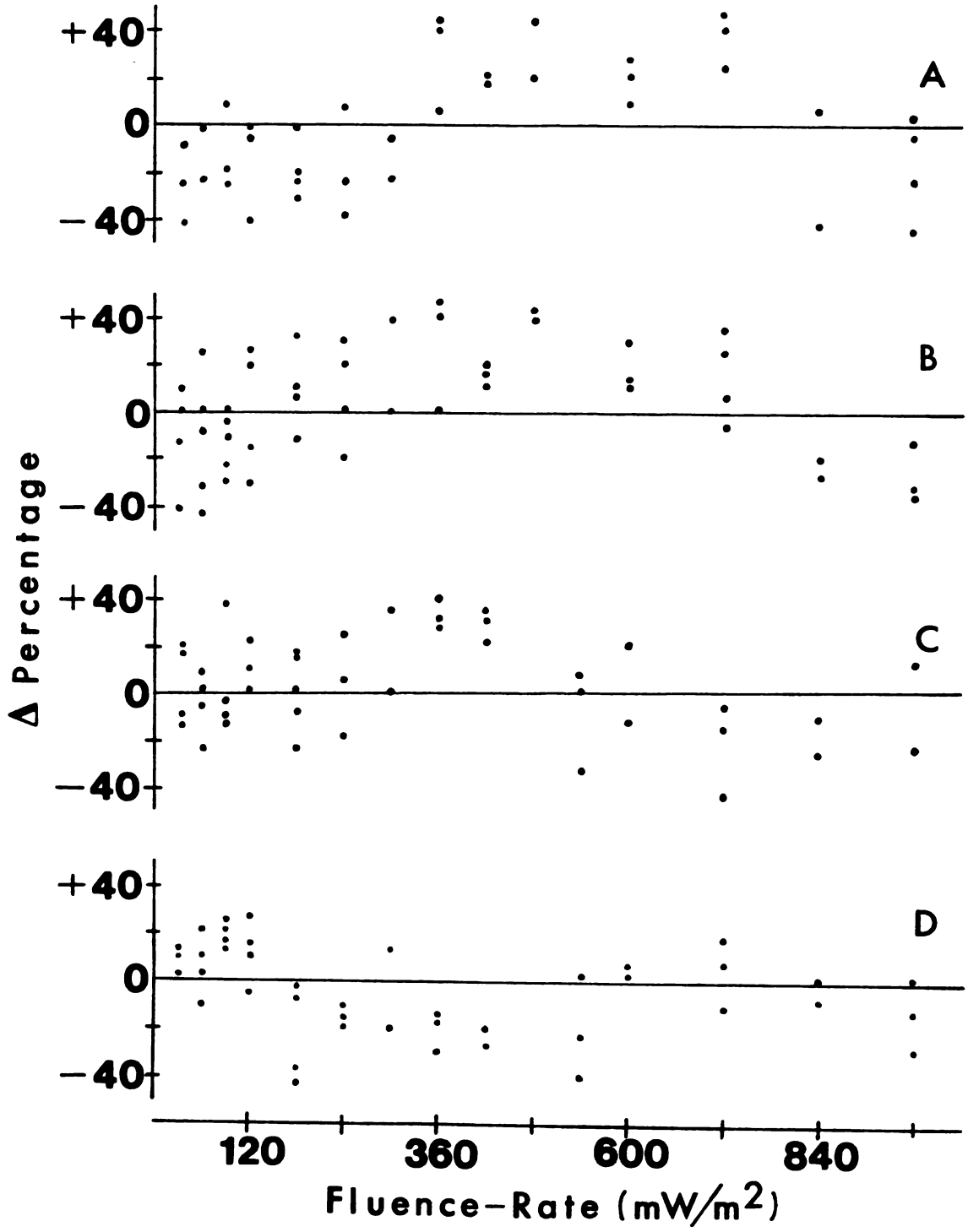


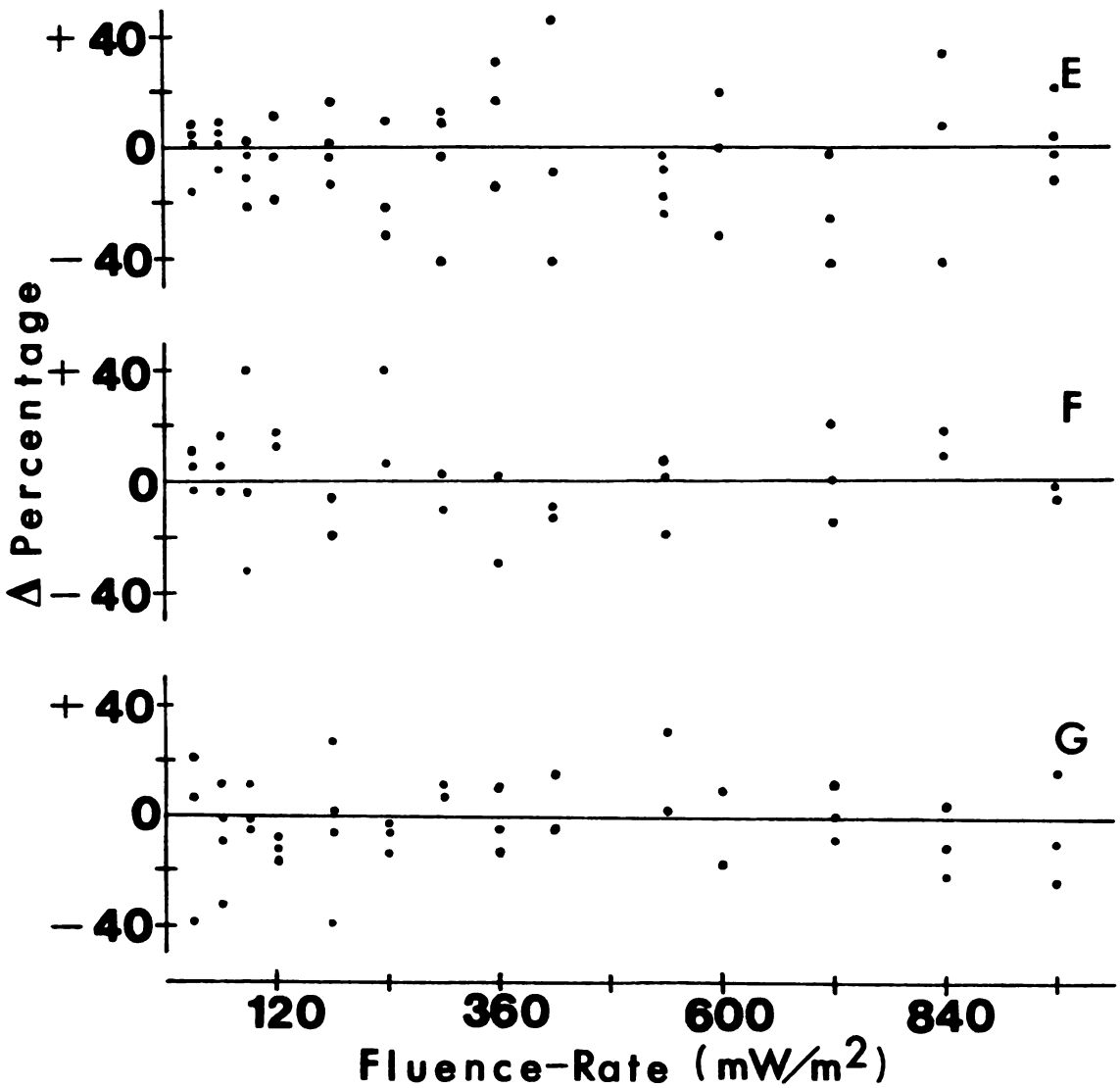
Figure 15. Relative absorbance of Ax 2 amoebae at 638nm (P_{638}) at different titers of culture.

Fluence-response curves at different culture ages have been measured, and a possibility of P_{638} as a phototactic photoreceptor pigment was investigated. At about 2.2 x 10^6 cells/ml, light intensities lower than about 300 mW/m^2 induced negative phototaxis of amoebae, and intensities between 300 to 800 mW/m^2, there was possible negative phototaxis (Figure 16A). Negative phototaxis at light intensities lower than about 100 mW/m^2, positive phototaxis between about 100 mW/m^2 to 700 mW/m^2, and negative phototaxis above 700 mW/m^2 were apparent at about 3.5 x 10^6 cells/ml (Figure 16B). At about 5.0 x 10^6 cells/ml, positive phototaxis could be observed at lower than about 450 mW/m^2 and above it there was negative phototaxis (Figure 16C). Positive phototaxis at light intensities lower than about 130 mW/m^2 and negative phototaxis between 130 mW/m^2 to 560 mW/m^2 were observed at the titer of about 6.5 x 10^6 cells/ml (Figure 16D). Cultures as old as, or older than, about 8.0 x 10^6 cells/ml showed no significant phototaxis (Figures 16E,F,G) in the light intensity range examined. Figure 17 shows the summarized results of fluence-responses and intracellular P_{638} content at different cell ages.

Absorbance at 638nm is not dependent on oxidizing or reducing reagents, such as ferricyanide and hydrosulfite, or by incubation with proteases, such as papain and trypsin (unpublished data), although four hours of incubation of cells in a phosphate buffer, pH 6.1 and 15 mM, at room temperature itself usually decreases the absorbance at 638nm

Figure 16. Fluence-response curves of D. discoideum, strain Ax 2, amoebal phototaxis at different culture ages. A) 2.2 x 10^6 cells/ml, B) 3.5 x 10^6 cells/ml, C) 5.0 x 10^6 cells/ml, D) 6.5 x 10^6 cells/ml, E) 8.0 x 10^6 cells/ml, F) 9.4 x 10^6 cells/ml, G) 12.0 x 10^6 cells/ml. At least two separate experiments were performed at each titer.


+40
0
−40
A
B
C
D
Δ Percentage
120
360
600
840
Fluence-Rate (mW/m2)


+40
0
−40
E
+40
0
−40
F
+40
0
−40
G
Δ Percentage
120
360
600
840
Fluence-Rate (mW/m2)

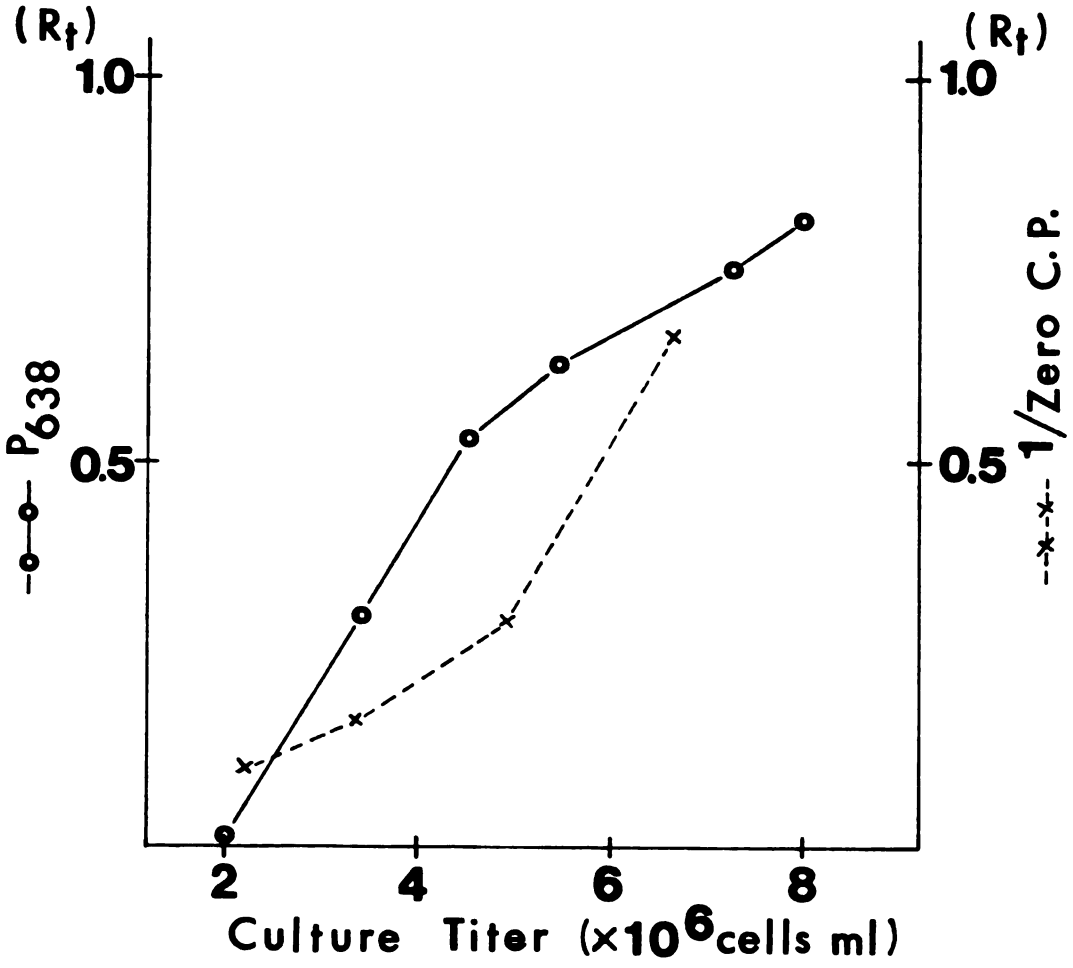


Figure 17. Comparison of changes in the intracellular P_{638} concentration and the fluence-rates for the "zero-crossing points" at different culture ages in Ax 2 amoebae.

(which results in a stronger absorption at 630nm). Incubation of the amoebae at higher pH maintains the absorption peak at 638nm, and incubation of cells at low pH or with detergents, such as sodium dodecyl sulfate (SDS), decreases P_{638} but increases P_{630}, i.e., a photoreceptor pigment which has an absorption peak at 630nm (Figure 18). A concurrent increase of absorbancy at 630 nm with a decrease of P_{638} indicates that P_{638} is converted to P_{630}. Growing amoebae, strain Ax 2, in the HL 5 liquid medium with decreased surface area/volume ratio causes a decrease in absorbance not only at 638nm but also at 630nm (Figure 19).

While these experiments were in progress, a compound was extracted from D. discoideum amoebae, axenic strain Ax 2, in the stationary stage, which is soluble in organic solvents. This compound was identified as protoporphyrin by means of thin layer chromatography, absorption and fluorescence spectroscopy. The extracted protoporphyrin has absorption maxima at 404, 502, 534, 575, 603 and 630 nm in ethyl ether, and P_{638} was suggested as an aggregated form of protoporphyrin (Lagarias, personal communication). A low temperature, 77°K, absorption spectrum of protoporphyrin IX $(Na)_2$ has a broad maximum at around 425 nm and sharp peaks at about 510, 540, 575 and 630 nm in 70 mM cetyl trimethyl ammonium bromide (CTAB) (Figure 20A), and a very broad maximum throughout the blue region of spectrum with sharp peaks at about 535, 585 and 638 nm in pH 8.7, 0.2 M Tris buffer (Figure 20B). The amoebae, ultrasonically

Figure 18. Low temperature, 77°K, absorption spectra of amoebae in different conditions. A) amoebae just after harvest; B) incubation of A for 4 hr at room temperature in pH 6.1, 15 mM phosphate buffer; C) amoebae in pH 2.3, 0.2 M phosphate buffer; D) amoebae in pH 6.5, 0.2 M phosphate buffer; E) amoebae in pH 10.2, 0.2 M phosphate buffer; F) amoebae in pH 6.1, 15 mM phosphate buffer; G) incubation of amoebae from the conditions in 18F transferred for 1 hr to 2% SDS.

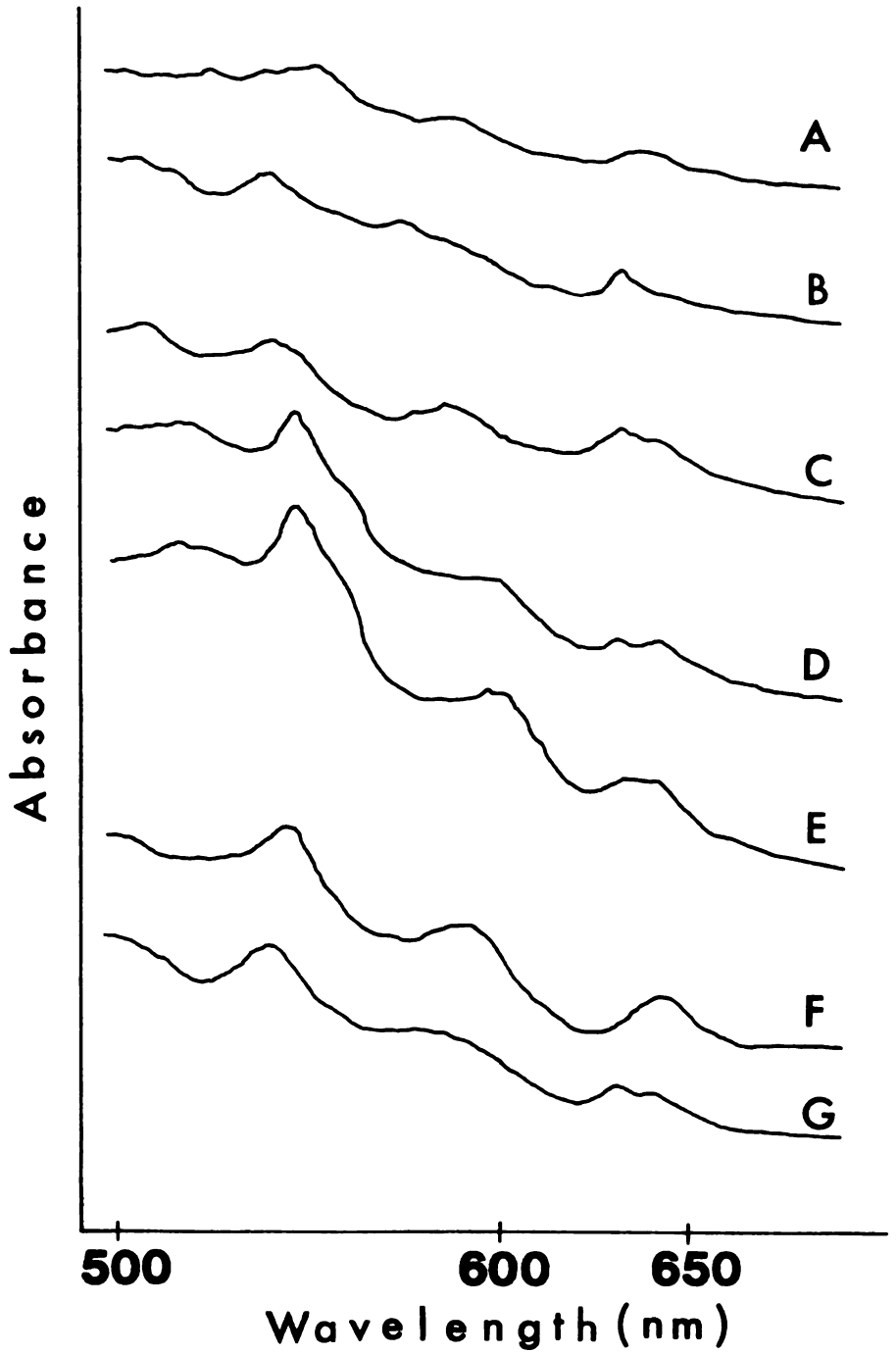
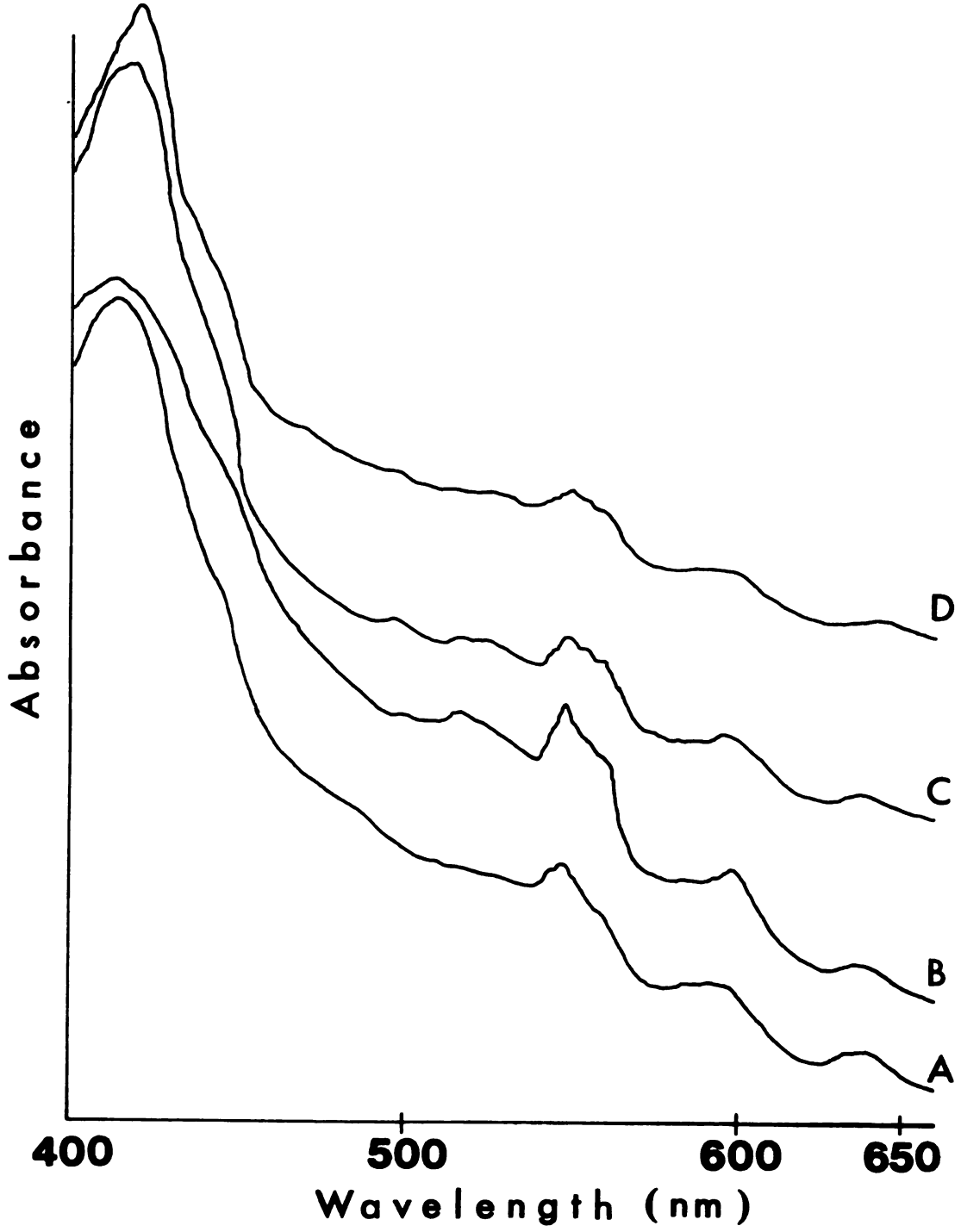

A
B
C
D
E
F
G
Absorbance
500
600
650
Wavelength (nm)

Figure 19. Low temperature, 77°K, absorption spectra of amoebae grown in HL 5 liquid medium with different surface area/volume ratios. A) 200 ml of medium; B) 300 ml of medium; C) 500 ml of medium; D) 600 ml of medium. Flask size was equally 2,000 ml.


Absorbance
D
C
B
A
400
500
600
650
Wavelength (nm)

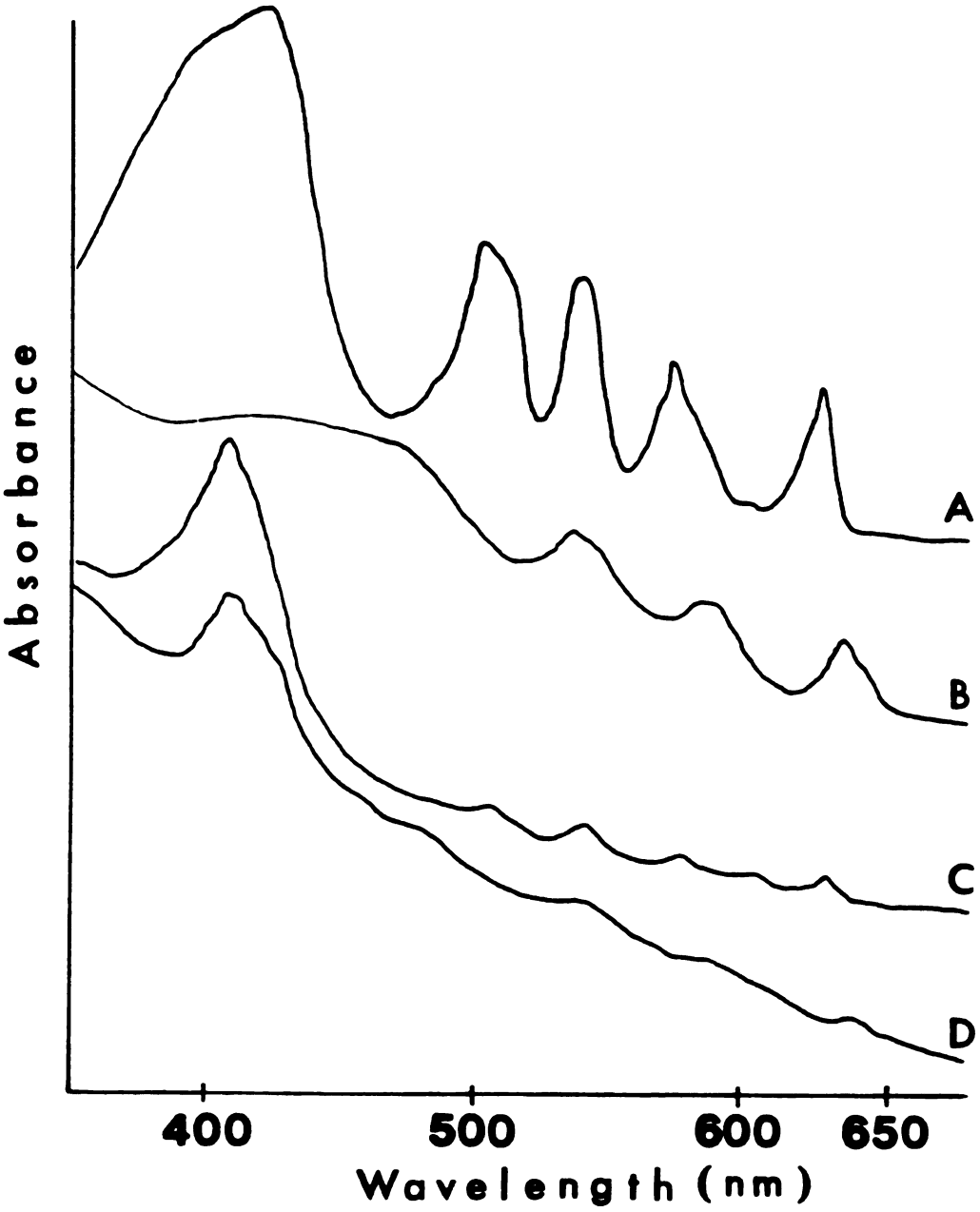
disrupted (1x at 70 W for 5 sec.) and pelleted by centrifugation at 1,000 x g for 10 min., have an absorption, low temperature (77°K), maximum at about 410 nm and secondary absorption peaks at about 510, 540, 577 and 630 nm in 70 mM CTAB (Figure 20C), and has a maximum at about 410 nm with secondary absorption peaks at about 490, 535, 585 and 638 nm in pH 8.7, 0.2 M Tris buffer (Figure 20D). These similarities in absorbancy were expected results as suggested (Lagarias, personal communication).

5.2. Subcellular localization of P_{638}

Materials and Methods

D. discoideum amoebae, strain Ax 2, were cultured axenically in HL 5 liquid medium as described in Chapter 4. Cells in the log phase were harvested with centrifugation, washed with phosphate buffer, and suspended in the extraction buffer, 0.2 M tris buffer with 1 mM EDTA and 0.25 M sucrose at pH 8.7. Cells (2×10^9 cells in 7 ml of the extraction buffer) were ultrasonically disrupted. Each disruption was at 70 W for 5 sec. One application at this strength gave about 70% cell breakage, and two or more ultrasonifications appeared to result almost 100% cell breakage. The ultrasonically disrupted cells were centrifuged at 1,000 x g for 10 min to pellet unbroken cells. The supernatant was layered on the top of a 20%-55% continuous sucrose density gradient and centrifuged at 10,000 x g for 3 hr at 4°C.

Figure 20. Low temperature, 77°K, absorption spectra of protoporphyrin IX $(Na)_2$ and Ax 2 amoebae, ultrasonically disrupted and pelleted by centrifugation at 1,000 x g for 10 min. Protoporphyrin IX $(Na)_2$ was dissolved in 70 mM cetyl trimethyl ammonium bromide (in pH 8.0, 50 mM Tris-HCl buffer) A), and in pH 8.7, 0.2 M Tris buffer B). The amoebae were suspended in 70 mM CTAB (in pH 8.0, 50 mM Tris-HCl buffer) C), and in pH 8.7, 0.2 M Tris buffer D).


Absorbance
A
B
C
D
400
500
600
650
Wavelength (nm)

Following centrifugation, the gradients were fractionated and the fractions stored at -20°C until the next experiment.

The activities of marker enzymes for organelles, the density of sucrose, and an absorption spectrum of each fraction were measured (Figure 21). Marker enzymes used were as follows: cytochrome c oxidase and malate dehydrogenase for mitochondria, cytochrome c reductase for endoplasmic reticulum, alkaline phosphatase for plasma membrane, acid phosphatase for vacuole and urate oxidase for peroxisomes. The density of sucrose was measured by means of a hand refractometer (Bausch and Lomb) and an absorption spectrum at 77°K was measured with the spectrophotometer on line with a small computer as described in Chapter 1.

An extraction buffer pH of 8.7 was chosen because of the ease in breaking the cells (a pH over 10 usually resulted in lysis of the amoebae) (Figure 22) and stability of P_{638} at that pH. All the experiments were performed in a cold room under dim white light and samples were kept at 0°C. For the room temperature absorption measurements, a Cary 15 spectrophotometer and a Gilford 410 spectrophotometer were used.

Each enzyme has been assayed as follows: Cytochrome c oxidase for mitochondrial membranes was measured following Appelmans et al. (1955) with a slight modification. Horse heart cytochrome c (0.53 mg/ml) in 50 mM tris-acetate buffer (pH 7.4) containing about 0.05% Triton X-100 was reduced

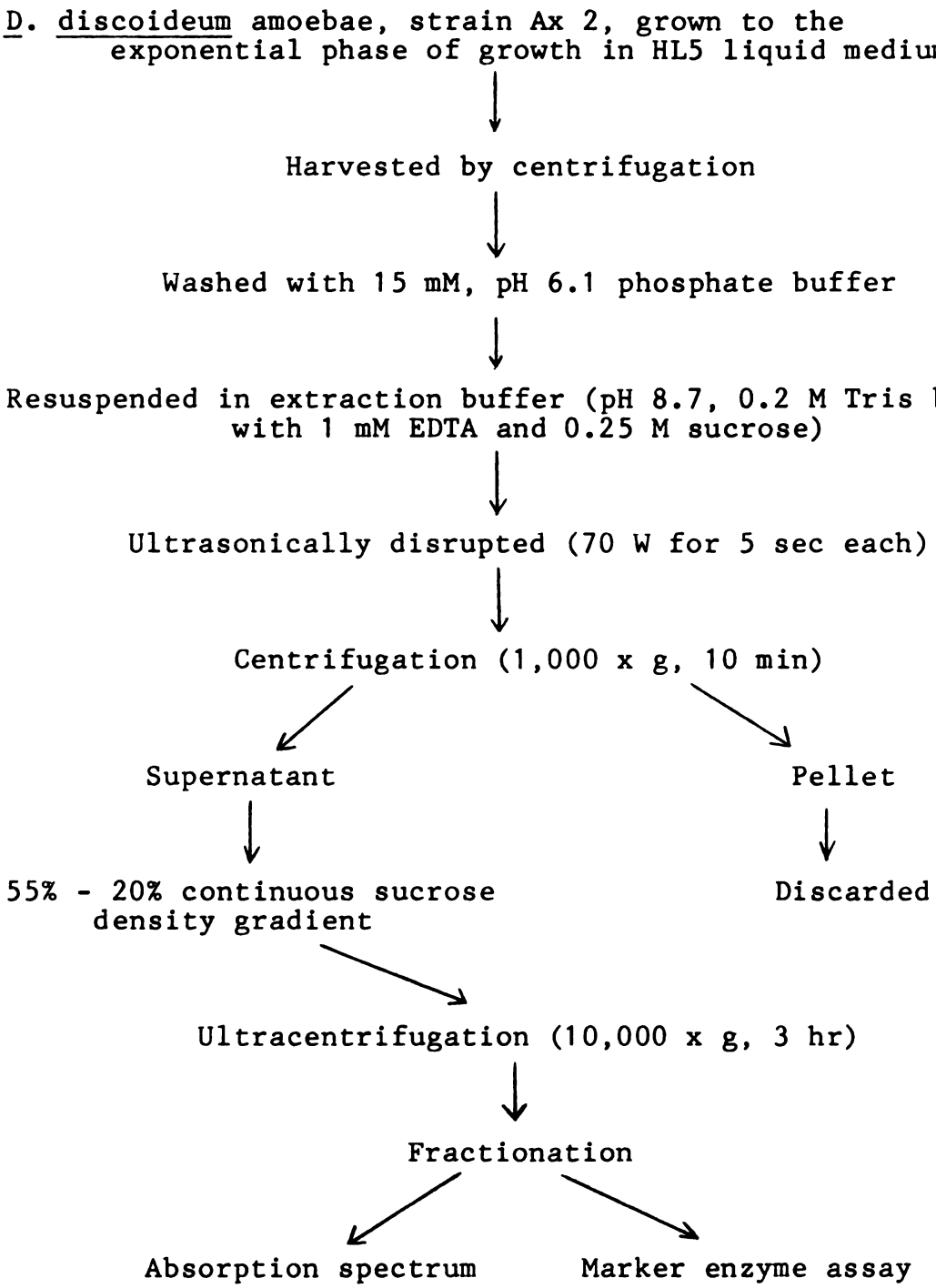


Figure 21. Flow sheet for the fractionation of the subcellular organelles.

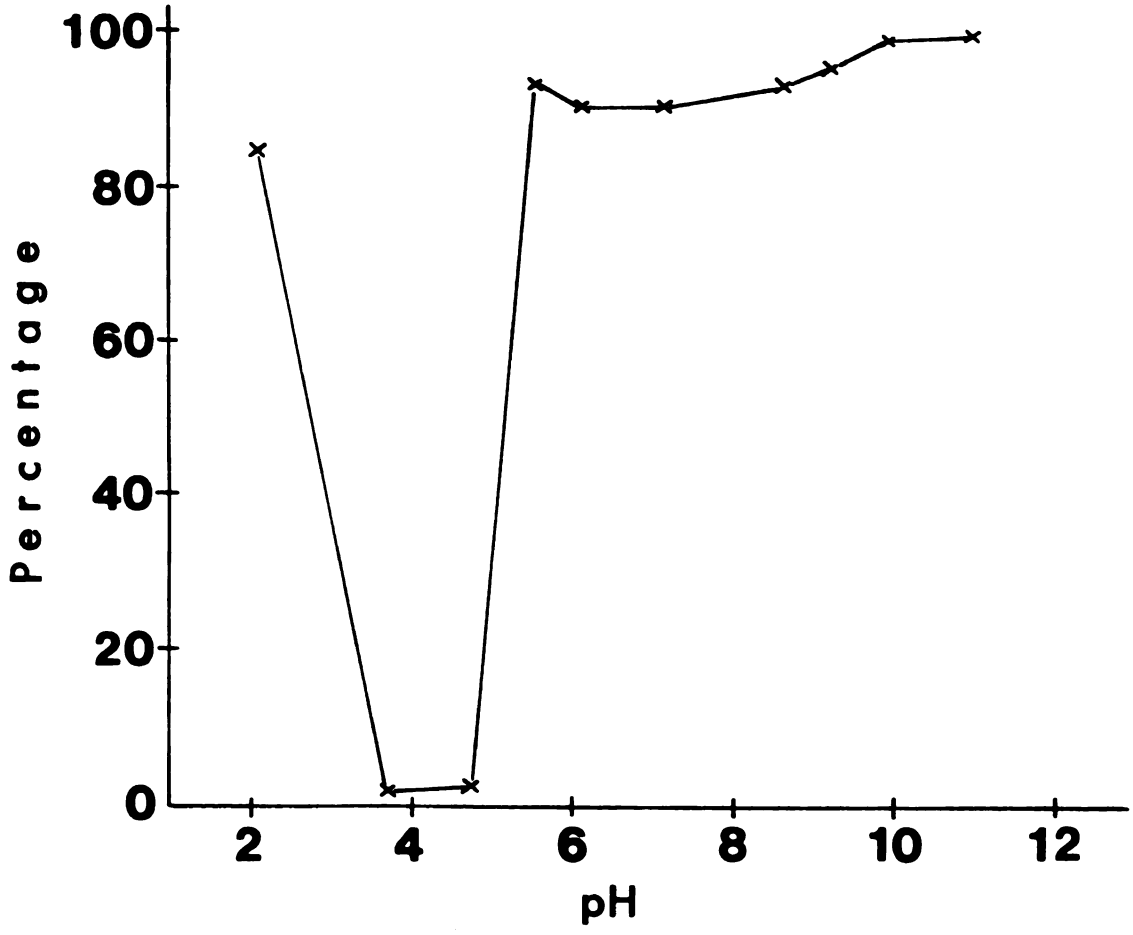


Figure 22. Percentage of breakage of Ax 2 amoebae by ultrasonification (1x at 70 W for 7 sec) at different pH's.

with 1 ul/ml sodium hydrosulfite solution (1M) prepared with distilled water immediately before addition. To 1 ml of this solution in a 1.4 ml cuvette 20 ul of sample was added. After rapid mixing, the rate of oxidation of cytochrome c was followed at 550nm with the Gilford 410 recording spectrophotometer. The slope of the recorded response was measured and used for the relative activity of this enzyme.

Malate dehydrogenase for mitochondrial matrix was measured by the method of Ochoa (1955) with a slight modification. Two tenths of ml of 0.00037 M NADH, freshly prepared in 0.05 M, pH 7.4, tris-HCl buffer, was mixed with 2.6 ml of 0.1 M, pH 7.4, phosphate buffer. One tenth of ml of 0.006 M oxalacetic acid, freshly prepared in 0.1 M, pH 7.4, phosphate buffer, and 20 ul of sample was then added. The absorbance change at 340nm was also followed with the Gilford spectrophotometer.

NADH-dependent cytochrome c reductase for endoplasmic reticulum was measured according to the methods of Lord et al. (1973) with a slight modification. A 1 ml aliquot of horse heart cytochrome c (0.53 mg/ml) in 50 mM tris-acetate buffer (pH 7.4) containing 2 mM KCN was mixed with 40 ul of 50 mM NADH in a 1.4 ml cuvette. The reaction was initiated by the addition of 20 ul of sample. The reduction of cytochrome c was followed at 550nm in the Gilford spectrophotometer.

Alkaline phosphatase for plasma membrane was measured following the methods of Green and Newell (1974) with a

slight modification. The assay system contained in the 1.4 ml cuvette: 40 mM ethanolamine-HCl, pH 9.5, 5 mM $MgSO_4$, 2 mM KF (total volume of these reaction mixtures was 1 ml), 0.1% (v/v) Triton X-100 and 40 ul of sample. $MgSO_4$ was replaced by 10mM-EDTA in the control cuvette. After a 5 min incubation at room temperature, the reaction was initiated by adding p-nitrophenyl phosphate such that the final concentration was 10 mM. The EDTA-sensitive hydrolysis was measured at 420nm by means of the Gilford spectrophotometer. The inclusion of an EDTA control was intended to compensate for the hydrolysis of p-nitrophenyl phosphate by other phosphatases, such as glucose 6-phosphatase, which do not require Mg^{+2} ions (Hübscher and West, 1965).

Acid phosphatase was measured as described in Müller and Møller (1969) with a slight modification. The reaction mixture contained 0.02 M p-nitrophenyl phosphate in 0.1 M, pH 5.0, acetate buffer. 40 ul of sample was then added to this 1 ml reaction mixture, and incubated at 25°C for 5 min. The reaction was stopped by the addition of 0.5 ml of 1 M NaOH, and the liberated p-nitrophenol was determined at 410nm with the Gilford digital spectrophotometer.

Urate oxidase for peroxisomes was also measured as described in Müller and Møller (1969) with a slight modification. The 40-ul sample was added to 1 ml, 0.05 M, pH 9.4, glycine-NaOH buffer and incubated at room temperature for 5 min to achieve a stable absorbance at 292nm. Then 20 ul

buffered urate solution (21 mg uric acid is dissolved in 10 ml of 0.02 M NaOH and 10 ml of 0.1 M glycine-NaOH buffer of pH 9.4 added) was added. After careful mixing the absorbance changes at 292nm were recorded for 5 min or longer with the Gilford spectrophotometer.

Fractionation was done by using 2112 Redirac Fraction Collector (LKB Bromma) in the cold room in darkness, and Braunsonic 1510 ultrasonificator was used to disrupt the amoebae.

Chemicals used: Biochemicals were the products of Sigma, Aldrich, Calbiochem, or Fisher.

Results

Biosynthesis of protoporphyrin (at least at the late steps) occurs in the mitochondria from various systems, such as mammalian liver, tobacco leaves and Rhodopseudomonas spheroids (see Frydman, et al., 1978 for a recent review about biosynthesis of protoporphyrin). Therefore, marker enzymes for mitochondria were the main interest during the localization of P_{638}.

Ultrasonic disruption of the amoebae three times at 70 W for 5 sec each resulted in two peaks of P_{638}, one major peak at about 46.2% of sucrose and a broad secondary peak from 37.2% to 39.8% of sucrose. The peak of cytochrome c oxidase activity appeared at much lower concentration of sucrose than the peaks of P_{638} (Figure 23).

Ultrasonical disruption of the amoebae twice at 70 W for 5 sec resulted in two sharp peaks of P_{638} at about 45.3% and 37.2% of sucrose (Figure 24A). Under this condition, activity of cytochrome c oxidase showed two major peaks, one at about 46.2% and the other at about 37.5% of sucrose. The main activity of cytochrome c reductase was below 34% of sucrose (Figure 24B). The major peaks of alkaline phosphatase were at about 35% of sucrose, while the majority of activity of acid phosphatase was in the soluble fractions (Figure 24C).

Ultrasonical disruption of the amoebae once at 70 W for 5 sec resulted in a single peak of P_{638} at about 44.6% of sucrose, which coincided with the peaks of cytochrome c oxidase and malate dehydrogenase activity, indicating that intact, relatively well preserved mitochondria have been obtained. The main activity of cytochrome c reductase was at below 34% of sucrose as before. Substantial acid phosphatase activity was found in the soluble fractions with a secondary maximum of activity at about 37.4% of sucrose. Alkaline phosphatase showed its major activity at 33% sucrose, while the maximum urate oxidase activity was found at 42% sucrose (Figure 25). Some examples of liquid nitrogen temperature absorption spectra at different concentrations of sucrose are shown in Figure 26.

Figure 23. Absorbance at 638nm and activity of marker enzymes in each fraction from sucrose density gradient. D. discoideum amoebae, strain Ax 2, were ultrasonically disrupted, three times at 70 W for 5 sec.

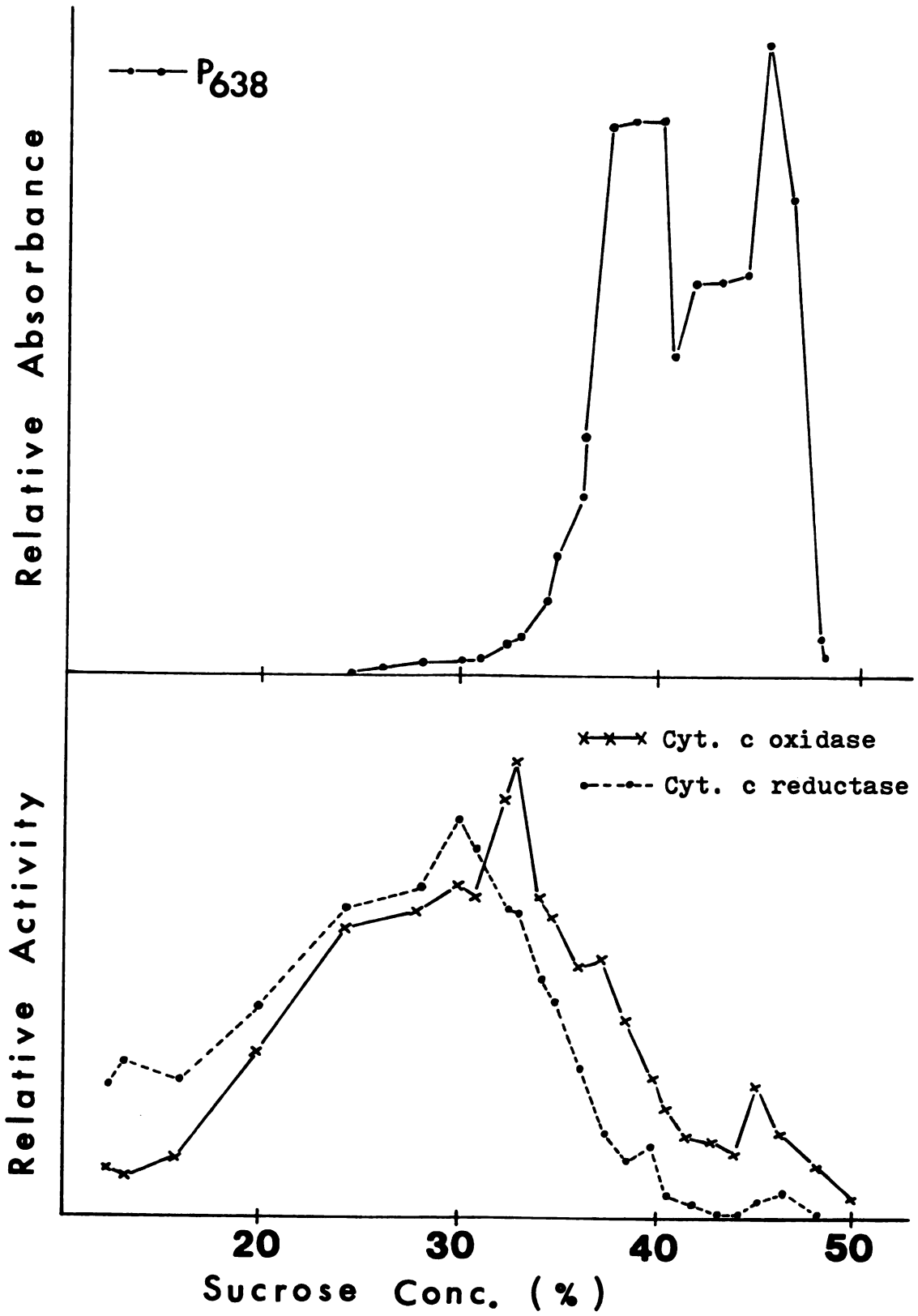

P638
Relative Absorbance
Cyt. c oxidase
Cyt. c reductase
Relative Activity
20
30
40
50
Sucrose Conc. (%)

Figure 24. Absorbance at 638nm (A) activity of marker enzymes (B,C) from sucrose density gradient. B) cytochrome c oxidase and cytochrome c reductase; C) acid phosphatase and alkaline phosphatase. D. discoideum amoebae, strain Ax 2, were ultrasonically disrupted, twice at 70 W for 5 sec.

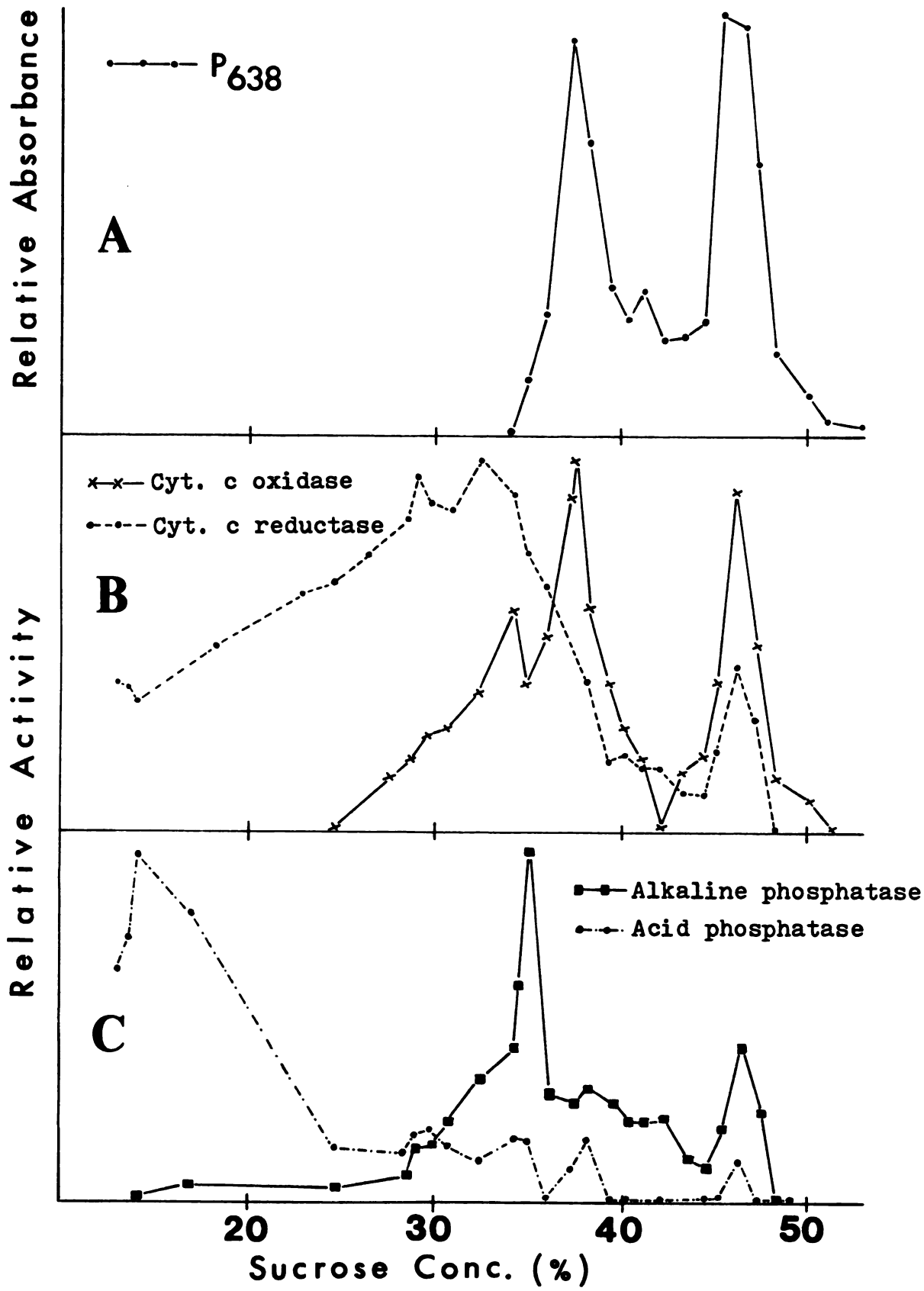

Relative Absorbance
P638
A
Relative Activity
Cyt. c oxidase
Cyt. c reductase
B
Alkaline phosphatase
Acid phosphatase
C
20
30
40
50
Sucrose Conc. (%)

Figure 25. Absorbance at 638nm and activity of marker enzymes from sucrose density gradient. A) cytochrome c oxidase, B) malate dehydrogenase and C) cytochrome c reductase; D) alkaline phosphatase, E) urate oxidase and F) acid phosphatase. D. discoideum amoebae, strain Ax 2, were ultrasonically disrupted, at 70 W for 5 sec.

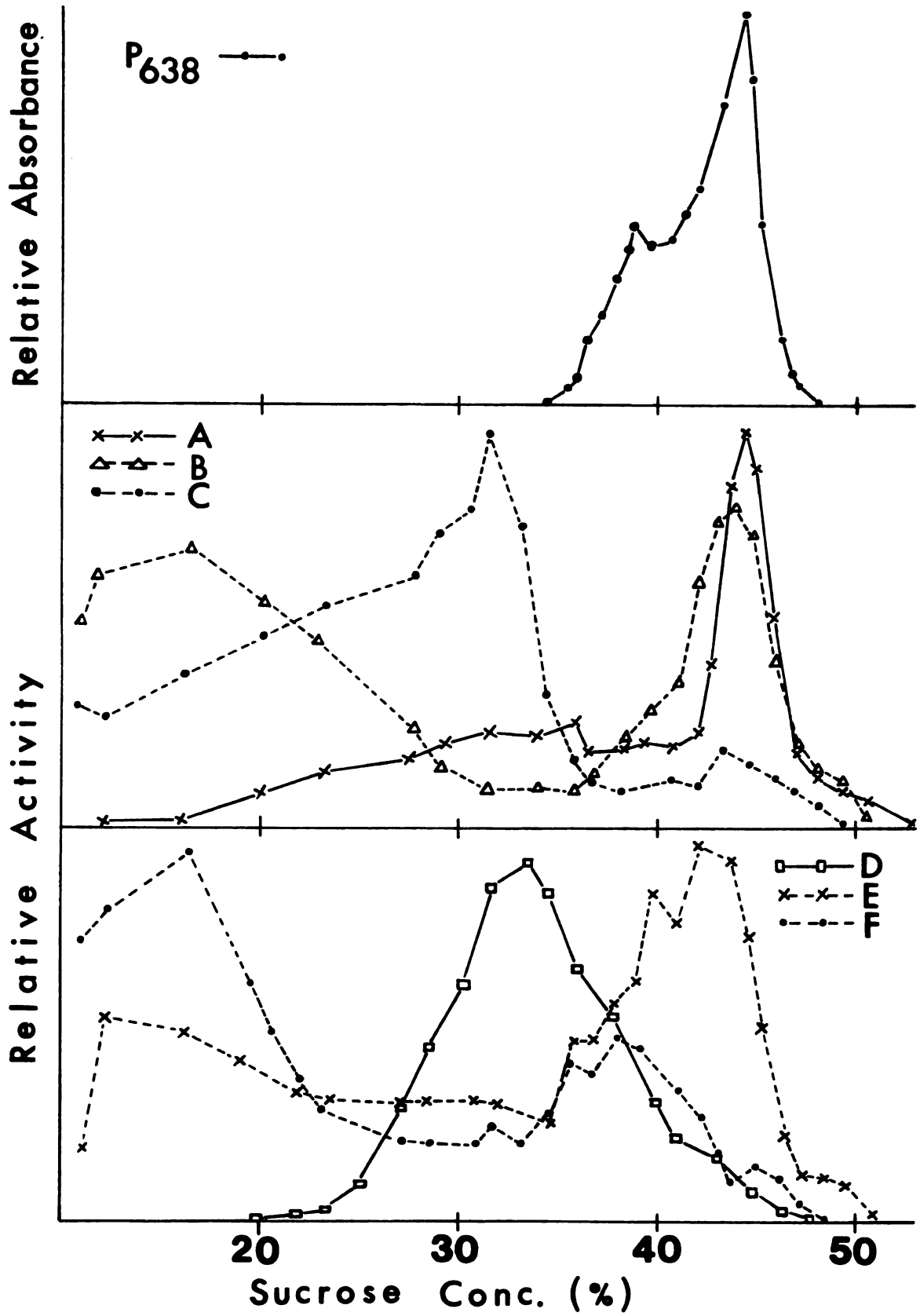
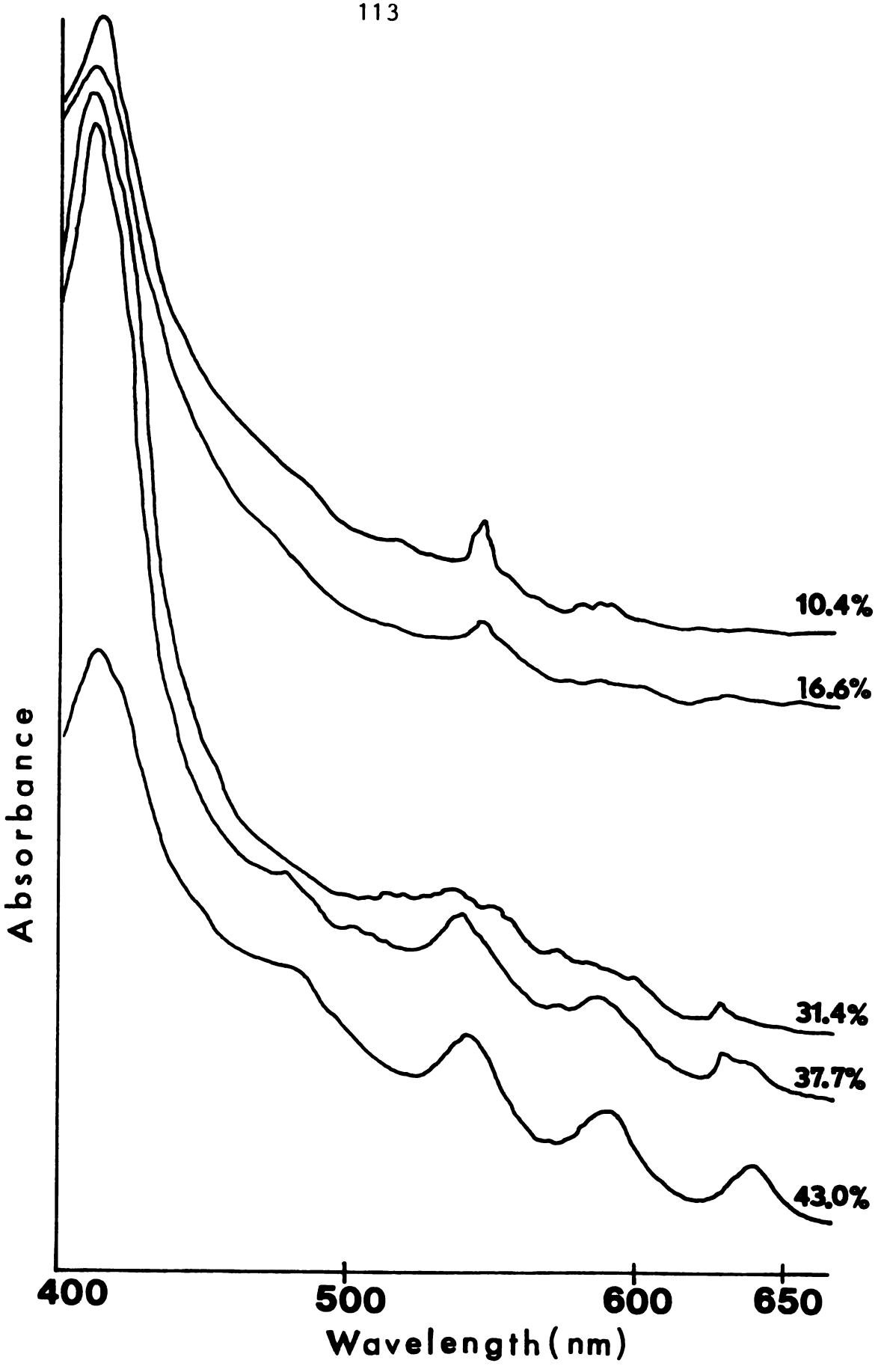

Relative Absorbance
P638
Relative Activity
A
B
C
D
E
F
20
30
40
50
Sucrose Conc. (%)

Figure 26. Low temperature (77°K) absorption spectra of fractions from sucrose density gradient. Sucrose concentration of each fraction is marked on the right side of the absorption spectra.


Absorbance
10.4%
16.6%
31.4%
37.7%
43.0%
400
500
600
650
Wavelength (nm)

Discussion

The concentration of amoebae used in these experiments--about 10^4 cells/cm^2--may not be low enough to totally eliminate the known chemotactic effects of amoebae (Gingle, 1976; Grutsch and Robertson, 1978). However, there were no noticeable chemotactic responses observed under these experimental conditions. In addition, it is not certain how the chemotactic responses, if there were any, would affect phototactic response of amoebae. Shortly after the depletion of food, the amoebae enter an interphase which usually lasts around six hours. During this interphase, amoebae are negatively chemotactic to the unidentified "repellent(s)" described by Kakebeeke et al. (1979). Under these experimental conditions, since the amoebae are free of bacteria, no chemotactic response should be given to folic acid. Moreover, amoebae in interphase are not chemotactically responsive to cAMP (Cripps and Rutherford, 1981). Negative chemotaxis of amoebae to the "repellent(s)" could interfere with the amoebal phototaxis in these experiments. However, it is not well documented what conditions would be necessary for the negative chemotaxis. For example, the concentration of amoebae, about 10^4 cells/cm^2, could be in the range where the "repellent(s)" are ineffective. If phototaxis of amoebae under this condition is affected by the "repellent(s)", it would probably lower the phototactic response of amoebae rather than making it more obvious, i.e., it would be reasonable

to assume negative effect(s) on this stage in the absence of a known positive cooperativity between the two tactic responses. Effects of cAMP during the final stage of the experiment will be negligible because: 1) amoebae have by then been widely dispersed and, 2) the period during which cAMP is effective (at the most one or two hours) is not long enough to significantly affect the result. Because a collimated light beam was used as a light source, it is certain that we can eliminate any light induced temperature gradient along the glass microscope slide.

A heme protein with about 240,000 molecular weight, named phototaxin, was isolated and purified from D. discoideum amoebae and was suggested as the photoreceptor pigment controlling phototaxis in D. discoideum slugs (Poff et al., 1974). Since P_{638} is not sensitive to oxidizing and reducing reagents, it cannot be phototaxin. A decrease of P_{638} correlates with an increase in absorbance at 630nm, i.e., an increase of P_{630}, at low pH. Incubation of amoebae with SDS, CTAB, or in pH 6.1 phosphate buffer for 4 hr at room temperature also decreases P_{638} and increases P_{630} (Figures 18, 20). Most active photoreceptor pigments exist as a complex with a protein. The conversion of P_{638} to P_{630} could be an indication of a change in the linkage of P_{638} to a protein, a conformational change in protein and chromophore complex, or a change in the chromophore itself. The lack of effects of proteases on P_{638} argues against the

possibility of a P_{638}-protein complex, i.e., if P_{638} forms a complex with protein(s), a change in protein by protease(s) would show up as an absorption change at 638 nm. Detergents convert P_{638} to P_{630} which probably suggests that P_{638} is in a hydrophobic environment and/or P_{638} itself is a hydrophobic molecule, i.e., detergents remove P_{638} from its surroundings and/or change the conformation of P_{638} thus resulting in the conversion of P_{638} to P_{630}.

Absorption spectra of protoporphyrin IX $(Na)_2$ and amoebae, disrupted ultrasonically and pelleted by centrifugation, shared many similarities in aqueous and organic solvents. In CTAB, both had five major peaks, and the positions of these peaks were almost identical. This similarity also holds in an aqueous environment (Figure 20). These results strongly support that P_{630} and P_{638}, both, are protoporphyrin in different forms as suggested (Lagarias, personal communication). It is well known that protoporphyrin is very hydrophobic and that it aggregates in aqueous environments, especially at higher pH levels (Gallagher and Elliott, 1973). Thus, it is very likely that P_{638} is an aggregate of protoporphyrin, and P_{630} is a monomer of protoporphyrin which has shown up in CTAB and conditions of more hydrophobicity, such as in SDS (Figure 18) or in membrane lipids. The higher stability of P_{638} at a higher pH (Figure 18) also can be interpreted as a higher tendency of protoporphyrin to aggregate in aqueous

environments at higher pH levels as previously documented (Gallagher and Elliott, 1973).

Fractionation of the subcellular components of amoebae by sucrose density centrifugation provides evidence that P_{638} is in mitochondria (Figure 25). This result was expected from other examples which showed that the later steps of protoporphyrin biosynthesis occur in mitochondria (Poulson and Polglase, 1974). Breakage of amoebae by two different strengths of sonification shows peaks of P_{638} coincides with maximum cytochrome c oxidase activity (Figures 24, 25). Stronger sonification of amoebae results in multiple peaks of P_{638} which do not coincide with the peaks of cytochrome c oxidase. The P_{638} peaks remain at higher sucrose concentrations even though the peak of cytochrome c oxidase, the marker enzyme for mitochondrial membrane, has been shifted to a much lower density (Figure 23). The reason for mismatching peaks of P_{638} and cytochrome c oxidase activity in Figure 23 is not certain, but it is possible that during the reverse of the mitochondrial membrane position by excessive breakage, i.e., during inside out vesicle formation from pieces of mitochondrial membrane, protoporphyrin does not move along with the mitochondrial membrane. These results suggest that protoporphyrin is produced in the mitochondria, and probably stays inside the mitochondria in the aggregated form, at least, in cultures in the early log-phase of growth, and

that this aggregated protoporphyrin is non-specifically bound to the membrane of mitochondria.

Action spectra of photoaccumulation, photodispersal (Häder and Poff, 1979 a,b) and phototaxis of amoebae (Chapter 4) certainly show an action peak at 638 nm. Although it is not known how protoporphyrin, P_{638}, works in those responses, it is clear that P_{638} is involved in these responses. Fluence-response curves of phototaxis of Ax 2 amoebae indicate, while they are in the log-phase of growth, that a lower fluence-rate is needed to induce positive and negative phototaxis as the titer of the culture increases and the intracellular P_{638} increases (Figure 17). It is however too early to draw a definite conclusion from these data because the increase of P_{638} is accompanied by an increase in other peaks such as cytochromes (Figure 14).

One of the well-known functions of protoporphyrin is as a photosensitizer which produces $^1O_2$ when illuminated (Burchard and Dworkin, 1966; Ito, 1978). The singlet oxygen can damage membrane as the component proteins or lipids of the membrane are photooxidized (Foote, 1976). This effect could be strong enough to result the lysis of the cell as can be seen in erythrocytes (Goldstein and Harber, 1972). Thus, this photodynamic effect of protoporphyrin could be one mechanism which can result phototaxis of amoebae. The main problem to apply the photodynamic effect in phototaxis of amoebae is the way singlet oxygen functions. Singlet

oxygen diffuses very rapidly (Ito, 1978) thus, it does not exhibit specificity in location where it acts. This nonspecificity argues against the hypothesis which explains phototaxis of amoebae based on the photodynamic effect of protoporphyrin.

Another possible function of protoporphyrin could be as a screening pigment. In this case, it is necessary to have another photoreceptor pigment which is directly related to the phototaxis of amoebae. Different numbers of quanta absorbed by the phototactic photoreceptor pigment at the proximal side to the distal side of the cell will be able to result in phototaxis of amoebae. Protoporphyrin in the mitochondria can screen the presumable phototactic photoreceptor pigment far from the light source and can result in different amounts of quanta absorbed by the pigment along the length of amoebae. The resultant gradient of photochemical reactions will be able to make the amoebae turn.

Although two possible functions of protoporphyrin in amoebal phototaxis have been proposed, they would have no more meaning than an attempt to build a working hypothesis. Further investigation is needed to explain precisely how protoporphyrin functions in amoebal phototaxis.

Summary

Phototaxis of D. discoideum amoebae is culture age dependent while the amoebae are in log-phase of growth. Cells from the younger culture need higher doses of light to

result in both positive and negative phototaxis than cells from older culture.

Low temperature, 77°K, absorption spectra of the amoebae show a characteristic peak at 638nm which is where the secondary maximum of the positive and negative phototaxis also is. This peak is not stable at room temperature or in low pH aqueous solution. This peak also can be lowered by incubating the amoebae with detergents, but not with proteases and oxidizing or reducing reagents. Decrease of a peak at 638nm correlates with an increase of a peak at 630nm. All these indications support "P_{638}", a photoreceptor pigment with an absorption peak at 638nm, as an aggregated protoporphyrin.

P_{638}, protoporphyrin, has been localized in mitochondria. Marker enzyme assay after fractionation of the organelles by sucrose density gradient shows P_{638} coincides with cytochrome c oxidase and malate dehydrogenase activities which indicate mitochondria.

Possible functions of protoporphyrin in mitochondria as a screen pigment and a photosensitizer have been discussed.

CHAPTER 6

IS PROTOPORPHYRIN, P_{638}, THE SOLE PHOTORECEPTOR PIGMENT REGULATING AMOEBAL PHOTOTAXIS IN D. DISCOIDEUM?

Introduction

Amoebae of D. discoideum feed on bacteria in the mulch of the forest floor. Once the food is depleted, they follow a developmental cycle in which the pseudoplasmodium and the fruiting body are formed. During this life cycle, D. discoideum has two motile stages, the amoebae and the slug, which are able to respond to the environmental stimuli by showing oriented movements.

Amoebae of D. discoideum are chemotactic to folic acid, an unidentified "repellent(s)", and cAMP, and are thermotactic and phototactic. Pseudoplasmodia of D. discoideum are also chemotactic to "STF", thermotactic and phototactic.

Photoaccumulation of the amoebae inside a light trap and photodispersal of amoebae from a trap were the first photoresponses detected in D. discoideum amoebae (Häder and Poff, 1979 a,b). Subsequent work demonstrated positive and negative phototaxis of the amoebae and suggested these responses to be the basis for photoaccumulation and photodispersal of the amoebae, respectively (Chapter 4). All of the action spectra for these responses (photoaccumulation,

photodispersal, positive phototaxis and negative phototaxis) and the low temperature, 77°K, absorption spectra are very similar. All have a primary maximum at 405 to 410nm, a broad band in the long-wavelength blue and green region, and secondary maximum at about 638nm. Of these, the peak at 638nm is most unique to this system, and a photoreceptor pigment, designated as P_{638}, has been identified as an aggregated form of protoporphyrin and localized in the mitochondrion (Chapter 5).

Although protoporphyrin would appear to be involved somehow in the phototaxis of D. discoideum amoebae, strain Ax 2, based on the action spectra, there are still important data which argue against the idea that protoporphyrin is the phototactic photoreceptor pigment. There are regions of the action spectra which can not be accounted for by protoporphyrin absorption. The action spectra do not have minima from about 430 to 550nm of the spectrum, although the absorption spectrum of protoporphyrin clearly shows a couple of minima. The major documented function of protoporphyrin is as a photosensitizer (Burchard and Dworkin, 1966; Ito, 1978) which should have a disruptive effect in the organism. It is difficult to think that a photoreceptor pigment with a disruptive effect is the primary photoreceptor pigment controlling phototaxis of amoebae.

The phototactic behaviour of amoebae of the previously reported thermotactic mutants (Chapter 3) has been investigated because of the possible interaction between phototaxis

and thermotaxis as has been described in slugs (Fontana, 1982). Understanding the phototactic behaviour of these mutants may assist toward an understanding of the function of protoporphyrin in phototaxis, the identities of the primary photoreceptor pigment, and an understanding of the relationship between thermosensory and photosensory transduction in D. discoideum amoebae.

A wide range of fluence-rates was used in the experiment presented in this chapter, because of the negative phototaxis noticed at low fluence-rates in amoebal phototaxis from very young cultures (Figures 16 A,B).

Materials and Methods

D. discoideum amoebae of the axenic strain, Ax 2, were cultured axenically in the liquid culture medium, HL 5, in rotary shake culture or nonaxenically in association with K. aerogenes on solid SM agar.

D. discoideum amoebae, nonaxenic strains HL 50, HO 428, HO 596, HO 813 and HO 1445 were grown in association with K. aerogenes on the solid SM agar. Cultures were maintained in darkness at 23.5 $\pm$ 0.3°C.

Axenically grown Ax 2 amoebae were harvested from cultures at the titer of about 4-5 x 10^6 cells/ml by centrifugation and washed twice with phosphate buffer as described in Chapter 4. Nonaxenically grown amoebae, strains Ax 2, HL 50, HO 428, HO 596, HO 813 and HO 1445, were also harvested by centrifugation and washed with

phosphate buffer until the supernatant was clear as described in Chapter 1.

The suspension of amoebae, at a final concentration of 1 x 10^7 amoebae/ml, was mixed with charcoal and linearly spotted on the 1% water agar (w/v) covering a 1 x 25 x 75 mm glass microscope slide by means of the micromanipulator as described in Chapter 2. The thickness of the water agar covering the microscope slide was approximately 1 mm and the concentration of charcoal was decreased by about 4x from the one used in Chapter 2 such that possible shadow by charcoal could be minimized. The slides were then kept in a transparent 40 x 100 x 25 cm plastic box while they were exposed to the light as described in Chapter 5.

All phototaxis experiments were performed at 23.5 $\pm$ 0.3°C for 7 hr while exposed only to the actinic light.

After 7 hr exposure to the actinic light, the amoebae which had moved out from the original spot line in both directions were viewed under the microscope and counted, and a Δ% was calculated. The relative value of Δ%, as described in Chapter 2, was used to build a fluence-rate response curve for each strain.

A slide cube projector (Bell and Howell, CP 40) with a 3.5 cm secondary filter of 5% $CuSO_4$ in aqueous solution (w/v) as an IR cut filter was used as a light source. The light beam was directed at the microscope slides from an angle of about 10° to 15° above the horizontal plane.

The light intensity was measured with a Kettering model 65 radiometer and/or an International Light Spectroradiometer (Newburyport, Mass) and controlled using neutral density filters (Balzer or Inconnel) between the light source and the sample.

Absorbance was measured using a single beam spectrophotometer on line with a small computer as described in Chapter 1. All the samples for absorbancy measurement contained 5×10^7 cells in 0.4 ml of 15 mM phosphate buffer at pH 6.1, and the time taken for the preparation of samples was kept short and as constant as possible. The purpose of these precautions in preparing samples, keeping the same number of cells in the same amount of buffer and spending equal lengths of time preparing each sample, was to permit comparisons of absorbance in amoebae of different strains.

Results

The absorption peak at 638nm which is characteristic for *D. discoideum* amoebae Ax 2, when grown axenically, disappears when Ax 2 amoebae are grown nonaxenically in association with *K. aerogenes* on SM agar plates (Figure 27). The absorption spectrum of Ax 2 amoebae grown in association with bacteria is almost identical with the absorption spectra of other nonaxenic strains (Figure 28). Low temperature, 77°K, absorption spectra of amoebae of these strains showed little if any absorption at 638nm. All have

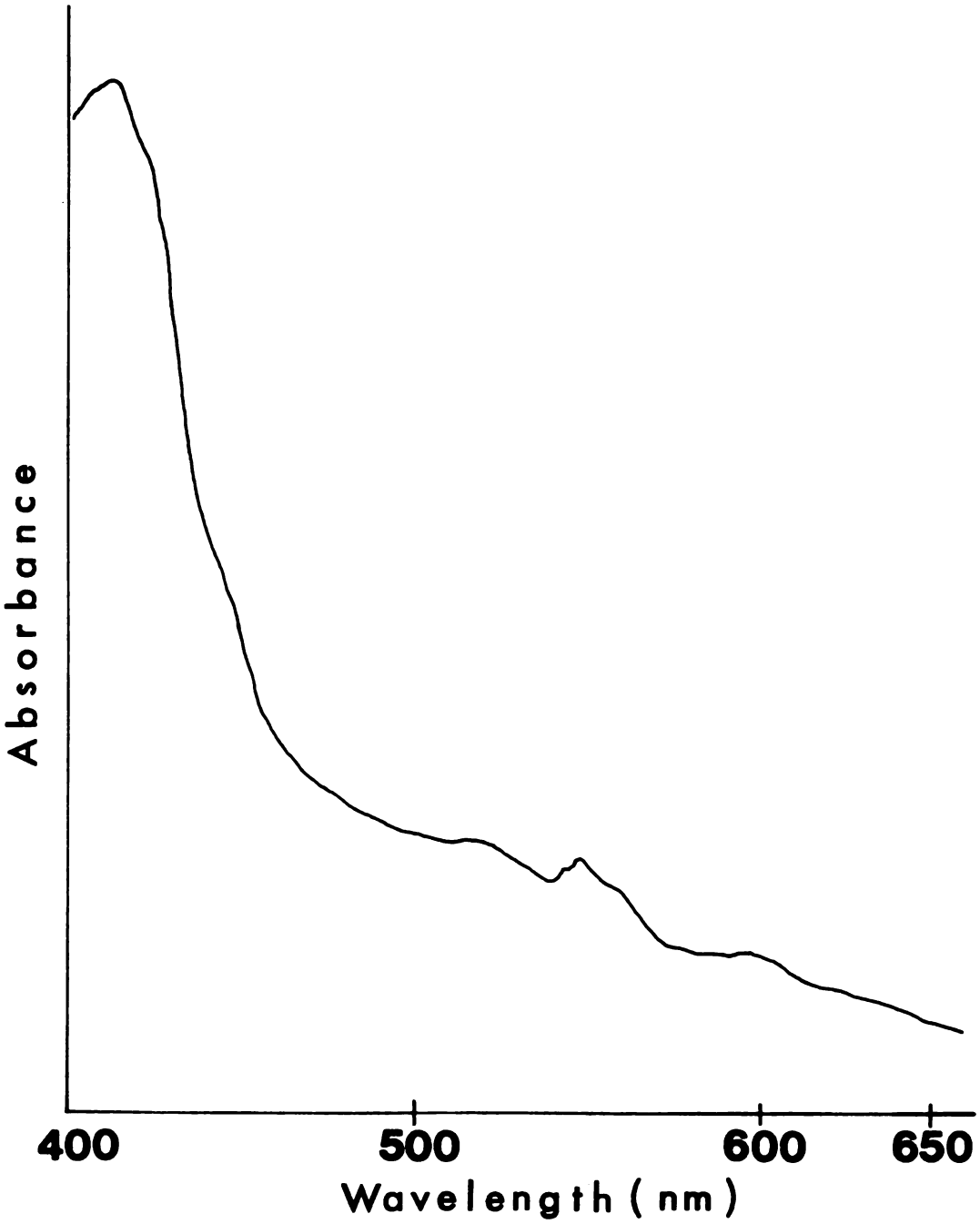


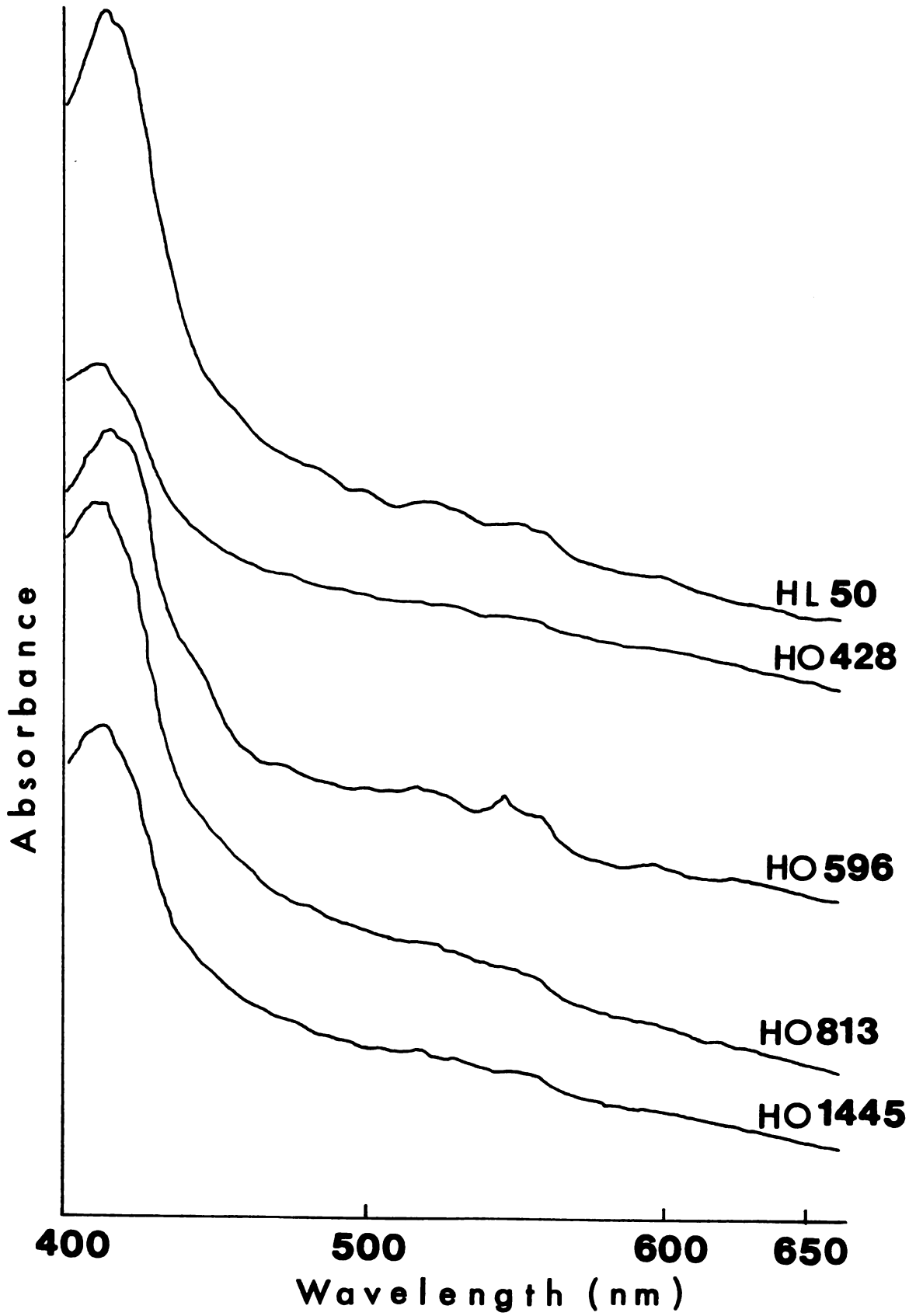
Figure 27. Low temperature, 77°K, absorption spectrum of Ax 2 amoebae grown in association with K. aerogenes.

a maximum at about 410nm with a shoulder at about 420nm, including the cytochromes which peak in green region of the spectrum. It appears that high intracellular concentration of protoporphyrin, P_{638}, disappears as Ax 2 amoebae are grown in association with bacteria on a SM agar plate. It was shown earlier, in Chapter 5, that the absorption peak at 638nm decreases as the surface area/volume ratio is decreased. This probably is due to an obligatory O_2 requirement in protoporphyrin biosynthesis (Frydman et al., 1978). Thus, it is likely that the unique absorption peak at 638nm in D. discoideum amoebae, grown axenically in HL 5 liquid medium, is a result of culture condition rather than inherent characters of this axenic strain.

A negative phototactic response in the fluence-rate range of about 10^{-6} W/m^2 to 8 x 10^{-3} W/m^2 was apparent in Ax 2 amoebae grown axenically in the liquid medium. A weakly positive phototaxis, or perhaps no response, was seen from 3 x 10^{-8} W/m^2 to 1 x 10^{-6} W/m^2. Sharp secondary peaks between 0.01 W/m^2 and 1 W/m^2, which indicate a second positive and negative phototactic response, were observed at a fluence-rate similar to that for the previously observed light response (Figure 29A).

HL 50 amoebae exhibited positive phototactic responses at the fluence-rate less than about 6 x 10^{-5} W/m^2. A stronger negative phototaxis was seen above 6 x 10^{-5} W/m^2 and lower than about 0.7 W/m^2. Above this fluence-rate, no significant responses could be observed (Figure 29A).

Figure 28. Low temperature, 77°K, absorption spectra of D. discoideum amoebae, strains HL 50, HO 428, HO 596, HO 813 and HO 1445.


HL 50
HO 428
HO 596
HO 813
HO 1445
Absorbance
400
500
600
650
Wavelength (nm)

Mutant strain HO 428 amoebae showed positive phototactic response at a wide range of fluence-rates, as high as about 6×10^{-4} W/m^2. The amoebae respond negatively from about 6×10^{-4} W/m^2 to about 7×10^{-2} W/m^2 and gave a possible second positive phototaxis at around 0.6 W/m^2 (Figure 29B).

Amoebae of strain HO 596 showed statistically significant positive phototactic responses at a relatively wider range of fluence-rate than did the other strains. The approximate transition from positive to negative phototaxis was at 9×10^{-5} W/m^2. No response was seen above 6×10^{-2} W/m^2 (Figure 29B).

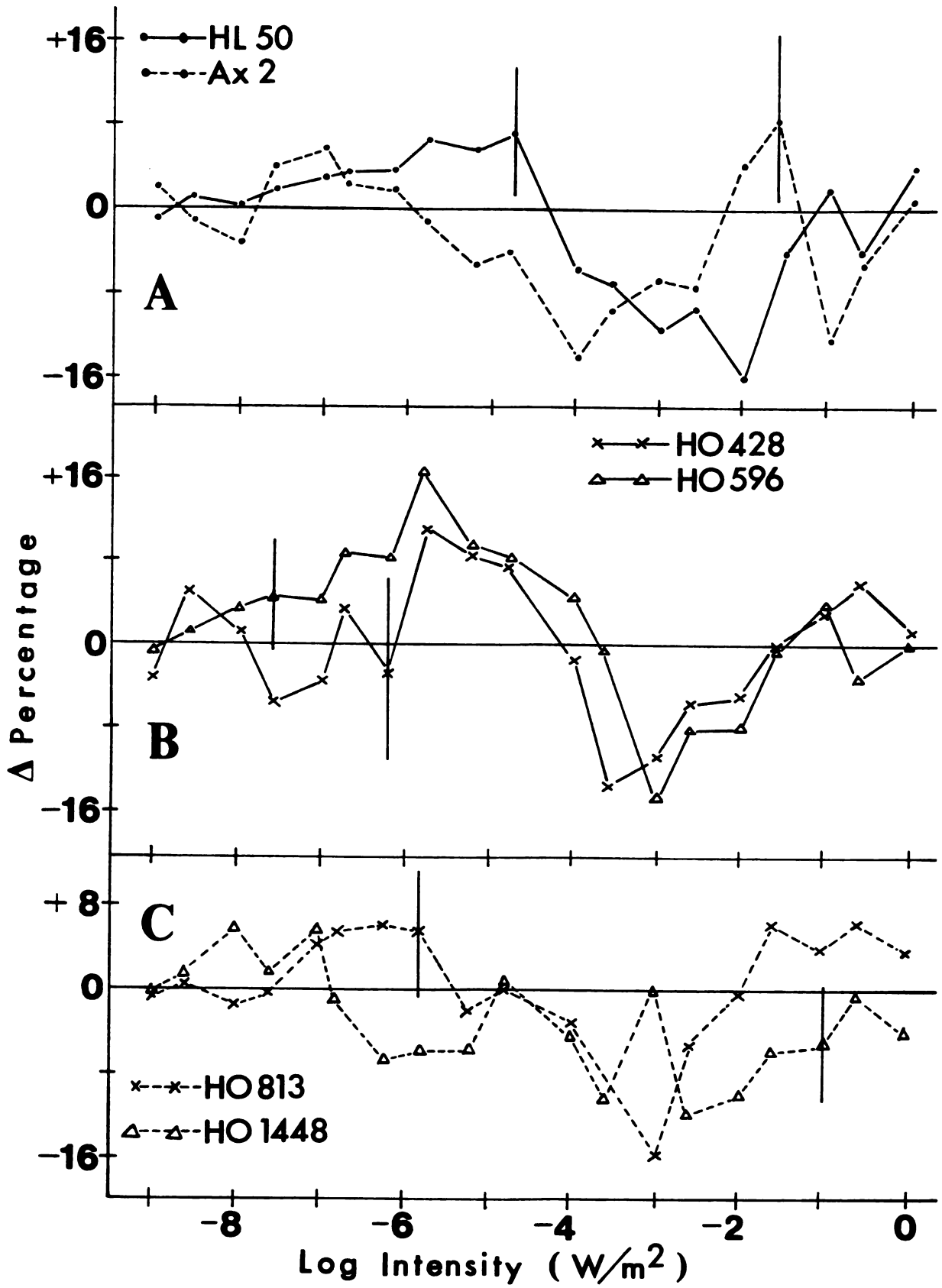
HO 813 amoebae showed a weak positive phototaxis at fluence-rates from about 7×10^{-8} W/m^2 to 8×10^{-6} W/m^2, negative phototaxis from about 6×10^{-5} W/m^2 to 1×10^{-2} W/m^2 and a second positive phototactic response above 1×10^{-2} W/m^2 (Figure 29C).

HO 1445 amoebae showed no significant positive phototactic responses throughout the fluence-rate checked. Mostly, a negative phototactic response prevailed (Figure 29C).

Discussion

The decrease of P_{638} in Ax 2 amoebae grown non-axenically, and in the amoebae of strain HL 50 and its mutants, could be a result of restricted O_2 supply (Frydman *et al.*, 1978). It is also possible that P_{638}

Figure 29. Fluence-response curves of D. discoideum amoebal phototaxis. A) HL 50, —•—•— ; Ax 2, ---•---•--- . B) HO 428, —×—×— ; HO 596, —△—△—. C) HO 813, --×--×--; HO 1445, --△--△--.


+16
0
−16
HL 50
Ax 2
A
HO 428
HO 596
B
+ 8
C
HO 813
HO 1448
Δ Percentage
−8
−6
−4
−2
0
Log Intensity (W/m2)

is overproduced by Ax 2 amoebae grown axenically in liquid culture.

Absorption peaks for the cytochromes (which contain heme) were undiminished in the nonaxenic cultures, while the characteristic peak of protoporphyrin at 638nm decreased in these cultures. Therefore, one can conclude that there was no significant change in the conversion step of protoporphyrin to heme (Dailey and Lascelles, 1977). Thus, it appears reasonable to assume that protoporphyrin is overproduced by Ax 2 amoebae in liquid culture, and that ferrochelatase cannot convert protoporphyrin to heme(s) as rapidly as the protoporphyrin is produced. The excretion of protoporphyrin into the liquid medium as the culture ages (Figure 17) is supporting evidence for this interpretation. It was shown in Figure 19 that the amount of P_{638} increases as the surface area/volume ratio of the culture medium increases. Thus, a possible explanation for the high content of protoporphyrin in Ax 2 amoebae in liquid culture is that the O_2 supply to the amoebae is higher than it is in the non-axenic cultures. This would result in larger protoporphyrin production in liquid culture while the rate of protoporphyrin consumption would be relatively identical in these two conditions. This explanation suggests that a high content of protoporphyrin would not be "normal" in D. discoideum amoebae. This clearly raised questions about the function of protoporphyrin as a phototactic photoreceptor

pigment. Is protoporphyrin the real photoreceptor pigment which brings about phototaxis in amoebae?

An additional point suggesting this question was raised in Chapter 5. Protoporphyrin probably stays in the mitochondria as an aggregate and binds nonspecifically to the mitochondrial membrane. Although such a situation is not mandated, it is reasonable to assume that a photoreceptor pigment must be specifically bound to some photoreceptor structure for a pigment to be a primary stimulus receptor. This brings up the possibility of another photoreceptor pigment, which is not protoporphyrin, in D. discoideum amoebal phototaxis.

Although all of the nonaxenic strains used in these experiments do not have a protoporphyrin peak at 638nm (Figure 28), they all show positive and negative phototaxis (Figure 29). Action spectrum for Ax 2 amoebal phototaxis was based on fluence-rates from approximately 1 x 10^{-2} W/m^2 to 1 W/m^2 (Figure 12). This action spectrum clearly showed a peak at about 638nm which indicates protoporphyrin is involved. The fluence-rate response curve in the phototaxis of Ax 2 amoebae in Figure 29A shows a secondary positive and negative phototactic response at around 1 x 10^{-2} w/m^2 to 1 W/m^2 which confirms those previous results. Thus, a phototactic action spectrum for these secondary positive and negative phototactic responses of Ax 2 amoebae should be identical to the previous one presented (Figure 12). It is likely that these secondary

positive and negative phototactic responses are somehow linked to the protoporphyrin based on these action spectra.

The phototactic responses of Ax 2 amoebae at low fluence-rates are stronger than those in the 10^{-2} to 10^{0} W/m^2 region. A strong negative phototaxis in response to a wide range of fluence-rates (about 10^{-6} to 8×10^{-3} W/m^2) and a possible weak positive response at very low fluence-rates (below 10^{-6} W/m^2) were observed in this strain. These phototactic responses at the very low fluence-rates are not unique to the Ax 2 strain. All other strains except HO 1445 showed strong phototactic responses at approximately the same range of fluence-rates. Fluence responses from these nonaxenic strains, which show no significant absorption peak at 638nm, strongly argue against protoporphyrin as the photoreceptor pigment responsible for the positive and negative phototaxis at those low fluence-rates.

If it is necessary to postulate a photoreceptor pigment other than protoporphyrin in D. discoideum amoebal phototaxis, then two possible candidates are phototaxin and flavin. The heme protein, phototaxin, has been suggested to be the photoreceptor pigment which results in slug phototaxis. This heme compound can be photooxidized, and evidence indicates that it resides in mitochondria (Poff et al., 1974). Low temperature, 77°K, absorption spectra of D. discoideum amoebae from all the strains used in these experiments showed a major peak of oxidized "phototaxin" at

about 410nm. Poff et al. (1974) also showed another photoreceptor pigment in D. discoideum whose physiological function is not known. This second photoreceptor pigment can reduce cytochrome b-559 when the pigment is photoreduced with the proper wavelength of light. Based on the action spectrum of the reduction of cytochrome b-559, this pigment was identified as flavin.

Without action spectra for the phototaxis of amoebae at the very low fluence-rates, one cannot draw conclusions concerning the identity of the photoreceptor pigment regulating phototaxis.

Other interesting points noticed in the fluence-rate response curves presented in Figure 29 are:

1. A possible second phototactic response is seen in HO 428 and HO 813 amoebae at high fluence-rates, and may be correlated to Ax 2 amoebal phototaxis. However, the statistical significance of these responses is questionable.
2. A comparison of the fluence-rate response curves among mutants indicates the strength of the positive phototactic response is related to the "zero-crossing point". HO 596 which has the strongest positive phototactic response has the highest "zero-crossing point", while HO 813, a mutant with a weaker positive phototaxis, has a lower "zero-crossing point". On the other hand, the maximum strength of the negative phototactic response is relatively constant among mutants. The meaning of this correlation between the

"zero-crossing point" and the strength of positive phototaxis is not clear at this stage.

3. Mutant HO 1445 is of special interest because it lacks positive phototaxis although no significant difference in absorption is seen compared to other strains. Thus, it is possible that HO 1445 is a mutant with a defect(s) only at positive phototactic transduction path in amoebae.

Summary

When D. discoideum amoebae were grown in association with K. aerogenes on the SM agar plates, the characteristic protoporphyrin absorption peak at 638nm was diminished. Fluence-rate responses were measured for the amoebae of the strain Ax 2 and several nonaxenic strains over much wider range of fluence-rates than the previously investigated. The response curve for Ax 2 amoebae showed a positive and a negative phototaxis between 0.01 W/m^2 to 1 W/m^2 which confirms the previous results, but in addition showed more prominent phototactic responses of Ax 2 amoebae at much lower fluence-rates. These very low fluence-rates also induced phototactic responses in all other nonaxenic strains. Because all these nonaxenic strains lacked P_{638}, the function of protoporphyrin in D. discoideum amoebal phototaxis has been questioned. It is likely that phototaxis by D. discoideum amoebae is regulated by a photoreceptor pigment(s) other than protoporphyrin.

Protoporphyrin may only be involved in phototaxis of Ax 2 amoebae grown in liquid culture.

CHAPTER 7

GENERAL DISCUSSION OF THE ROLE OF AMOEBAL THERMO- AND PHOTO-RESPONSES IN SLUG SENSING

Introduction

Amoebae of the cellular slime mold, D. discoideum, live and grow in the mulch on the forest floor when they feed on bacteria. Upon the depletion of food, the amoebae aggregate forming a multicellular pseudoplasmodium, slug, or glex which eventually develops into a sorocarp consisting of a stalk bearing a sorus containing spores. Under the proper conditions, the spores may germinate releasing amoebae, thus repeating the developmental cycle (Raper, 1940). Much of the work with this organism has centered upon its development and the fact that one can easily separate growth and cell division from development.

Dictyostelium is also being increasingly recognized as an ideal system for the study of sensory transduction in a eukaryotic organism, probably due to its simplicity and ease of culture along with its various responses toward environmental stimuli. Sensory responses thus far described are: chemotaxis by the amoebae to cAMP (Robertson et al., 1972), folate (Pan et al., 1975), and unidentified repellent(s) (Bonner, 1977); thermotaxis of the amoebae

(Chapters 2 and 3), both positive and negative phototaxis of the amoebae (Chapters 4,5 and 6), chemotaxis by the pseudoplasmodia to an endogenous "slug turning factor" (Fisher et al., 1981), phototaxis by the pseudoplasmodia (Bonner et al., 1950; Francis, 1964; Poff et al., 1973; Fisher et al., 1981; Fontana, 1982), and positive and negative thermotaxis by the pseudoplasmodia (Bonner et al., 1950; Whitaker and Poff, 1979; Fontana, 1982).

During this dissertation, thermotaxis of D. discoideum amoebae has been described (Chapters 2 and 3). The amoebae can sense a temperature gradient, 0.22°C/cm, and show positive or negative thermotactic responses which change as development proceeds. Phototaxis of D. discoideum amoebae has been re-evaluated (Chapters 4,5 and 6), and it has been proposed that some photoreceptor(s) other than protoporphyrin is the photoreceptor pigment contributing to amoebal phototaxis and the function of protoporphyrin may be as a screening pigment.

Ease in manipulating the unicellular and multicellular forms in one organism is one reason that D. discoideum has been a popular model system in many areas. In this chapter, possible interrelations between amoebal (unicellular) and slug (multicellular) thermo- and photo-sensing will be discussed. The first section of this chapter will describe the possible role of amoebal thermotaxis in slug thermotaxis. The second section will be concerned with the interrelationships between amoebal phototaxis and slug phototaxis.

7.1. Possible role of amoebal thermotaxis in slug thermotaxis.

Characterization of slug thermotaxis

For the thermal response curves of D. discoideum slugs, strains HL 50, HO 428, HO 596 and HO 813, grown and developed at 23.5°C, see Schneider et al. (1982). For the thermal response curve of the slug, strain HL 50, grown and developed at 27.5°C instead of 23.5°C, see Hong et al. (1982). The thermal response curve of the slug, strain HO 1445, grown and developed at 23.5°C is presented in the appendix C (Figure 33).

Slug thermotaxis was characterized as follows: The angular distribution of slugs was measured following a 24 hr-exposure on the temperature gradients formed as described in Chapter 1. The degree of the thermotactic response is presented as r, which is based on an empirical circular distribution, i.e., each slug may be represented by a point on the circumference of a circle. Because the calculation is based on the unit circle, r varies from zero to one with zero denoting a random population and one representing a perfectly directed population. See Batschelet (1965) for detailed statistical analysis.

Characterization of thermotaxis of amoebae, strain HL 50, grown at 27.5°C.

Amoebae of strain HL 50 were grown for about 22 hr at 27.5°C on the SM agar plates in association with K. aerogenes. These plates were usually about 50% cleared,

and amoebae were harvested, mixed with charcoal, spotted linearly on 1% water agar covering a glass microscope slide, and exposed on the thermal gradients for 16 hr. Amoebae were scored using a microscope as described in Chapter 2.

Amoebal thermotaxis is the basis for slug thermotaxis.

After 16 hr on the thermal gradients, amoebae of D. discoideum (strain HL 50) showed negative thermotaxis on the gradients with midpoint temperatures of 14°, 16° and 18°C and positive thermotaxis when the midpoint temperature was above 20°C (Figure 3E). The maximum response was at 23°C, with the transition from a negative to a positive response occurring at about 19.1°C. This transition is comparable to that observed with slugs of this strain which have been grown and allowed to develop at 23.5°C. With these slugs, the transition occurs at 18.7°C and the maximum positive response is at 23°C (Schneider et al., 1982).

Amoebae of strain HO 428 showed positive thermotaxis throughout the entire temperature range (Figure 4C) after 16 hr on the gradients. Its strongest thermotactic response for the amoebae was at a midpoint temperature of 18°C, and there was a sharp drop in response at midpoint temperatures above 21°C. Pseudoplasmodia of this strain also showed only a positive response throughout the entire temperature range (Schneider et al., 1982). The drop in response at high midpoint temperatures in amoebae is also consistent with

recent stimulus-response results with slugs of HO 428 (Fontana, 1982).

Temperature response curves of strain HO 596 amoebae after 16 hr on the temperature gradients are very similar to the temperature response curve of slug of this strain. The highly weakened positive thermotactic response and the higher transition temperature of HO 596 amoebal temperature response curve (Figure 5C) is similar to the temperature response curve for slugs of this strain (Schneider et al., 1982). Similarly, strong correlations between amoebal thermotaxis and slug thermotaxis are also seen in the temperature response curves of slugs of strain HO 813 (Schneider et al., 1982) and amoebae of this strain. HO 813 amoebae, after 16 hr on the gradients, show a significantly higher transition temperature than the parental strain, HL 50 (Figure 6C); this is also the case for the slugs of this strain.

The higher transition temperature of HO 1445 amoebae after 16 hr on the temperature gradient (Figure 7C) was not comparable with the transition temperature of the slugs of this strain. This may be explained by the upward shift in transition point in most strains as development proceeds. HO 1445 amoebae might have a more rapid expression of the negative response in comparison with the other strains.

The similarities observed in temperature response curves of amoebae and slugs in the shape of curves, the transition points, and the relative strength of the positive

and negative responses are all consistent with the conclusion that slug thermotaxis is a direct result of amoebal thermotaxis.

Dictyostelium slug thermotaxis is adaptable to a new growth and development temperature. If the growth and development temperature of the slug is increased, the temperature response curve of the slug is shifted to a higher temperature, or vice versa (Whitaker and Poff, 1980). HL 50 amoebae grown at 27.5°C, rather than 23.5°C, had a transition temperature about 2°C higher than that of amoebae grown at 23.5°C (Figure 30). Strain HL 50 which was grown and allowed to develop into slugs at 27.5°C, also demonstrated both negative and positive thermotaxis with the transition between the two responses occurring at around 20.4°C (Hong et al., 1982). This similarity in upward shift of transition point suggests that adaptation in slug thermotaxis is also a result of adaptation by the component amoebae to a new growth temperature.

The one difference found between amoebal and slug thermotaxis is in thermal sensitivity. The slugs have a strong response on a thermal gradient of 0.11°C/cm while the amoebae demonstrate a weaker response on a gradient of 0.22°C/cm. This difference is easily explained when one considers that there are 10^4 to 10^5 amoebae per slug. It is expected that the combined response of this many amoebae would be greater than that of one amoeba.

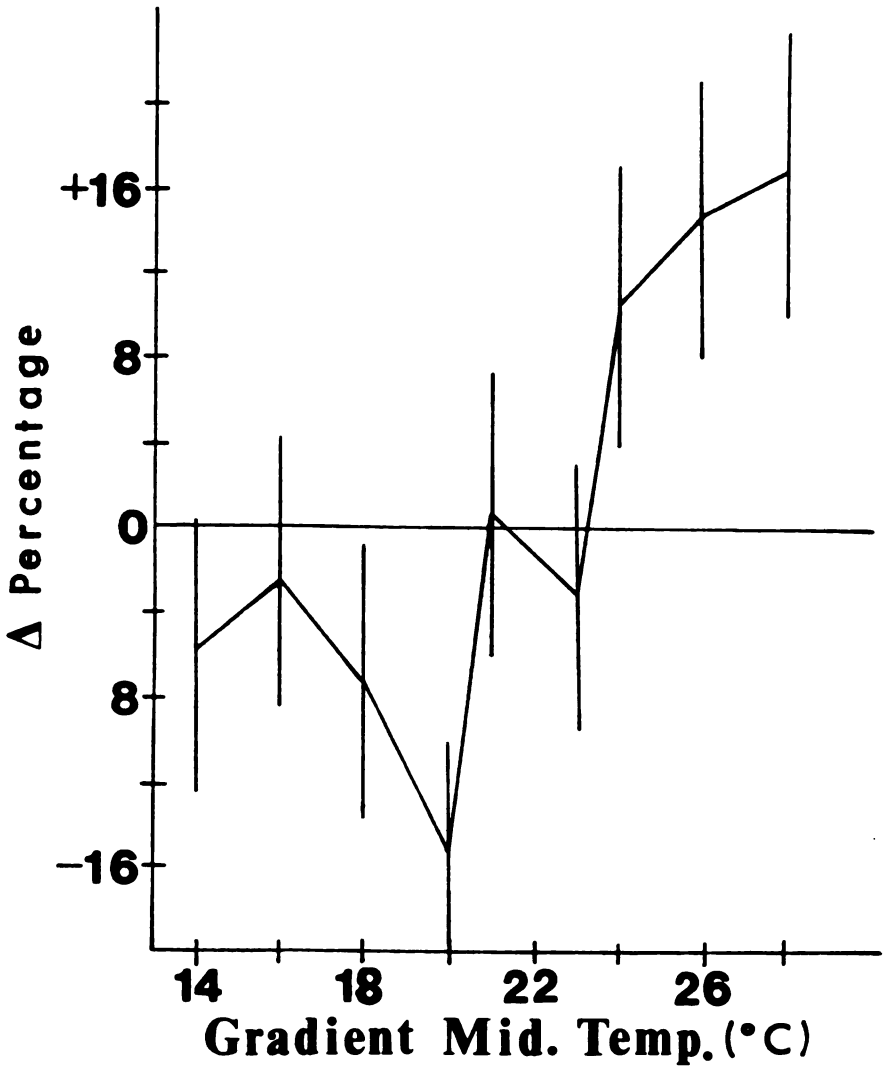


Figure 30. Temperature response curve of D. discoideum amoebae, strain HL 50, grown at 27.5°C. Vertical bars represent the 95% confidence interval, and each datum point is the average of 8 replicates.

7.2. Possible connection between amoebal phototaxis and slug phototaxis

Many results suggest a different mechanism of sensing in amoebal and in slug phototaxis. For example, the action spectrum for slug phototaxis (Poff et al., 1973) differs significantly from that for amoebal phototaxis (Chapter 4); and amoebae show both positive and negative phototaxis (Chapter 4) but slugs show only positive phototaxis (Poff et al., 1973).

No data presently are available to suggest a correlation between amoebal and slug phototaxis. However, as discussed in Chapters 5 and 6, there are still many questions left concerning amoebal phototaxis. It may not be an exaggeration to say that we have seen only the tip of the iceberg in amoebal phototaxis. Given this lack of solid information, it is interesting to speculate concerning possible connections between amoebal and slug phototaxis.

It is not certain whether or not the action spectrum of amoebal phototaxis produced for Ax 2 amoebae grown in liquid medium can accommodate phototactic responses of other strains (Chapter 6).

Although free amoebae show no major peak attributable to reduced heme, it is possible that once they are aggregated compactly in a slug, there will be an increase of reduced heme content as O_2 supply is limited.

Amoebal thermotaxis changes as development proceeds (Chapters 2 and 3). Thus, comparison must not be made

between the thermotactic responses of slugs and amoebae recently depleted of food. Similarly, different phototactic responses may be seen in the amoebae long after food depletion, and the comparison between the phototactic responses of slugs and of amoebae recently depleted of food may not be valid.

Slugs of some strains move at some discrete angle with respect to the incident light rather than moving directly toward the light. Several suggestions for the basic mechanism of this "bidirectional phototaxis" have been proposed (Fisher and Williams, 1981; Fontana, 1982). One of these suggestions is based on the assumption that slugs show both negative and positive phototaxis (Fisher and Williams, 1981). According to this hypothesis, directionality of slug phototaxis is determined by the competition between positive and negative phototaxis in the slug. If positive phototaxis were totally dominant, a slug would move directly toward the light. Bidirectional phototaxis of a slug would result in an angle with respect to the incident light where positive and negative phototaxis responses were balanced. If this hypothesis be correct, a connection may be made between negative amoebal phototaxis and negative slug phototaxis.

The discussion in this section is conjectural and intended to propose possible avenues for further work.

Summary

The possible role of amoebal thermo- and photo-sensing in slug sensing has been discussed.

1. General features of the temperature response curve of amoebae, strain HL 50 and mutants, after 16 hr on the temperature gradient are very similar to the slug's. The similarities strongly suggest that amoebal thermotaxis is the basis for slug thermotaxis.

2. There is no evidence available at present which suggests a role of amoebal phototaxis in slug phototaxis. However, several potential connections are discussed.

CONCLUSIONS

Organisms are able to survive in a very narrow range of temperature and light intensities generally using light as the sole energy supply. It is thus natural that the organism has a very efficient way to sense and adapt to these environmental stimuli. It is also reasonable to assume that organisms have specific mechanisms for processing these important signals from the surroundings. These mechanisms will have some particular molecular basis which eventually brings a change in the whole organism. All organisms on earth whether they are macro- or micro-organisms have evolved a way to deal with these environments. In no case have we accumulated enough knowledge to understand even a small portion of this postulated mechanism.

Some microorganisms may provide us with a model system through which we can achieve an understanding of sensory transduction in eukaryotic organisms. Dictyostelium discoideum has been one of those microorganisms investigated, and during the last decades, we have learned a great deal about its sensory responses. Two attributes of Dictyostelium are particularly attractive for a model sensory system. This organism is extremely sensitive to small fluctuations in its environment. Both the amoebae and the

slugs respond to sensory stimuli permitting us to study sensory physiology as a function of development in a single organism.

The objectives of this study were: (1) to investigate thermosensing in D. discoideum amoebae; (2) to investigate the possible connections between amoebal and slug thermosensing; (3) to directly confirm positive and negative phototactic responses in D. discoideum amoebae; (4) to study the nature of the photoreceptor pigment regulating amoebal phototaxis.

Amoebae of D. discoideum, strain HL 50, show only positive thermotaxis throughout the physiological temperature range when checked shortly after food depletion. Negative thermotactic responses become apparent at the lower physiological temperatures after 12 hr on the thermal gradients. After 16 hr on the thermal gradients, amoebae of D. discoideum, strain HL 50, show a distinct negative thermotaxis at 14°, 16°, and 18°C and positive thermotaxis at higher temperatures. The development of negative amoebal thermotaxis at low temperatures is probably induced by food depletion and/or exposure on the thermal gradients. Amoebae of the mutant strain HO 428 showed positive thermotaxis which is strong at lower temperatures and drops sharply above the growth temperature of amoebae. The temperature response of HO 428 amoebae was not affected by the length of exposure of amoebae on the gradients. HO 596 amoebae showed both positive and negative thermotactic responses shortly

after food depletion. A longer exposure of these amoebae on the thermal gradients induced a stronger negative response at lower temperatures. A similar time-dependent change was observed in HO 1445 amoebae. Based on the steady positive thermotactic response by HO 428 amoebae and the change in temperature response at higher temperatures by HO 596 amoebae, a model for the temperature response of vegetative D. discoideum amoebae has been proposed. The main features of the model are: a positive response at the thermal gradients with midpoint temperatures lower than the growth temperature of amoebae and a negative response above it.

After 16 hr on the thermal gradients, HL 50, HO 596 and HO 813 amoebae show temperature responses which are very similar to that of the slugs of each strain. The transition temperature from the negative to the positive response is approximately the same and, in some cases, the position of maximum response is very similar. HO 428, a mutant with no negative thermotactic response in slug thermotaxis, also shows no negative amoebal thermotaxis. These similarities strongly suggest that amoebal thermotaxis is the basis for slug thermotaxis. It is therefore unnecessary to postulate a thermosensing mechanism which includes the complexities of the slug. Rather, one may now assume that the sensing mechanism is at or below the cellular level.

Amoebal phototaxis has been re-investigated with direct observation of the response of amoebae. The results basically confirm the previous reports. The amoebae showed

positive response to light below 100 mW/m^2, and negative response above this light intensity. An action spectrum, based on the "zero-crossing" points in fluence-response curves for monochromatic light, shows a major peak at about 405nm and secondary maxima at about 450, 520, 580 and 638nm. This action spectrum resembles the action spectra for accumulations of amoebae in and dispersal from light traps and that of inhibition of aggregation by light.

Low temperature, 77°K, absorption spectra of the amoebae clearly resemble the action spectra presented previously. The most distinctive peak in the action spectrum for phototaxis (about 638nm) is similar to the most distinctive peak in the low temperature absorption spectrum (638nm). The absorption at 638nm is not stable in a cell extract or in disrupted cells at room temperature or in low pH aqueous solution. This peak also can be lowered by incubating the amoebae with detergents, but not with proteases or oxidizing or reducing reagents. Decrease of the absorption peak at 638nm correlates with an increase of a peak at 630nm. These data support the conclusion that "P_{638}", a photoreceptor pigment with an absorption peak at 638nm, results from an aggregated protoporphyrin. Subcellular fractionation of the amoebae showed that P_{638} co-separates with cytochrome c oxidase and malate dehydrogenase. This indicates that P_{638} is probably localized in the mitochondria.

When D. discoideum amoebae were grown in association with K. aerogenes on SM agar plates, the characteristic protoporphyrin absorption peak at 638nm was diminished. These nonaxenically grown amoebae still have a strong absorption in the blue and green regions of spectrum. The fluence-rate response has been checked for the amoebae of the strain Ax 2 and some nonaxenic strains over a wide range of fluence-rates. The response curve of Ax 2 amoebae shows a positive and a negative phototaxis between 0.01 W/m^2 to 1 W/m^2 which confirms the previously described results. This curve also shows more prominent phototaxis of Ax 2 amoebae at much lower fluence-rate. These very low fluence-rates also induced phototactic responses in all other nonaxenic strains. Because these non-axenic strains lack spectrophotometrically observable P_{638}, the function of protoporphyrin in D. discoideum amoebal phototaxis may be questioned. It is likely that D. discoideum amoebae have another photoreceptor pigment(s), which is not protoporphyrin, for phototaxis, and that protoporphyrin either is involved in the phototaxis of Ax 2 amoebae grown in the liquid culture, or that protoporphyrin has some secondary function in amoebal phototaxis such as acting as a screening pigment.

Conclusions concerning the role of protoporhyrin or even of "P_{638}" would be far easier to draw given action spectra for amoebal phototaxis in the non-axenic strains.

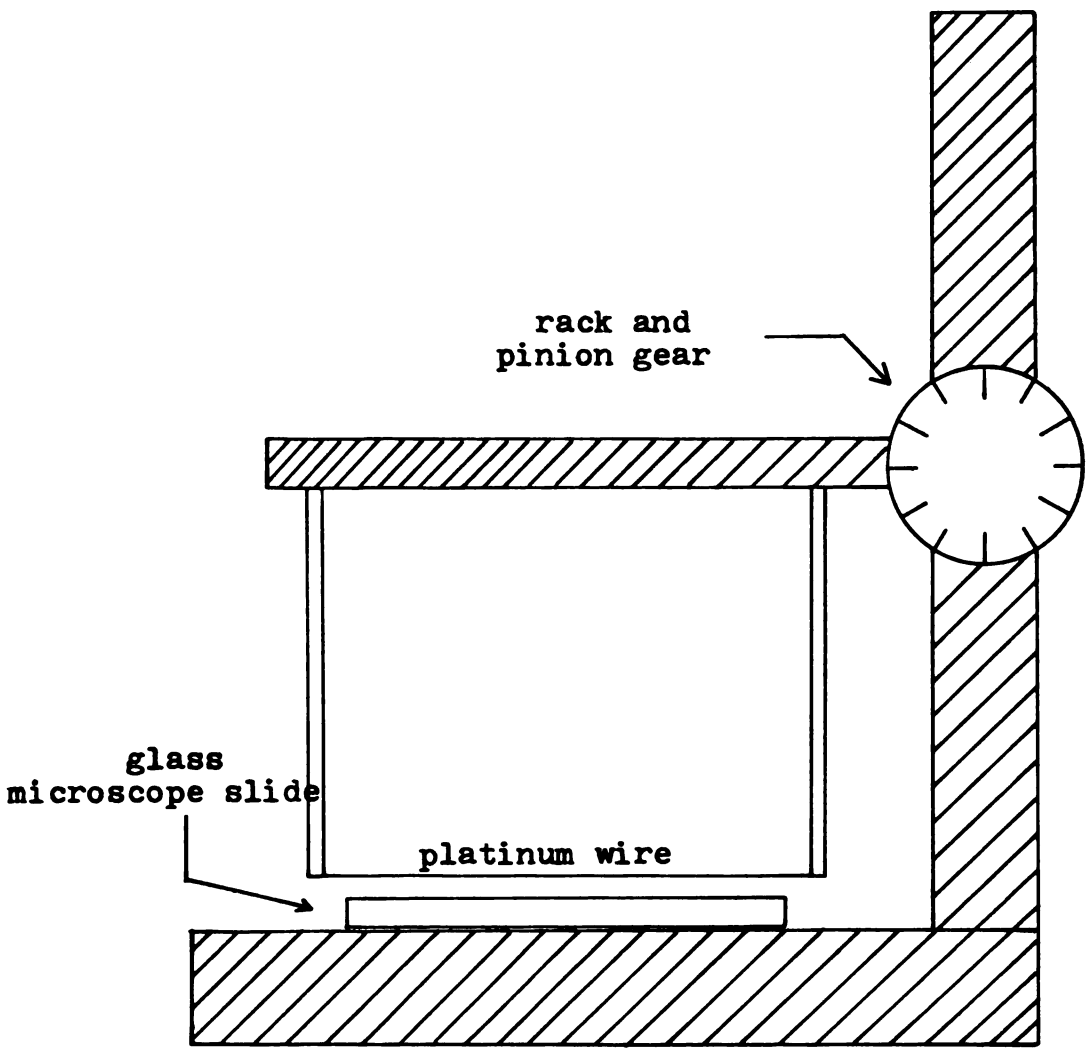
In particular, one wishes to see whether or not non-axenic amoebae show an action maximum at about 638nm.

Available mutants in thermosensing and possibly photosensing should be of great advantage in studying the mechanisms of sensory transduction in Dictyostelium. These mutants should be manipulated to obtain strains showing only the particular lesion dealing with thermosensing or photosensing. Only with such material can one be reasonably confident that the characteristic being studied is indeed under the control of a single gene. Once this has been achieved, the possibilities are great for understanding the mechanisms or thermosensing in Dictyostelium.

APPENDICES

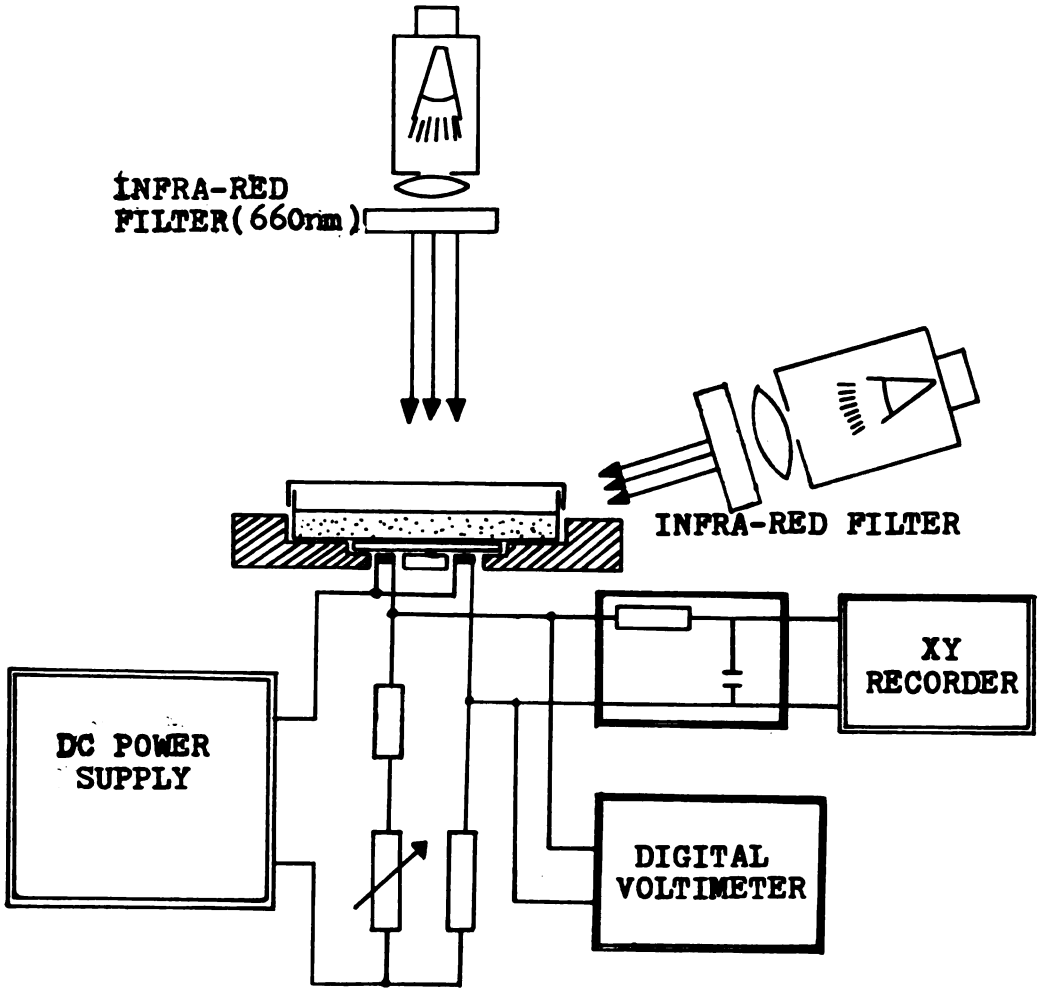
APPENDIX A

Figure 31. Diagram of a micromanipulator used to spot the amoebae suspension on the 1% water agar (w/v) covering a glass microscope slide. The micromanipulator was constructed of two pieces of fine platinum wire, 0.1mm in diameter, which were attached at their ends to two metal bars such that the wires were appressed together. The bars were attached to a rack and pinion gear such that the wires could be raised and lowered.


rack and
pinion gear
glass
microscope slide
platinum wire

APPENDIX B

Figure 32. Diagram of an apparatus used to measure movement of D. discoideum amoebae in response to unilateral light. The two PIN diodes, connected in a Wheatstone bridge, change their resistance with incident light intensity from the monochromatic measuring beam (660nm). Any mass movement of the amoebae toward or away from the actinic light results in a changed incident lighting on the two diodes and thus an electrical signal from the bridge circuit.


INFRA-RED
FILTER(660nm)
INFRA-RED FILTER
XY
RECORDER
DC POWER
SUPPLY
DIGITAL
VOLTIMETER

APPENDIX C

THERMOTACTIC MUTANTS OF D. DISCOIDEUM SLUG

Introduction

Slugs of D. discoideum are phototactic (Bonner et al., 1950; Poff et al., 1973), thermotactic (Bonner et al., 1950; Whitaker and Poff, 1980) and chemotactic (Fisher et al., 1981). Schneider et al. (1982) isolated and characterized three classes of thermotaxis mutants of slugs: a) the mutant only shows positive thermotaxis, b) the mutants show a weakened positive thermotactic response but an unimpaired negative response, and c) a mutant with weakened negative and positive responses. Additional mutants have been obtained and characterized, and mutants with different thermotactic characters from those previously described and some mutants with similar characters are reported here.

Mutant Isolation

Amoebae of D. discoideum (HL 50) were mutagenized with MNNG (N-methyl 1-N'-nitro-N-nitrosoguanidine), cloned, and screened for aberrations in thermotaxis on gradients of 15.5°-16.5°C and 22.75°-23.25°C as previously described (Schneider et al., 1982) with a slight modification.

Three mutageneses were performed with increasing incubation time with the mutagene. The first mutagenesis resulted in a killing rate of about 95%, the second mutagenesis resulted in a killing rate about 99% and the third mutagenesis gave a killing rate of about 99.5%.

Mutant Characterization

Putative thermotaxis mutants were characterized on 0.11°C/cm temperature gradients with midpoint temperatures of 16°, 18°, 20°, 22°, 24° and 26°C. After 1 or 2 days migration, the plates were shadow-graphed, and the angular distribution of the pseudoplasmodia was measured and used to calculate the directness of slug movement. See Whitaker and Poff (1980) for detailed methods.

Results and Discussion

A. Of the 600 clones screened, twenty six showed apparently altered thermotactic responses. The first mutagenesis produced three mutants from about 200 clones screened, and among these three mutants, one appeared to be a new mutant. The second mutagenesis produced six mutants from about 200 clones screened, and they showed similar thermotactic behaviours to the mutants described by Schneider et al. (1982). Sixteen apparent thermotaxis mutants were selected from the mutagenesis with 99.5% killing rate. Of these, six mutants showed thermotactic behaviours different from those previously described.

B. HO 1175 (Figure 33A), HO 1228 (Figure 33B) and HO 1445 (Figure 33C) are the some examples from each mutagenesis which can be classified into the three classes of mutants described previously (Schneider et al., 1982). HO 1175 and HO 1445 have both weakened positive and negative thermotaxis from the parental strain, HL 50. HO 1228 is a mutant which is similar to HO 596. It shows weakened positive response but an unimpaired negative response.

C. HO 1155 (Figure 33D), a product of the first mutagenesis, HO 1462 (Figure 33E), HO 1488 (Figure 33F) and HO 1484 (Figure 33G) all have similar thermotactic characters. They appeared to have both unimpaired positive and negative thermotactic responses. However they differ from the parental strain in that they show a shifted transition temperature. HO 1155, HO 1462 and HO 1488 showed a transition temperature which is lower than the parental strain, and HO 1484 has a transition temperature about 2°C higher than HL 50.

D. HO 1458 shows a slightly weakened negative thermotaxis but an apparently unimpaired positive thermotaxis (Figure 33H).

E. HO 1475 shows no negative thermotaxis and a weakened positive thermotaxis (Figure 33I).

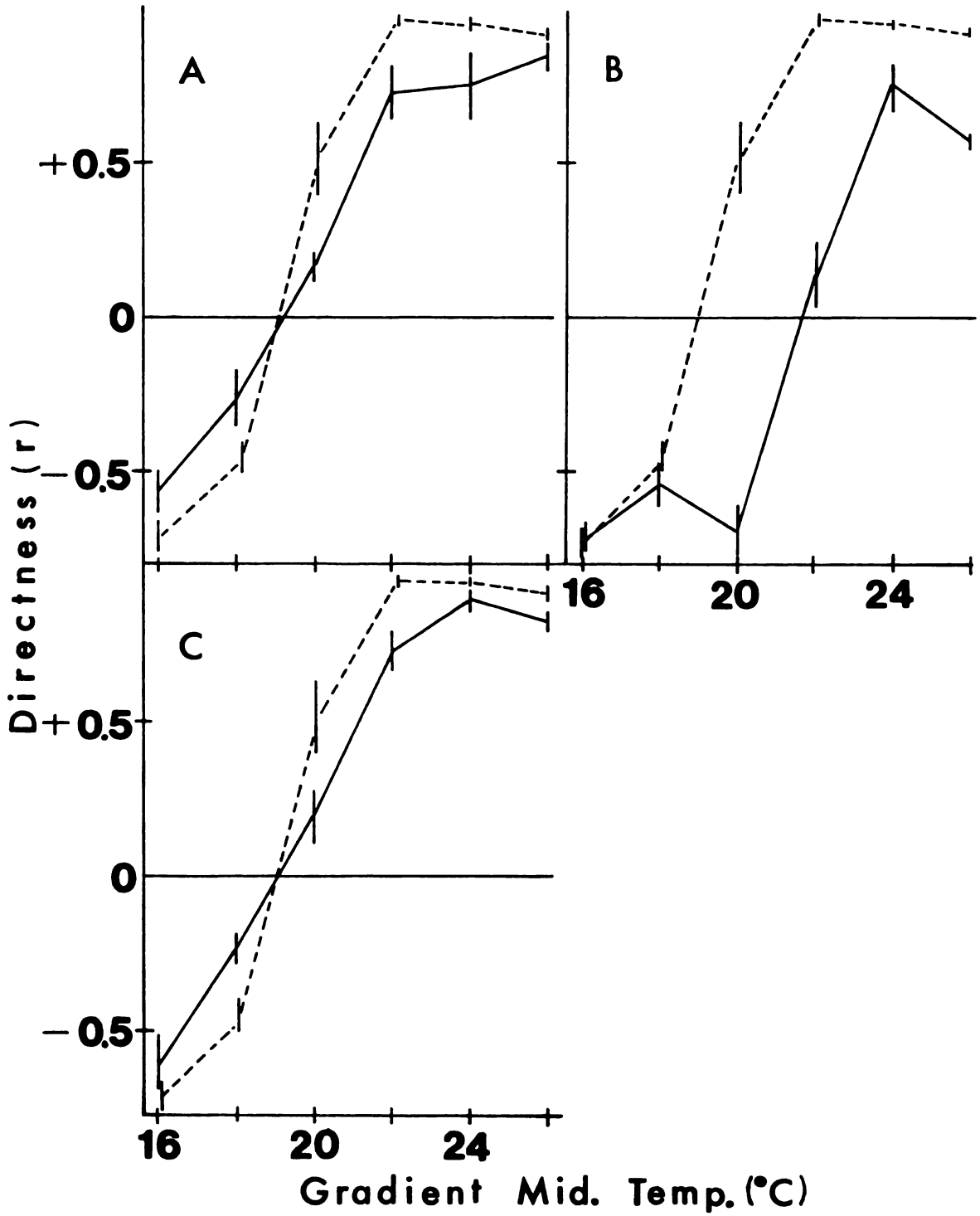
F. HO 1392 shows little if any thermotactic response on any of the temperature gradients (Figure 33J).

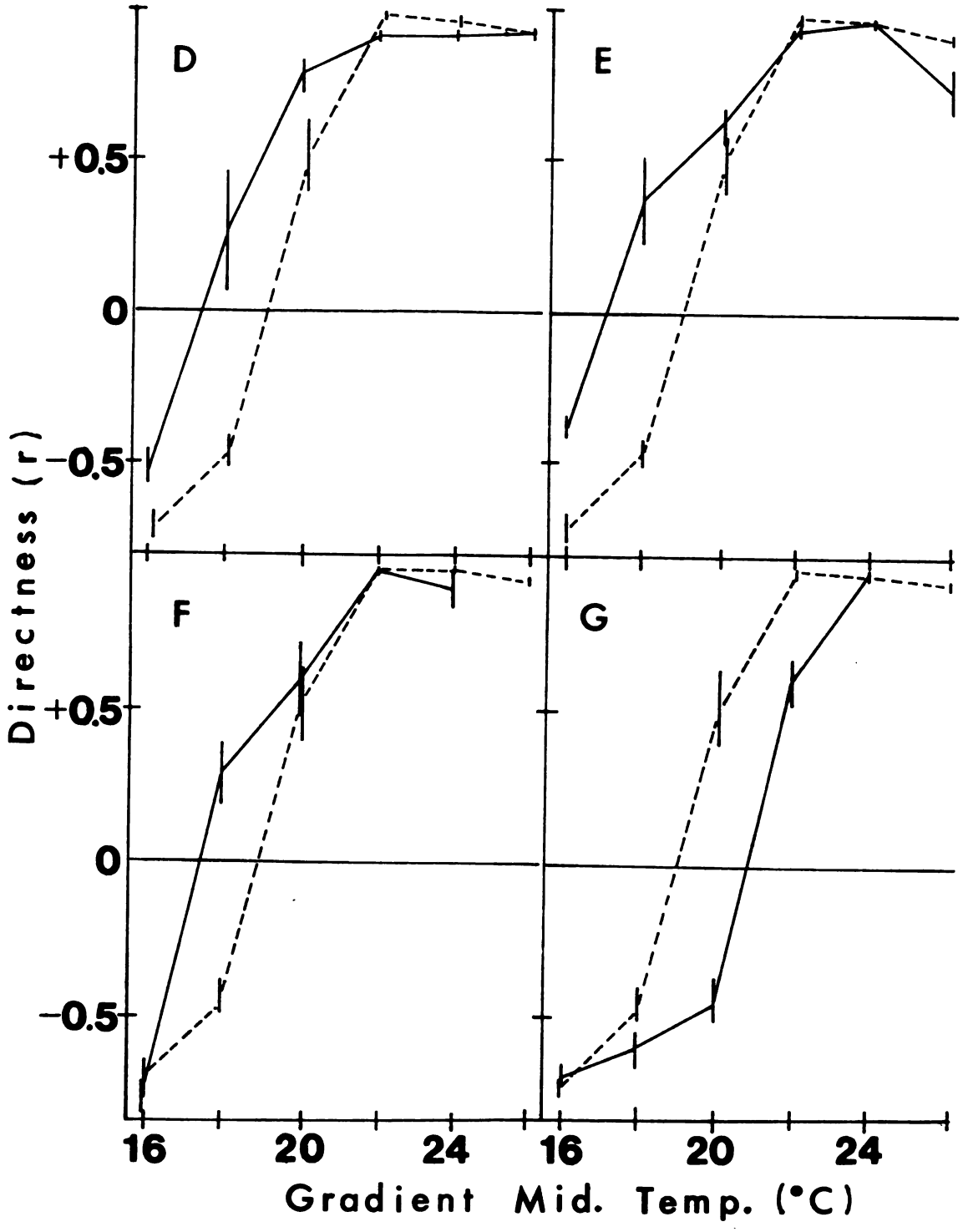
G. Because of the high killing rate employed, most mutants reported here cannot be considered as the ones with

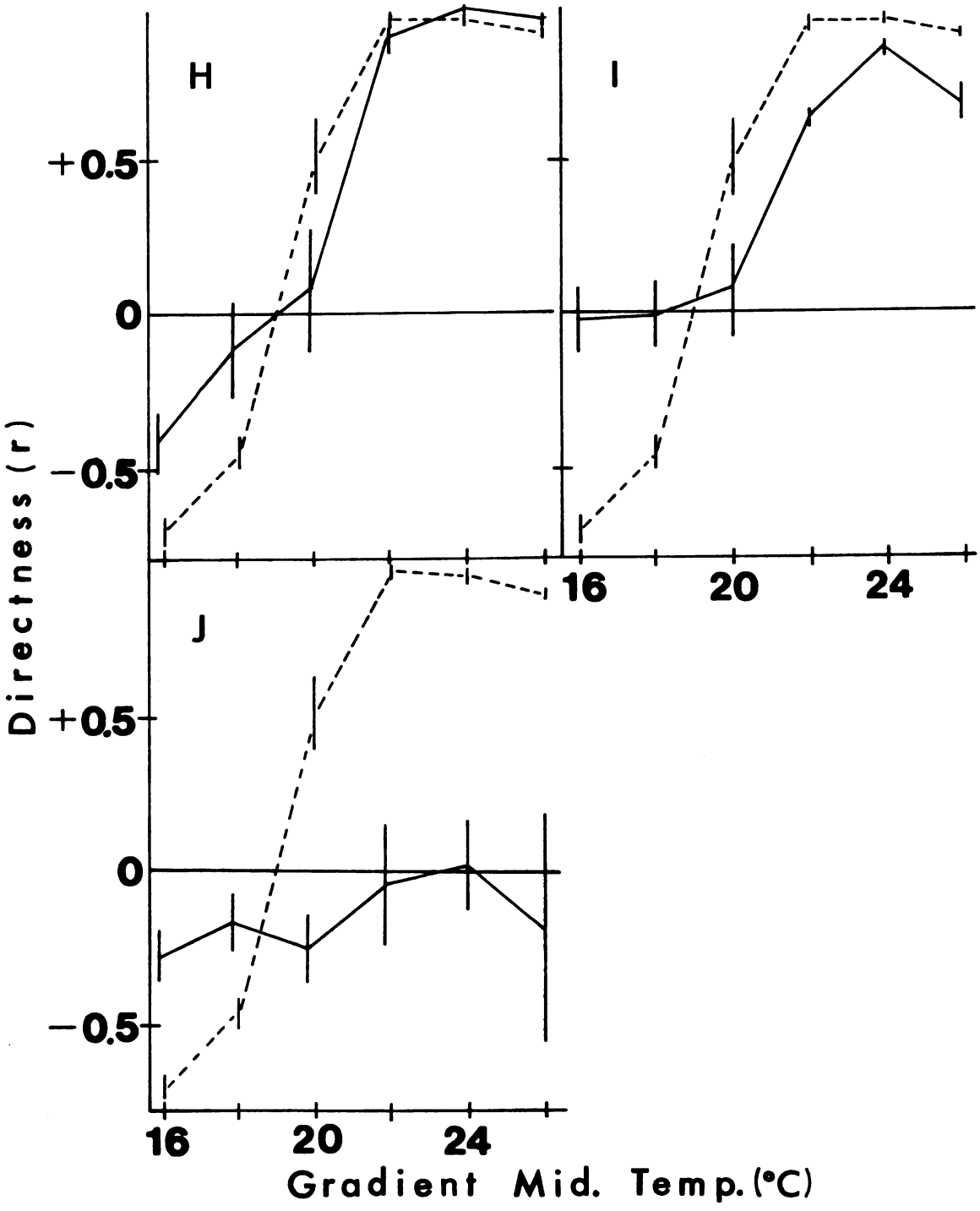
only one defect in the genome which controls thermosensory transduction.

H. The mutants reported here, in addition to the previously reported mutants, will be very helpful in dealing with thermotactic sensing in D. discoideum slug, especially if they can be cleared genetically to be mutants with just one lesion which affects the thermotactic response.

Figure 33. Temperature response curves of thermotactic mutants. A) HO 1175, B) HO 1228, C) HO 1445, D) HO 1155, E) HO 1462, F) HO 1488, G) HO 1484, H) HO 1458, I) HO 1475, and J) HO 1392. Vertical bars represent $\pm$ one standard error. Each data point represents an average of six to eight replicates. The temperature response curve for the parental strain, HL 50, is presented as a broken line in each figure.


A
B
C
+0.5
0
−0.5
Directness (r)
16
20
24
Gradient Mid. Temp. (°C)


D
E
F
G
+0.5
0
−0.5
Directness (r)
16
20
24
Gradient Mid. Temp. (°C)


H
I
J
+0.5
0
−0.5
Directness (r)
16
20
24
Gradient Mid. Temp. (°C)

BIBLIOGRAPHY

BIBLIOGRAPHY

Alemany, S., M.G. Gil, and J.M. Mato (1980) Regulation by Guanosine 3': 5'-Cyclic Monophosphate of Phospholipid Methylation during Chemotaxis in Dictyostelium discoideum. Proc Natl Acad Sci USA 77:6996-6999.

Appelmans, F., R. Wattiaux, and C.D. Duve (1955) Tissue Fractionation Studies. 5. The Association of Acid Phosphatase with a Special Class of Cytoplasmic Granules in Rat Liver. Biochem J 59:438-445.

Batschelet, E. (1965) Statistical Methods for the Analysis of Problems in Animal Orientation and Certain Biological Rhythms. Am Inst of Biol Sci, Washington, D.C., pp. 1-57.

Bazari, W.L. and M. Clarke (1981) Characterization of a Novel Calmodulin from Dictyostelium discoideum. J Biol Chem 256:3598-3603.

Bonner, J.T. (1967) The Cellular Slime Molds. Princeton University Press, Princeton, N.J., pp. 1-205.

Bonner, J.T. (1977) Some Aspects of Chemotaxis Using the Cellular Slime Molds as an Example. Mycologia 69:443-459.

Bonner, J.T., D.S. Barkley, E.M. Hall, T.M. Konijn, J.W. Mason, III G. O'Keefe, and P.B. Wolfe (1969) Acrasin, Acrasinase, and the Sensitivity to Acrasin in Dictyostelium discoideum. Devel Biol 20:72-87.

Bonner, J.T., W.W. Clarke, Jr., C.L. Neely, Jr., and M.K. Silfkin (1950) The Orientation to Light and the Extremely Sensitive Orientation to Temperature Gradients in the Slime Mold Dictyostelium discoideum. J Cell Comp Physiol 36:149-158.

Bozzone, D. and J. Bonner (1982) Cell Interactions During Early Stages of Macrocyst Development in Dictyostelium discoideum. Int Cellular Slime Mold Conf (Abstr.).

Burchard, R.P. and M. Dworkin (1966) Light-Induced Lysis and Carotenogenesis in Myxococcus xanthus. J Bacteriol 91:535-545.

Burkart, U. and D.-P. Häder (1980) Phototactic Attraction in Light Trap Experiments: A Mathematical Model. J Math Biol 10:257-269.

Clarke, M., W.L. Bazari, and S.C. Kayman (1980) Isolation and Properties of Calmodulin from Dictyostelium discoideum. J Bacteriol 141:397-400.

Cripps, M.M. and C.L. Rutherford (1981) A Stage-Specific Inhibitor of Adenylate Cyclase in Dictyostelium discoideum. Exp Cell Res 133:309-316.

Dailey, H.A., Jr. and J. Lascelles (1977) Reduction of Iron and Synthesis of Protoheme by Spirillum itersonic and Other Organisms. J Bacteriol 129:815-820.

Feinleib, M.E. and G.M. Curry (1967) Methods for Measuring Phototaxis of Cell Populations and Individual Cells. Physiol Plant 20:1083-1095.

Fisher, P.R., E. Smith, and K.L. Williams (1981) An Extracellular Chemical Signal Controlling Phototactic Behavior by Dictyostelium discoideum Slugs. Cell 23:799-807.

Fisher, P.R. and K.L. Williams (1981) Bidirectional Phototaxis by Dictyostelium discoideum Slugs. FEMS Microbiol Lett 12:87-89.

Fisher, P.R. and K.L. Williams (1982) Thermotactic Behaviour of Dictyostelium discoideum Slug Phototaxis Mutants. J Gen Microbiol 128:965-971.

Fontana, D.R. (1982) Thermotactic and Phototactic Responses of Dictyostelium discoideum Pseudoplasmodia. Ph.D. Dissertation, Submitted to Michigan State University, E. Lansing, MI.

Foote, C.S. (1976) Photosensitized Oxidation and Singlet Oxygen: Consequences in Biological Systems. In: Free Radicals in Biology. (Pryor, W.A., ed) Academic Press, New York, Vol 2, pp. 85-133.

Forward, R.B., Jr. (1976) Light and Diurnal Vertical Migration: Photobehavior and Photophysiology of Plankton. Photochem Photobiol Rev 1:157-209.

Francis, D.W. (1964) Some Studies on Phototaxis of Dictyostelium. Cell Comp Physiol 64:131-138.

Frydman, R.B., B. Frydman, and A. Valasinas (1978) Protoporphyrin: Synthesis and Biosynthesis of Its Metabolic Intermediates. In: The Porphyrins (Dolphin, D., ed.) Academic Press, New York, Vol. VI. pp. 67-115.

Futrelle, R.P., W.G. McKee, and J. Traut (1980) Response of Dictyostelium discoideum to Localized cAMP Stimuli; Computer Analysis of Cell Motion. J Cell Biol 87:CI 415 (Abstr.).

Gallagher, W.A. and W.B. Elliott (1973) Ligand-Binding in Porphyrin Systems. Ann N.Y. Acad Sci 206:463-482.

Gerisch, G. (1982) Chemotaxis in Dictyostelium. Ann Rev Physiol 44:535-552.

Gerish, G., Y. Maeda, D. Malchow, W. Roos, U. Wick, and B. Wurster (1977) Cyclic AMP Signal and the Control of Cell Aggregation in Dictyostelium discoideum. In: Development and Differentiation in the Cellular Slime Molds (Cappuccinelli, P. and J.M. Ashworth, eds.) Elsevier/North Holland Biomedical Press, Amsterdam, pp. 105-123.

Gingle, A.R. (1976) Critical Density for Relaying in Dictyostelium discoideum and Its Relation to Phosophodiesterase Secretion into the Extracellular Medium. J Cell Sci 20:1-20.

Gingle, A.R. and A. Robertson (1976) The Development of the Relaying Competence in Dictyostelium discoideum. J Cell Sci 20:21-27.

Goldstein, B.O. and L.C. Harber (1972) Erythropoietic Protoporphyria: Lipid Peroxidation and Red Cell Membrane Damage Associated with Photohemolysis. J Clin Invest 51:892-902.

Green, A.A. and P.C. Newell (1975) The Isolation and Subfractionation of Plasma Membrane from the Cellular Slime Mould Dictyostelium discoideum. Biochem J 140:313-322.

Green, A.A. and P.C. Newell (1975) Evidence for the Existence of Two Types of cAMP-Binding Sites in Aggregating Cells of Dictyostelium discoideum. Cell 6:129-136.

Grutsch, J.F. and A. Robertson (1978) The cAMP Signal from Dictyostelium discoideum Amoebae. Develop Biol 66:285-293.

Häder, D.-P. and K.L. Poff (1979a) Light-Induced Accumulations of Dictyostelium discoideum Amoebae. Photochem Photobiol 29:1157-1162.

Häder, D.-P. and K.L. Poff (1979b) Photodispersal from Light Traps by Amoebae of Dictyostelium discoideum. Exp Mycol 3:121-131.

Häder, D.-P. and K.L. Poff (1979c) Inhibition of Aggregation by Light in the Cellular Slme Mould Dictyostelium discoideum. Arch Microbiol 123:281-285.

Häder, D.-P. and K.L. Poff (1980) Effects of Ionophores and $TPMP^+$ on Light-Induced Responses in Dictyostelium discoideum. Arch Microbiol 126:97-101.

Häder, D.-P., B.D. Whitaker, and K.L. Poff (1980) Responses to Light by a Nonphototactic Mutant of Dictyostelium discoideum. Exp Mycol 4:382-385.

Hedgecock, E.M. and R.L. Russell (1975) Normal and Mutant Thermotaxis in the Nematode Caenorhabditis elegans. Proc Natl Acad Sci USA 72:4061-4065.

Henderson, E.J. (1975) The Cyclic Adenosine 3',5'-Monophosphate Receptor of Dictyostelium discoideum. J Biol Chem 250:4730-4736.

Hong, C.B., D.R. Fontana, and K.L. Poff (1982) Thermotaxis of Dictyostelium discoideum Amoebae and Its Possible Role in Slug Thermotaxis. Proc Natl Acad Sci USA in press.

Hong, C.B., M.A. Häder, D.-P. Häder, and K.L. Poff (1981) Phototaxis in Dictyostelium discoideum amoebae. Photochem Photobiol 33:373-377.

Hübscher, G. and G.R. West (1965) Specific Assay of Some Phosphatases in Subcellular Fractions of Small Intestinal Mucosa. Nature (London) 205:799-800.

Ito, T. (1978) The Dependence of Photosensitizing Efficacy of Acridine Orange and Toluidine Blue on the Degree of Sensitizer-Cell Interaction. Photochem Photobiol 31:565-570.

Jacobson, A. and H.F. Lodish (1975) Genetic Control of Development of the Cellular Slime Mold, Dictyostelium discoideum. Ann Rev Genetics 9:145-185.

Juliani, M.H. and C. Klein (1981) Photo Affinity Labelling of the Cell Surface Adenosine 3',5'-Monophosphate Receptor of Dictyostelium discoideum and Its Modification in Down-Regulated Cells. J Biol Chem 256:613-619.

Kakebeeke, P.I.J., R.J.W. Dewit, S.D. Kohtz, and T.M. Konijn (1979) Negative Chemotaxis in Dictyostelium and Polysphondylium. Exp Cell Res 124:429-432.

Konijn, T.M., J.G.C. van de Meene, J.T. Bonner, and D.S. Barkley (1967) The Acrasin Activity of Adenosine-3',5'-Cyclic Phosphate. Proc Natl Acad Sci USA 58:1152-1154.

Koshland, D.E., Jr. (1976) Bacterial Chemotaxis as a Model Behavioral System. (Raven, New York, 1980); J Adler Sci Am 234:40-47.

Koshland, D.E., Jr. (1979) A Model Regulatory System: Bacterial Chemotaxis. Physiol Rev 59:812-862.

Kuczmarski, E.R. and J.A. Spudich (1980) Regulation of Myosin Self-Assembly: Phosphorylation of Dictyostelium Heavy Chain Inhibits Formation of Thick Filament. Proc Natl Acad Sci USA 77:7292-7296.

Lagarias, J.C. (1981) Personal Communication.

Loomis, W.F. (1975) Dictyostelium discoideum: A Developmental System. Academic Press, N.Y. pp. 1-214.

Lord, J.M., T. Tagawa, T.S. Moore, and H. Beevers (1973) Endoplasmic Reticulum as the Site of Lecithin Formation in Castor Bean Endosperm. J Cell Biol 57:659-667.

Maruta, H., W. Baltes, G. Gerisch, P. Dieter, and D. Marmi (1980) Signal Transduction in Chemotaxis of Dictyostelium discoideum. Role of Ca^{2+} and Calmodulin in the Regulation of Myosin Heavy Chain Kinases and Other Protein Kinases. In: Higher Plant Membranes (Marmer, D., ed.) N.Y.: Elsevier, North Holland Biochemical Press.

Mato, J.M., B. Jastorff, M. Morr, and T.M. Konijn (1978) A Model for Cyclic AMP-Chemoreceptor Interaction in Dictyostelium discoideum. Biochim Biophy Acta 544:309-314.

Mato, J.M. and T.M. Konijn (1979) Chemotactic Signal and Cyclic GMP Accumulation in Dictyostelium discoideum. In: Development and Differentiation in Cellular Slime Molds. (Cappuccinelli, D. and J.M. Ashworth, eds) Elsevier/North Holland Biomedical Press, Amsterdam, pp. 93-103.

Mato, J.M., F.A. Krens, P.J.M. van Haastert, and T.M. Konijn (1977) 3':5'-Cyclic AMP-Dependent 3':5'-Cyclic GMP Accumulation in Dictyostelium discoideum. Proc Natl Acad Sci USA 74:2348-2351.

Mato, J.M. and D. Marin-Cao (1979) Protein and Phospholipid Methylation during Chemotaxis in Dictyostelium discoideum and Its Relationship to Calcium Movements. Proc Natl Acad Sci USA 76:6106-6109.

Mato, J.M. H. Woelders, and T.M. Konijn (1979) Intracellular Cyclic GMP-Binding Protein in Cellular Slime Molds. J Bacteriol 137:169-172.

McMahon, D., M. Miller, and S. Long (1977) The Involvement of the Plasma Membrane in the Development of Dictyostelium discoideum. I. Purification of the Plasma Membrane. Biochim Biophy Acta 465:224-241.

McNab, R.M. and D.E. Koshland, Jr. (1972) The Gradient-Sensing Mechanism in Bacterial Chemotaxis. Proc Natl Acad Sci USA 69:2509-2512.

Müller, M. and K.M. Møller (1969) Urate Oxidase and Its Association with Peroxisomes in Acanthamoeba species. Eur J Biochem 9:424-430.

Nultsch, W.E. and G. Throm (1975) Effect of External Factors on Phototaxis of Chlamydomonas reinhardtii. I. Light. Arch Microbiol 103:175-179.

Ochoa, S. (1955) "Malic" Enzyme. In: Methods in Enzymology. (Colowick, S.P. and N.O. Kaplan, eds). Academic Press, N.Y. Vol. 1, pp. 739-741.

Pan, P., E.M. Hall, and J.T. Bonner (1975) Determination of the Active Portion of the Folic Acid Molecule in Cellular Slime Mold Chemotaxis. J Bacteriol 122:185-191.

Poff, K.L. and W.L. Butler (1974) Absorbance Changes Induced by Blue Light in Phycomyces blackesleeanus and Dictyostelium discoideum. Nature 248:799-801.

Poff, K.L., W.L. Butler, and W.F. Loomis, Jr. (1973) Light-Induced Absorbance Changes Associated with Phototaxis in Dictyostelium. Proc Natl Acad Sci USA 70:813-816.

Poff, K.L. and W.F. Loomis, Jr. (1973) Control of Phototactic migration in Dictyostelium discoideum. Exp Cell Res 82:236-240.

Poff, K.L., W.F. Loomis, Jr., and W.L. Butler (1974) Isolation and Purification of the Photoreceptor Pigment Associated with Phototaxis in Dictyostelium discoideum. J Biol Chem 249:2164-2167.

Poff, K.L. and M. Skokut (1977) Thermotaxis by Pseudoplasmodia of Dictyostelium discoideum. Proc Natl Acad Sci USA 74:2007-2010.

Poulson, R. and W.J. Polglase (1974) Aerobic and Anaerobic Coproporphyrinogenase Activities in Extracts from Saccharomyces cerevisiae. J Biol Chem 249:6367-6371.

Raper, K.B. (1940) Pseudoplasmodium Formation and Organization in Dictyostelium discoideum. J Elisha Mitchell Sci Soc 56:241-282.

Robertson, A., D.J. Drage, and M.H. Cohen (1972) Control of Aggregation in Dictyostelium discoideum by an Internal Periodic Pulse of Cyclic Adenosine Monophosphate. Science 175:333-335.

Schneider, M.J., D.R. Fontana, and K.L. Poff (1982) Mutants of Thermotaxis in Dictyostelium discoideum. Exp Cell Res 140:411-416.

Schuchart, H. (1975) Phototaxis Methodology. In: Proc Inst School Badia Fiesolana (Colombetti, G., ed.) Pisa, Lito Felici, pp. 85-108.

Smith, E., P.R. Fisher, W.N. Grant, and K.L. Williams (1982) Sensory Behavior in Dictyostelium discoideum Slugs: Phototaxis and Thermotaxis are Not Mediated by a Change in Slug Speed. J Cell Sci 54:329-339.

Steel, R.G.D. and J.H. Torrie (1980) Confidence Interval. In: Principles and Procedures of Statistics. 2nd ed. McGraw-Hill, N.Y. pp. 1-560.

Sussman, M. (1966) Biochemical and Genetic Methods in the Study of Cellular Slime Mold Development. In: Methods in Cell Physiology (Prescott, D., ed.) Academic Press, New York, Vol. 2, pp. 397-410.

Tomchik, K.J. and P.N. Devreotes (1981) Adenosine 3', 5'-Monophosphate Waves in Dictyostelium discoideum: A Demonstration by Isotope Dilution-Flurography. Science 212:443-446.

Whitaker, B.D. (1979) Studies of Thermotaxis by Pseudoplasmodia of Dictyostelium discoideum. Ph.D. Dissertation, Submitted to Michigan State University, E. Lansing, MI.

Whitaker, B.D. and K.L. Poff (1980) Thermal Adaptation of Thermosensing and Negative Thermotaxis in Dictyostelium. Exp Cell Res 128:87-93.

Wurster, B. and U. Butz (1980) Reversible Binding of the Chemoattractant Folic Acid to Cells of Dictyostelium discoideum. Eur J Biochem 109:613-618.

Wurster, B., K. Schubiger, and G. Gerisch (1977) Cyclic GMP in Dictyostelium discoideum. Oscillations and Pulses in Response to Folic Acid and Cyclic AMP Signals. FEBS Lett 76:141-144.