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THE EFFECTS OF HEAT SHOCK ON DEVELOPMENT
AND PROTEIN SYNTHESIS OF THE MYXOMYCETE,
PHYSARUM POLYCEPHALUM.

A thesis presented in partial fulfilment of the
requirements for the degree of Master of Science
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ABSTRACT

When *Drosophila* embryos and pupae, undergoing differentiation, are subjected to a brief heat shock at an elevated temperature, specific abnormalities are produced in the adult organism. The type of abnormality produced is dependant upon the stage in the differentiation process at which the heat shock is administered. In addition, *Drosophila* cells respond to heat shock with the rapid cessation of all pre-existing transcription and translation, and the simultaneous transcription of a specific set of heat shock genes. Heat shock mRNAs are subsequently translated preferentially into a novel set of heat shock proteins. This response of *Drosophila* is independant of the type of tissue and the developmental state. Many other organisms display a similar protein synthetic heat shock response.

Physarum plasmodia can be readily induced experimentally to undergo a process of differentiation leading to the development of mature sporangia. Heat shocks administered during this differentiation have been reported to result in the formation of abnormal sporangia. This thesis is concerned with the detailed investigation of normal sporangial development in *Physarum* and the effects on subsequent development of heat shocks administered at a number of different stages of development, using a combination of light microscopy, scanning electron microscopy and transmission electron microscopy. The more immediate response of *Physarum* protein synthesis to heat shock is also investigated, using radioactive labeling of proteins, SDS-polyacrylamide gel electrophoresis and fluorography.

Heat shocks administered during the early stages of *Physarum* sporangial development induce a delay in subsequent development but normal mature sporangia are produced. Heat shocks administered late in development induce the formation of grossly abnormal sporangia, with the type of abnormality induced being dependant upon the stage of development attained at the time of the heat shock. Heat shocks administered at a mid-point in development induce a complete, though not permanent, developmental arrest.

Heat shocks at a number of different stages of *Physarum* sporangial development induce a considerable

reduction in pre-existing protein synthesis, while the synthesis of a novel set of heat shock proteins is induced by each heat shock. The heat shock proteins of *Physarum* have approximate molecular weights of 85,000, 78,000, 75,000, 73,000, 69,000, 18,000, and 14,000 daltons, with the predominant heat shock protein being that of 69,000 daltons. *Physarum* plasmodia undergoing active growth synthesize the set of heat shock proteins typical of plasmodia undergoing development but, in this case, the pre-existing protein synthesis continues during the heat shock.

The effects of heat shock on both the development and protein synthesis of *Physarum* plasmodia are discussed and relationships between these two phenomena are proposed. Also discussed are the similarities between heat shock-induced abnormalities in *Drosophila* and *Physarum*, and the similarities between the protein synthetic heat shock responses of these two organisms. In both organisms, a similar molecular basis probably underlies abnormality production, while the major heat shock proteins of both organisms are remarkably similar in molecular weight.

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SECTION 1. INTRODUCTION

It has long been established that the imposition of an environmental stress during the development of *Drosophila* can induce mutant-like abnormalities, described as phenocopies, in the mature adult. Thus a brief shift from the normal growth temperature to an elevated, but not lethal temperature (heat shock) at specific stages of embryological development or metamorphosis induces specific phenocopies (Goldschmidt, 1935, cited in Mitchell and Lipps, 1978). The cause of the abnormality has been suggested to be a heat shock-induced conformational change in specific proteins (Milkman, 1963). However, it has since become clear that phenocopy production results from the direct effect of heat shock on transcription of a gene that must be expressed in a specific time interval for normal development (Seybold *et al.*, 1975). Additional work on the induction of phenocopies in *Drosophila* led to the hypothesis that the transitory repression of a transcriptional activity is equivalent to a lack of function or an abnormal function in a mutant, and the time of phenocopy production corresponds to the time of expression of the normal allele of the mutant gene (Mitchell and Lipps, 1978). As well as affecting the transcription of structural genes directly, heat shock may also interfere in some general way with the action of regulatory elements in the development of *Drosophila* (Santamaria, 1979).

Heat shock is also effective in inducing developmental abnormalities in other organisms. When *Physarum polycephalum* plasmodia undergoing sporulation are heat shocked at specific stages of development, many abnormal sporangia are subsequently produced (Sauer *et al.*, 1969). Similarly, when *Naegleria gruberi* amoebae are heat shocked during differentiation into swimming flagellates, an increase occurs in the average number of flagella per cell from approximately 2 to either 4.5 (Dingle, 1970) or between 5 and 6 (Walsh, 1980).

During the normal development of the larval salivary gland of *Drosophila*, considerable changes occur in the pattern of puffing activity in the polytene chromosomes. Puffing at sites on these chromosomes represents a measure of transcriptional activity and thus changes in puffing

activity reflect changes in gene expression during development. However when salivary glands are heat shocked, a unique set of puffs is rapidly induced while most pre-existing puffs regress (Ritossa, 1962; Ashburner and Bonner, 1979). In addition, it has been possible to isolate a unique set of messenger RNAs that are induced by heat shock (Spradling *et al.*, 1977; Mirault *et al.*, 1978). In parallel with these changes in transcription, heat shock also redirects protein synthesis from the production of a broad spectrum of proteins which are characteristic of the various tissues and cells of *Drosophila*, to the production of a unique set of proteins, the heat shock proteins (hsp's), which are not tissue specific. The set of hsp's of *Drosophila* includes at least seven different proteins with apparent molecular weights of 82,000, 70,000, 68,000, 27,000, 26,000, 23,000, and 22,000 daltons (for review see Ashburner and Bonner, 1979). Of these, the 70,000 dalton protein (hsp 70) has been found to represent approximately 80% of the total proteins synthesized by *Drosophila* in response to heat shock (Velazquez *et al.*, 1980). The response to heat shock at the level of transcription is extremely rapid such that after only 60 seconds at the elevated temperature, 2.2% of the nuclear RNA is hsp 70 gene transcripts (Findly and Pederson, 1981). Within 8 to 12 minutes, heat shock mRNAs have been processed, transported to the cytoplasm, and translated into protein, while the synthesis of the normal complement of proteins and mRNAs is rapidly curtailed (Lindquist, 1980).

Similar protein-synthetic responses to heat shock have been reported for many other eukaryotic organisms including chicken embryo fibroblasts, mouse L cells, and baby hamster kidney cells (Kelley and Schlesinger, 1978); *Chironomus tentans* (Vincent and Tanguay, 1979); Chinese hamster ovary cells (Bouche *et al.*, 1979); *Saccharomyces cerevisiae* (McCalister and Finkelstein, 1979; Miller *et al.*, 1979); *Dictyostelium discoideum* (Loomis and Wheeler, 1980); *Polysphondilium pallidum* (Francis and Lin, 1980); *Naegleria gruberi* (Walsh, 1980); plant cells (Barnett *et al.*, 1980); *Tetrahymena pyriformis* (Fink and Zeuthen, 1980; Guttman *et al.*, 1980); *Tetrahymena thermophila* (Hauser and Levy-

Wilson, 1981); quail myoblasts (Atkinson, 1981); HeLa cells (Slater *et al.*, 1981); and developing sea urchin embryos (Roccheri *et al.*, 1981). The strict dependence of hsp synthesis on new transcription at the elevated temperature, as indicated by the inhibition of hsp synthesis by actinomycin D, has been demonstrated for *Drosophila* (Lewis *et al.*, 1975), *Dictyostelium* (Loomis and Wheeler, 1980), *Naegleria gruberi* (Walsh, 1980), *Tetrahymena pyriformis* (Fink and Zeuthen, 1980), HeLa cells (Slater *et al.*, 1981), and sea urchin embryos (Roccheri *et al.*, 1981). Although the sets of hsp's of a number of these organisms differ in molecular weight, hsp 70 or a protein of approximately the same molecular weight predominates in *Chironomus*, *Dictyostelium*, *T. pyriformis*, as it does in *Drosophila*. In addition, hsp 70 of *Drosophila* has been shown to co-migrate on SDS acylamide gels with the hsp 70 of *Dictyostelium* (Loomis *et al.*, unpublished results cited in Loomis and Wheeler, 1980, and Velazquez *et al.*, 1980) Such observations seem to suggest that the heat shock response is a truly universal eukaryotic phenomenon. *Escherichia coli* has also been reported to respond to an increase in temperature with a transient increase in the synthesis of a number of proteins (Lemaux *et al.*, 1978; Yamamori *et al.*, 1978) and this further suggests that prokaryotes may be subject to a similar type of response.

Although heat shock itself is the primary inducer of the heat shock response, the mechanisms at the molecular level by which the heat shock loci are specifically selected for expression remain to be elucidated. It has been possible to partially induce the heat shock puffs in individual *Drosophila* salivary gland nuclei by micro-injection of mitochondrial supernatant, obtained from mitochondria subjected to the elevated temperature, into the salivary gland cells (Sin, 1975). Similarly, incubation of isolated salivary gland polytene nuclei in cytoplasm prepared from *Drosophila* tissue culture cells which had been heat shocked before disruption, induces puffs at the heat shock loci (Compton and Bonner, 1978; Compton and McCarthy, 1978; Bonner, 1981; Craine and Kornberg, 1981). The active factor in the cytoplasmic extracts has been partially

4

purified and characterized and found to be a protein (Craine and Kornberg, 1981). The observation that the heat shock loci are induced even in the absence of protein synthesis (Ashburner, 1970) suggests that proteins involved in gene activation must be components of cells during normal growth which are modified or translocated, or both, after heat shock. It remains a possibility that such proteins normally reside within mitochondria.

Additional controls in the heat shock response appear to operate at the level of translation. In parallel with the changes that occur in the patterns of proteins synthesized by *Drosophila* in response to heat shock, pre-existing polysomes disappear and are replaced by a population of polysomes containing RNA synthesized after the elevation in temperature (McKenzie *et al.*, 1975). When polysomal RNA is extracted from heat shocked *Drosophila* cells, it is found to direct the *in vitro* synthesis of heat shock proteins (McKenzie and Meselson, 1977). However mRNAs that are normally synthesized and translated remain in the cytoplasm of heat shocked cells and can be translated *in vitro* also (Mirault *et al.*, 1978). Furthermore, lysates prepared from heat shocked *Drosophila* cells preferentially translate the heat shock mRNAs, while the lysates prepared from normally growing *Drosophila* cells indiscriminately translate both normal mRNAs and heat shock mRNAs (Storti *et al.*, 1980; Krüger and Benecke, 1981; Scott and Pardue, 1981). *Drosophila* cells can therefore discriminate at the level of translation between two populations of mRNA which co-exist in the cell. The addition of crude ribosome fractions from normal cell lysates to lysates from heat shocked cells rescues translation of normal mRNA, suggesting that the discriminating elements are associated with ribosomes (Scott and Pardue, 1981). Upon the return of heat shocked cells to the normal temperature the normal proteins are again synthesized, initially from stored mRNAs (Lindquist, 1980; Storti *et al.*, 1980). The hsp's themselves are apparently not involved in translational control since *Drosophila* embryos, at a stage in development where they are unable to synthesize hsp's, still suffer an arrest or substantial reduction of normal protein synthesis (Dura, 1981).

On the basis of the heat shock-induced repression of normal protein synthesis, it has been proposed that phenocopy induction in *Drosophila* results from a non-coordinated recovery of the normal protein synthesis pattern following heat shock (Chomyn *et al.*, 1979). The process of differentiation in *Drosophila* pupae is accompanied by rapid changes in the patterns of synthesis of all the most abundant proteins and these changes, which are regulated at the level of transcription, can be related to phenocopy sensitivity on a temporal basis (Mitchell and Petersen, 1981). Although not a developmental abnormality in the sense of phenocopy induction, a delay in the onset of mitosis induced by heat shock occurs in a number of organisms. Often such delays can be related to morphological abnormalities within nuclei. In *Physarum polycephalum*, heat shock-induced mitotic delays with variable durations dependant upon the actual stage in the cell cycle at the time of the heat shock have been reported (Brewer and Rusch, 1968; Kauffman and Wille, 1975; Lomagin, 1978; Wright and Tollon, 1978; Tyson *et al.*, 1979; Wolf *et al.*, 1979). Similar cell cycle-dependant, heat shock-induced mitotic delays have been reported for *Tetrahymena* and *Schizosaccharomyces* (Zeuthen, 1974; Polanshek, 1977). In *Drosophila*, mitosis is blocked during heat shock and takes almost 3 hours at the normal incubation temperature before normal cell division occurs again (Arrigo *et al.*, 1980). There is some evidence to suggest that mitosis in both *Physarum* and *Schizosaccharomyces* is dependant upon protein synthesis at the sensitive stages of their cell cycles (Sachsenmaier *et al.*, 1972; Polanshek, 1977; Tyson *et al.*, 1979) and it is possible that the delays in mitosis onset are caused by the heat shock-induced repression of normal protein synthesis and therefore may have a molecular basis similar to that of phenocopy induction. It is interesting to note that division in *Escherichia coli* depends upon the accumulation of a specific protein throughout the cell cycle and that heat shock delays division increasingly with the age of the cell (Smith and Pardee, 1970).

While the physiological function of the heat shock response remains unclear, it is known that heat is not alone

in its ability to induce the transcriptional and translational changes associated with the heat shock response. Included amongst the many other effective inducing agents are anaerobiosis, uncouplers of oxidative phosphorylation, and inhibitors of various enzymes and other cellular functions (Ashburner and Bonner, 1979). Agents other than heat shock are also effective inducers of heat shock-type responses in a number of organisms besides *Drosophila*. Both deciliation and release from anoxia induce the same set of proteins in *Tetrahymena pyriformis* as does heat shock (Guttman *et al.*, 1980). Similarly, anaerobic treatment of maize seedlings results in the synthesis of a novel set of proteins, the major one being identified as alcohol dehydrogenase, an important enzyme for anaerobic survival (Sachs and Freeling, 1978).

Therefore it is likely that the heat shock response itself is but an example of a more general biological reaction to environmental stress. The proteins synthesized in response to that stress probably serve in some way to alleviate the effects of the stress on the organism, an hypothesis which is supported by a number of studies. When *Drosophila* embryos are heat shocked at any stage preceding the migration of the nuclei to the periphery of the egg, they fail to produce hsp's and stop cleaving, whereas embryos heat shocked at later stages do produce hsp's and continue developing (Graziosi *et al.*, 1980). Similarly, sea urchin eggs heat shocked prior to hatching fail to produce hsp's and do not survive the heat shock whereas those heat shocked after hatching do produce hsp's and survive (Roccheri *et al.*, 1981). *Dictyostelium* cells are protected from an otherwise lethal temperature following a pretreatment at a lower temperature which induces the heat shock response (Loomis and Wheeler, 1980). A mild heat shock pretreatment of *Drosophila* cells also greatly enhances survival and the recovery of protein synthesis after a higher temperature heat shock (Mitchell *et al.*, 1979; Petersen and Mitchell, 1981). In addition, pretreatment of *Drosophila* pupae leads to both increased survival of an otherwise lethal heat shock (Milkman, 1963; Mitchell *et al.*, 1979) and protection against phenocopy induction (Mitchell

et al., 1979; Petersen and Mitchell, 1981). When *Drosophila* cells are subjected to a gradual rise in temperature, the temperature range of the heat shock response is greatly extended (Lindquist, 1980) in a manner resembling that of pretreatment. Clearly a relationship does exist between an effective heat shock response and both survival and protection from heat shock-induced developmental abnormalities.

The importance of hsp's can also be demonstrated by inhibiting their synthesis. Thus when the synthesis of hsp's by *Drosophila* cells is inhibited by the addition of cycloheximide to the cells prior to heat shock, normal transcription is strongly inhibited following the heat shock (Arrigo, 1980; DiDomencio *et al.*, cited in Velazquez *et al.*, 1980). Although the hsp's are not responsible for the repression of genes active before the heat shock, their synthesis is necessary for the resumption of RNA synthesis following the heat shock (Arrigo, 1980). The addition of cycloheximide to *Saccharomyces cerevisiae* cells prior to heat shock also prevented the recovery of the pre-heat shock mRNA pattern of these cells on subsequent incubation at the normal temperature (McAlister and Finkelstein, 1980). With *Dictyostelium* cells, the protective action of pretreatment was prevented if the cells were incubated with cycloheximide during the pretreatment (Loomis and Wheeler, 1980).

Therefore it is apparent that the hsp's do in some way protect the cell against the effects of the elevated temperature. A number of observations indicate that the actual site of this protective action is within the nucleus. Using cell fractionation procedures, it has been shown that some hsp's may become localized within nuclei (Mitchell and Lipps, 1975; Vincent and Tanguay, 1979). Further work using this technique has shown that approximately 80% of hsp's 22, 23, 26, and 27, and 30% of hsp's 68 and 70 become localized within nuclei of *Drosophila* cells following a one hour heat shock, whereas most of hsp 84 is found in the cytoplasm (Arrigo *et al.*, 1980). When heat shocked cells are returned to the normal temperature, most of the hsp's migrate from the nuclei into the cytoplasm (Arrigo *et al.*, 1980). Electron microscope autoradiography of *Drosophila* cells has

also demonstrated that a major portion of the proteins synthesized during heat shock is rapidly transported to the nucleus and very little appears in cytoplasmic vacuoles or in mitochondria; once inside the nucleus the hsp's become quantitatively associated with chromosomes (Velazquez *et al.*, 1980). A more detailed biochemical fractionation study has confirmed the association of hsp's with the nuclei of heat shocked *Drosophila* cells and has indicated that the hsp's become associated with an intranuclear scaffold rather than being directly associated with chromatin (Levinger and Varshavsky, 1981). Another study also locates a substantial fraction of the lower molecular weight hsp's within nuclei of heat shocked *Drosophila* cells and identifies the most prominent basic hsp as histone H2b (Sanders, 1981). One of the hsp's of *Tetrahymena pyriformis* also accumulates within the nucleus and may be associated with structural or scaffolding proteins in the nuclear matrix (Guttman *et al.*, 1980). Thus, although the actual molecular interactions remain unclear, the relationship between the hsp's and the nucleus must be an important one for cell survival at elevated temperatures.

In the study presented here, the myxomycete *Physarum polycephalum* is utilized to investigate the effects of heat shock on both development and protein synthesis. The life cycle of *Physarum* (Fig. 1) encompasses two vegetative phases of growth: uninucleate, haploid amebae, which grow and divide by binary fission, and a multinucleate plasmodium, which grows without cell division. In the presence of an adequate food supply, the plasmodium continues to grow indefinitely, undergoing synchronous mitoses. The entire plasmodium can also migrate around by means of protoplasmic streaming. When starved, plasmodia can undergo a reversible transformation to a resistant encysted form, the sclerotium. Starvation can also induce sporulation, for which a period of illumination is also necessary, and which involves a complex sequence of developmental changes. These changes include the morphogenesis of a fruiting-body (sporangium), the cleavage of the cytoplasm to form uninucleate cells, the hardening and darkening of spore walls around these cells, and both mitotic and meiotic divisions of the nuclei. In

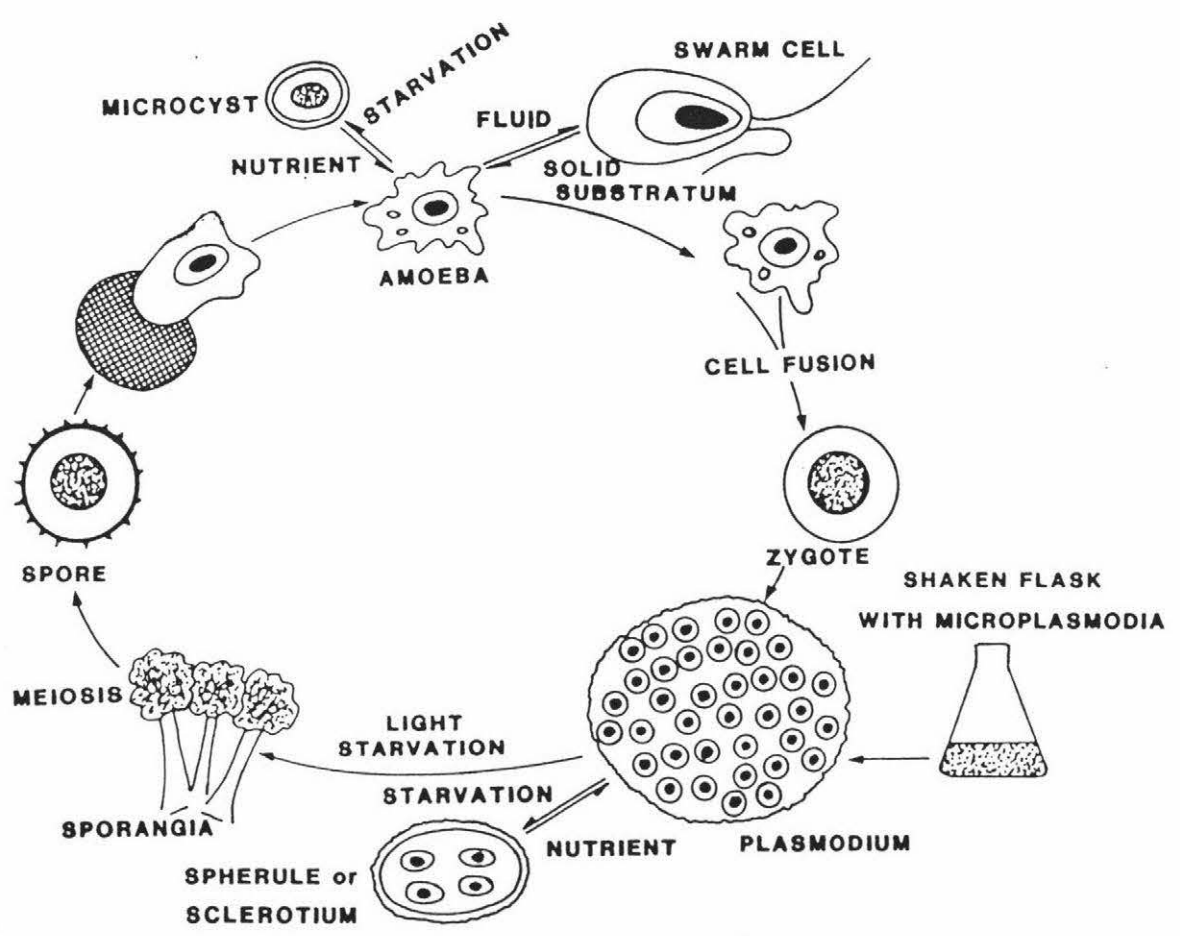


Figure 1. Diagrammatic representation of the life cycle of *Physarum polycephalum* (from Gorman and Wilkins, 1980).

moist conditions the spores germinate to release amoebae. When conditions become unfavourable, amoebae can also form a reversible encysted stage, the microcyst, and in very moist conditions they can form flagellated swarm cells. In heterothallic strains of *Physarum*, the transition from the amoebal to the plasmodial phase of the life cycle requires the fusion of two amoebae of different mating types, with the subsequent fusion of the two nuclei. However there are some strains, most notably the Colonia (CL) strain, in which the transition occurs apogamically within clones of amoebae to produce haploid plasmodia. The CL-2 strain, as used in the present study, is a diploid derivative of the CL strain produced by heat shocking CL plasmodia. (See Dee, 1975; Alexopoulos and Mims, 1979; Gorman and Wilkins, 1980). In the laboratory, the plasmodial phase is routinely cultured on a semidefined medium on a rotary shaker and under these conditions it breaks up into small pieces, the microplasmodia.

The biochemical, morphological, and ultrastructural changes that occur during the process of differentiation from plasmodia to mature spores are known in some detail (Guttes *et al.*, 1961; Sauer *et al.*, 1969; Rusch, 1970; Laane *et al.*, 1976; Goodman, 1980). In addition, it has been established that heat shocks at specific stages of this development result in subsequent developmental abnormalities (Sauer *et al.*, 1969). In the present study, these findings are extended by examining in greater detail the changes in the morphology and ultrastructure of the developing sporangia produced by heat shocks at specific stages of development.

To determine whether *Physarum* responds to heat shock with the changes in protein synthesis typical of *Drosophila* and, if so, whether this response is independent of developmental state, the changes in protein synthesis induced by heat shocks of sporulating plasmodia at different stages of development and of growing plasmodia are examined. A general suppression of translation in *Physarum* at an elevated temperature has been reported to occur (Bernstam, 1974; 1978). However it has yet to be established for *Physarum* whether this suppression of translation is

accompanied by the synthesis of specific hsp's. A heat shock induced repression of both normal transcription and translation, as has been established for *Drosophila*, could account for the developmental abnormalities reported to occur in *Physarum* (Sauer *et al.*, 1969). This possibility is investigated in this study by using heat shock as a probe of differences that occur in gene expression during sporulation.

SECTION 2. MATERIALS AND METHODS

2.1 Organism and culture media.

Physarum polycephalum strain CL-2, a derivative of the haploid, apogamic strain CL, was obtained from Dr. Adam Wilkins.

Liquid shake cultures of microplasmodia were grown in the standard, semidefined medium (Daniel and Baldwin, 1964). The sporulation medium (SM) used was that of Wilkins and Reynolds (1979).

2.2 Induction of sporulation.

The method used was essentially that of Wilkins and Reynolds (1979) with only minor modifications. 50ml cultures were grown to late log phase (approximately 72 hours) at 22°C from 3.0ml inocula in 1l flasks, with rotary shaking at 160 r.p.m. Microplasmodia were harvested by centrifugation at 500 × g for 1½ minutes and were then resuspended in an equal volume of SM. Aliquots of 0.5ml were placed on individual Whatman number 50 filter papers, each supported by a stainless steel grid, inside 50mm diameter petri plates. As soon as the complete set of plates was inoculated with microplasmodia, 3.3ml SM was introduced beneath each filter paper. The plates were then wrapped in aluminium foil to exclude all light and incubated at 22°C. The complete harvesting and plating out operation was carried out within 1 hour and the time of completion noted (0 hour of starvation).

Plasmodia were starved over SM for 72 hours in the dark and then illuminated at 22°C by placing them 30cm below 40 watt cool white fluorescent lights for 4 hours. At the end of the illumination period (0 hours postillumination), the plates were returned to the dark and all subsequent operations were conducted with the minimum of illumination.

2.3 Growing plasmodia.

For experiments involving growing plasmodia, 0.05ml of resuspended microplasmodia was plated out over 3.3ml of semidefined medium. Otherwise the harvesting and plating out operation was the same as that described in Section 2.2. Growing plasmodia were incubated at 22°C until such time as

each plasmodium covered most of the supporting filter paper.

2.4 Heat shock treatment.

Heat shocks were applied to growing plasmodia and to sporulating plasmodia at specified stages of development by aseptically transferring the plasmodia on the filter paper supports to fresh plates, preheated at the heat shock temperature, containing grids and 3.3ml of fresh SM. All heat shocks involved an incubation at 37°C unless otherwise stated. Control plasmodia were similarly transferred to fresh plates at 22°C.

2.5 Spore germination frequencies.

Mature spores of at least 5 days old were scraped from 1 or 2 plasmodia per treatment into 0.2ml of sterile PBS solution (Goodman, 1972). The spore suspension was mixed thoroughly with a sterile glass rod and then a further 2.0ml of PBS solution was added to it. The diluted spore suspension was vortexed and allowed to stand for approximately 3 hours. At the end of this time, the spore concentration was determined in an haemocytometer and dilutions were made to give final concentrations of $1 - 2 \times 10^3$ spores/ml (this allows for an assumed strain CL-2 spore germination of 0.5 - 1.0%; for spores from heat shocked plasmodia, a number of spore concentrations were used). 0.1ml of the final dilutions were plated out on 1/10 strength N+C (Daniel and Baldwin, 1964) pH 5.0 solidified with 1.5% agar, with 0.1ml of *E. coli* resuspended in 0.01M tris, pH 8.0/0.01M MgSO₄. The suspensions were spread evenly over the agar surface with a sterile glass spreader and the plates were incubated at 22°C for approximately 7 days, or until such time as plaques were clearly visible. From the number of plaques/plate and the dilutions made, the spore germination frequencies were calculated.

2.6 Light microscopy.

Developing sporangia were observed under a Leitz Ortholux microscope with oblique illumination, unless otherwise stated, and photographed with an attached Leitz Orthomat camera.

2.7 Transmission electron microscopy.

Developing sporangia were fixed for 3 hours at 4°C in a modified Karnovsky fixative (Karnovsky, 1965) containing 2% formaldehyde and 3% glutaraldehyde/0.1M phosphate buffer pH 7.2, by transferring plasmodia on the filter paper supports to 50mm Falcon plates containing 4.0ml of the fixative. Following this primary fixation, the plasmodia were washed quickly in 0.1M phosphate buffer pH 7.2 at 4°C, scraped off the filter paper supports into 10ml, disposable, glass vials, and then given two further washes with 4°C phosphate buffer of 30 minutes each. Where sporangia at later stages of development were to be subsequently fixed, early specimens were left in the final buffer wash, at 4°C, until such time as all specimens could be treated simultaneously. Specimens were then post-fixed in 1% osmium tetroxide/0.1M phosphate buffer pH 7.2 for 1 hour at 4°C, washed 3 × 10 minutes with deionized water, and then dehydrated in a graded ethanol series: 30 minutes in each of 25%, 50%, 75%, and 95%, and 2 × 30 minutes in 100% ethanol, all at room temperature. Following dehydration, the specimens were given two changes of propylene oxide of 15 minutes each and then left overnight in a mixture of 25% Durcupan ACM resin in propylene oxide on a stirrer with the vial tops removed. The following day the resin was replaced with fresh 100% resin and the specimens were left for a further 6 hours on a stirrer. At the end of this time, the specimens were embedded in fresh 100% resin in dried number 4 gelatin capsules and the capsules were then left in a 60°C oven for 72 hours to polymerize the resin.

Thick sections (0.5μ) were cut from polymerized blocks, floated from the sectioning knife onto water, and transferred to a drop of water on a glass slide. The slide was heated at approximately 65°C until all the water had evaporated and the sections were then stained for 1 minute at 65°C with 0.05% toluidine blue/H₂O. The sections were examined under a light microscope to identify areas of interest, to which the block was subsequently trimmed. Thin sections of silver appearance (0.06 - 0.09μ) were cut from the block and floated from the sectioning knife onto water, stretched by passing a copper grid dipped in chloroform

immediately above them, and then transferred to 300 mesh, unsupported, copper grids. All sectioning was carried out on a LKB Huxley Ultramicrotome using glass knives made on a LKB 7800 Knifemaker.

The thin sections were stained in a saturated solution of uranyl acetate in 50% ethanol/H₂O for 6 minutes, washed with 50% ethanol and then deionized water, counter-stained in lead citrate stain (Venable and Coggeshall, 1965), and then washed again with deionized water. The grids were treated either individually or in an apparatus designed to accommodate batches of grids (Forsdyke, 1979). Stained sections were examined and photographed in a Phillips EM 200 electron microscope.

2.8 Scanning electron microscopy.

Sporangia were fixed overnight in modified Karnovsky fixative (see Section 2.7), washed three times with 4°C 0.1M phosphate buffer pH 7.2, post-fixed in 1% osmium tetroxide for 4 hours at 4°C, and again washed three times with 4°C phosphate buffer. Dehydration was carried out in the same graded ethanol series as used for transmission electron microscopy (Section 2.7). Following dehydration, the sporangia were critical point dried from liquid CO₂ in a Polaron E3000 Series II critical point drier, glued to aluminium stubs with conductive silver paint, and then sputter coated with gold to a thickness of 0.01 - 0.02 μ in a Polaron E5100 sputter coating unit. The specimens were examined in a Cwiskscan 100 field emission scanning electron microscope.

2.9 Radioactive labeling of proteins.

For labeling experiments, plasmodia were transferred to fresh plates containing 3.3ml SM equilibrated at 22°C for controls and at 32°C or 37°C for heat shock treatments, and supplemented with either a mixture of (¹⁴C) amino acids (>50mCi/milliatom of carbon, Amersham CFB.25) or (³⁵S) methionine (>58mCi/mmol, Amersham SJ.123). Control plasmodia were labeled at 22°C for 1 hour and heat shocked plasmodia were labeled at either 32°C or 37°C for 1 hour, a group of 3 plasmodia being used for each treatment. In some experiments

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labeling began immediately upon transfer to the new temperature, while in other experiments a pretreatment step involving the incubation of plasmodia for 30 minutes at the new temperature before the addition of label was included.

Growing plasmodia were pretreated over SM at 22°C and at 37°C for 30 minutes and then labeled over SM for 1 hour.

2.10 Protein extraction.

Immediately after the end of the labeling period, the 3 plasmodia of each treatment were rinsed in ice-cold SM, scraped from the filter paper supports into a 25ml flask containing 6ml of 0.1M tris buffer pH 7.5 containing 0.05% Cleland's reagent, and then frozen in liquid air. After thawing, the plasmodial suspensions were sonicated in a MSE 100 watt Ultrasonic Disintegrator and then placed on ice for 30 minutes. To the resulting crude plasmodial extract was added twice its volume of glacial acetic acid containing 0.75% 2-aminoethanethiol to precipitate polysaccharides. The extracts were kept on ice for 30 minutes and were then centrifuged at 27,000 × g for 15 minutes (Sorvall Superspeed RC 2-B), the pellets were discarded and the supernatants, containing the acetic acid soluble proteins, were retained. 10ml of 20% trichloroacetic acid (TCA) was added to 10ml aliquots of each supernatant fraction to precipitate the proteins and the extracts were placed on ice for 1 hour. At the end of this time, the extracts were centrifuged at 27,000 × g for 15 minutes. The resulting supernatants were discarded and the pellets were washed gently with 5% TCA followed by acetone and were then air dried. Finally, the pellets were dissolved in 0.2 - 0.5ml of sodium dodecyl sulphate (SDS) sample buffer (Laemmli, 1970). Duplicate 5 or 10µl aliquots of the protein/SDS samples were added to 10ml of scintillation fluid (66.7% v/v toluene, 33.3% v/v Triton X-100, 0.4% w/v 2,5-diphenyloxazole, and 0.01% w/v 1,4-bis-2-(5-phenyloxazolyl)benzene) and these were counted in a Beckman LS 7000 liquid scintillation counter. 1ml aliquots of the acetic acid soluble proteins were precipitated with 20% TCA and centrifuged as above and the resulting pellets were dissolved in 1ml 1M NaOH for subsequent protein assays (Lowry *et al.*, 1951), using bovine serum albumin as

standards. Duplicate 10 μ l aliquots of the protein/1M NaOH samples were counted as above and the number of counts incorporated per μ g of plasmodial protein calculated. Protein samples in both sample buffer and 1M NaOH were stored at -70°C when not examined immediately.

2.11 SDS-polyacrylamide disc. gel electrophoresis.

SDS slab gels 1.5mm thick and 120mm long were set up using a 10% resolving gel and a 4.5% stacking gel (Maizel, 1971; Studier, 1973). Protein samples dissolved in sample buffer were boiled for 2 minutes and then applied to the gel, with an equal number of counts being applied to each well. Electrophoresis was carried out at 12 amps/gel until the bromophenol blue dye had entered the resolving gel, and then at 20 amps/gel until the dye had reached the bottom of the gel. Gels were fixed and stained in 0.04% Coomassie brilliant blue R in 25% isopropanol, 10% acetic acid/H₂O overnight at 37°C, and then destained in four changes of 10% acetic acid/H₂O of approximately 1 hour each.

2.12 Fluorography.

Immediately after destaining, gels were processed for fluorography (Bonner and Laskey, 1974; Laskey and Mills, 1975). The gels were then supported on Whatman 3MM filter paper and dried under vacuum over a boiling water bath for approximately 1 hour. Fluorographs were prepared by apposing dried gels to Kodak X-Omat S film (XS-5) within Cronex "conventional cassettes" at -70°C. To enable a quantitative interpretation of the subsequent film image to be made, some films were preflashed to an optical density of between 0.1 and 0.2 above non-preflashed film (Laskey and Mills, 1975; Laskey, 1980) using a Sunpak Auto Zoom 3600 flash unit (power ratio set at 1/48) with on Deep Orange, Kodak Wratten number 22 filter, and three Whatman number 1 filter paper filters. The distance from the flash unit to the film plane was approximately 100cm.

Exposed film was developed in Kodak D19 developer for 3½ minutes, placed in Kodak stopbath for 30 seconds, and fixed in Kodafix for 8 minutes.

2.13 Molecular weight determination of proteins.

The following proteins, with their respective molecular weights in parentheses, were used as molecular weight markers: bovine serum albumin (67,000), immunoglobulin G heavy chain (55,000), ovalbumin (43,000), and immunoglobulin G light chain (25,000). The molecular weight markers were co-electrophoresed in a parallel track with the samples on each gel. Following the staining and destaining procedures for Coomassie brilliant blue, the distance migrated by each of the marker proteins was measured, as was the distance migrated by the buffer front of each gel. A R_f value for each of the marker proteins was thus calculated (Majzel, 1971) and these were used, together with the molecular weight values, in a least squares fit analysis. The R_f values of unknown *Physarum* proteins were determined from the respective fluorographs and used in the equation derived from the least squares fit to calculate the approximate molecular weights.

2.14 Electron microscopy of polyphenol oxidase.

The methods used for the electron microscopic localization of the enzyme, polyphenol oxidase, were based on those of Czaninski and Catesson (1974), and Eppig, (1974).

Developing sporangia were fixed for 2 hours at 4°C in 2.5% glutaraldehyde/0.1M phosphate buffer pH 7.4, by transferring plasmodia on the filter paper supports to 50mm Falcon plates containing 5.0ml of the fixative. Following fixation, the plasmodia were washed quickly in 0.1M phosphate buffer pH 7.4 at 4°C, scraped off the filter paper supports into 10ml glass vials, and then given two further 4°C phosphate buffer washes of 30 minutes each. 1ml of 0.1M phosphate buffer pH 7.4 was then added to each vial and the vials were left at 4°C for 90 minutes. At the end of this time, the specimens were given two washes of 30 minutes each with 0.1M cacodylate buffer pH 7.4 and then left overnight, at 4°C, in 1ml of 2% (w/v) 1,3,4-dihydroxyphenylalanine (L-DOPA)/0.1M cacodylate buffer pH 7.4, L-DOPA being a substrate for polyphenol oxidase. The following day, the specimens were incubated in fresh L-DOPA/cacodylate buffer for a further 4 hours at 22°C and were then given two 10

minute washes with 0.1M phosphate buffer pH 7.4.

Two series of controls were incorporated for plasmodia at each time point/treatment. In one series, specimens were incubated for 90 minutes at 4°C in 1ml of 0.2M diethyldithiocarbamate (DDC, an inhibitor of polyphenol oxidase)/0.1M phosphate buffer pH 7.4, following the second post-fixation phosphate buffer wash. In the second series, specimens were incubated in cacodylate buffer without the addition of the L-DOPA substrate. Otherwise the treatment of controls was identical to that of experimental specimens.

Secondary fixation in 1% osmium tetroxide/0.1M phosphate buffer pH 7.2 was carried out at 4°C for 2 hours and this was followed by two washes in 0.1M phosphate buffer pH 7.2 of 10 minutes each. Subsequently, all specimens were dehydrated, embedded in resin, and sectioned in the manner described in Section 2.7. Unstained sections were examined and photographed in a Phillips EM 200 electron microscope.

SECTION 3. RESULTS

3.1 Effects of heat shock on development.

3.1.1 Introduction.

When *Physarum* microplasmodia are plated out over SM, fusion into one plasmodium occurs. Starvation in the dark over the following 72 hours, during which time the plasmodium assumes a stranded appearance and undergoes a number of mitoses, provides the plasmodium with the potential to sporulate. A subsequent period of 4 hours illumination is necessary before the plasmodium is actually competent to sporulate. Soon after the end of the illumination period, the morphogenetic changes associated with sporulation begin and fully mature sporangia are formed by approximately 19 hours after the end of the illumination period (postillumination).

The complex sequence of developmental changes associated with sporulation must require the expression of a sequence of genes within a developmental program. Heat shock, which has been shown to disrupt transcription and hence the developmental program of *Drosophila* (see Section 1), should provide a means of probing the actual changes in gene expression that occur during the sporulation of *Physarum*. In the study presented here, the developmental changes that occur during sporulation are investigated, as are the developmental changes that occur subsequent to heat shocks at specific stages of development. More specifically, the appearance in the developmental sequence of the enzyme polyphenol oxidase, an enzyme associated with melanin deposition in the spore wall, and the effects of heat shock on its appearance in the developmental sequence are also investigated.

3.1.2 Normal development.

The morphological changes that accompany the normal development of sporangia in *Physarum* following periods of 72 hours starvation and 4 hours illumination are shown in Figures 2 - 9. At 0 hours postillumination (Fig. 2), plasmodia have the typical stranded appearance of starved plasmodia. The first change in appearance of the plasmodia has occurred by 5 hours postillumination (Fig. 3) when

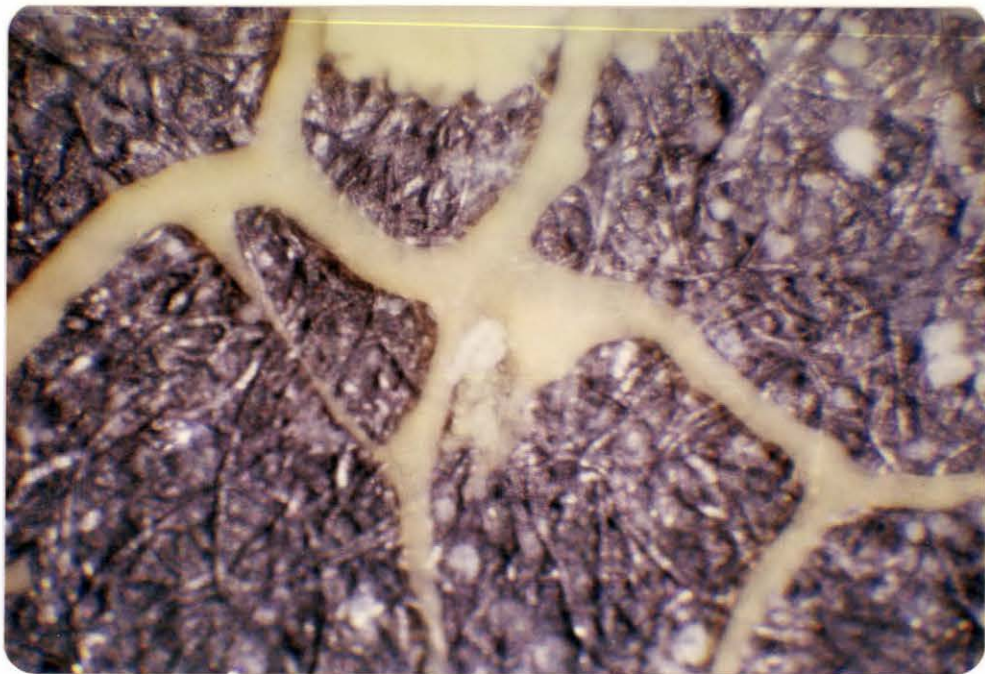


Figure 2. Normal development, 0 hours postillumination; 35 \times .

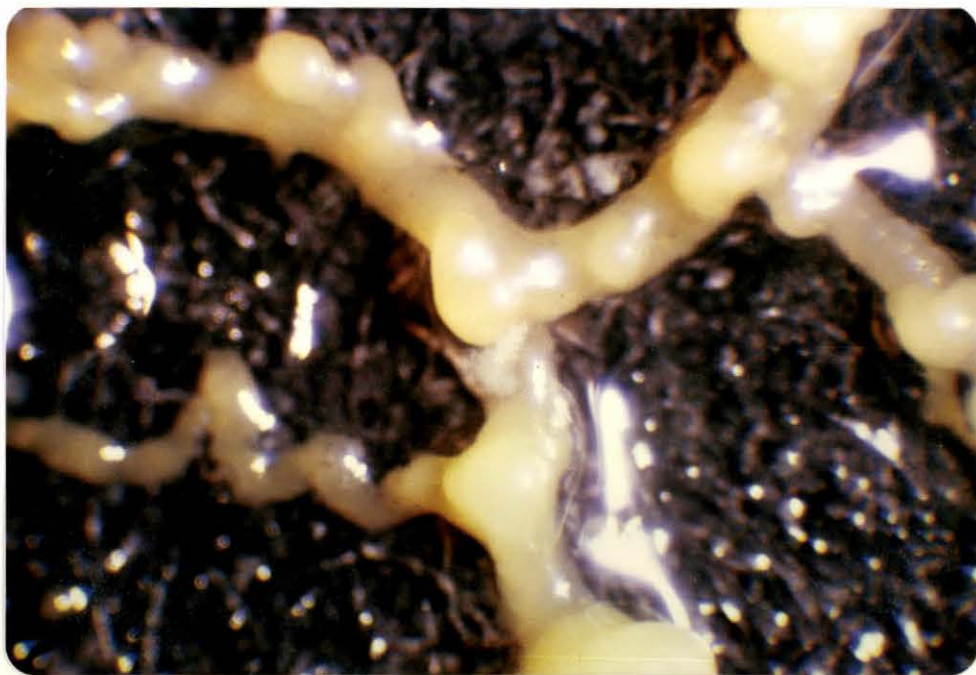


Figure 3. Normal development, 5 hours postillumination; 35 \times .

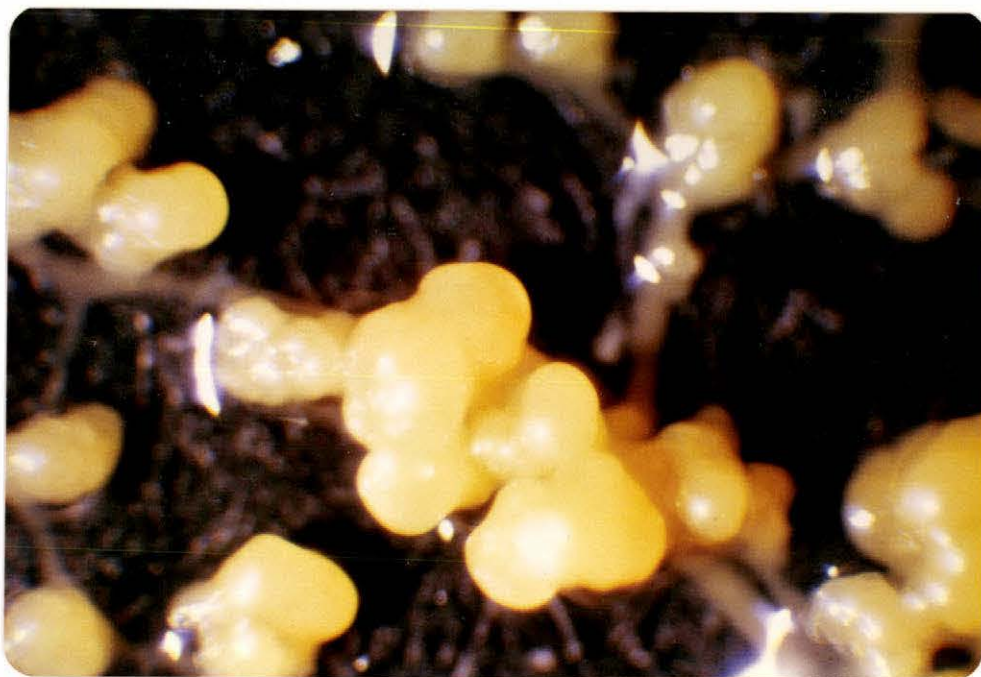


Figure 4. Normal development, 7 hours postillumination; 35 \times .

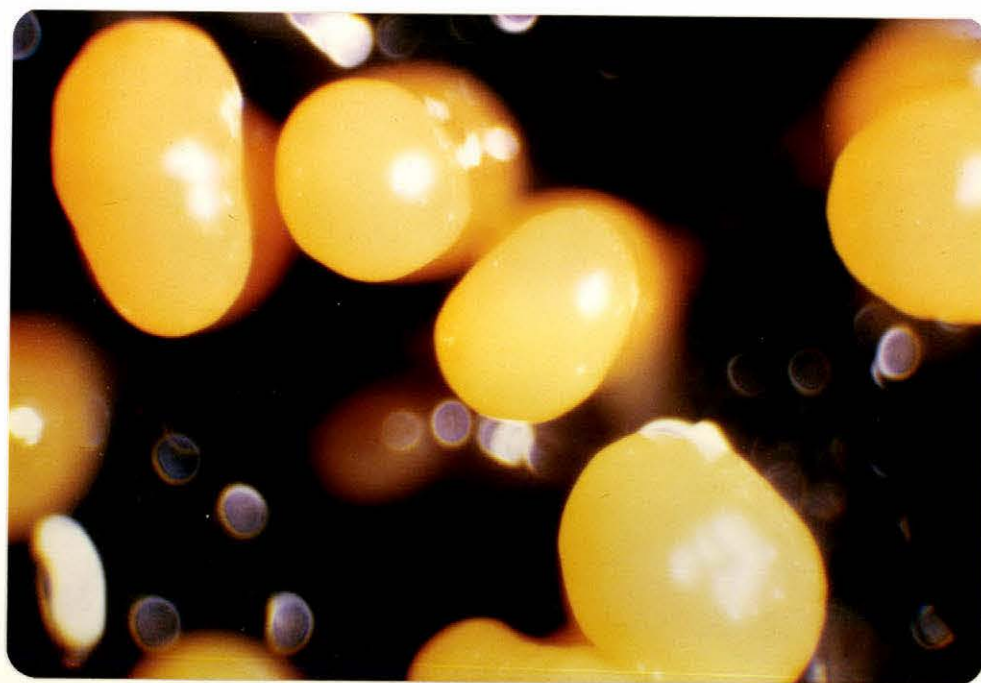


Figure 5. Normal development, 9 hours postillumination; 35 \times .

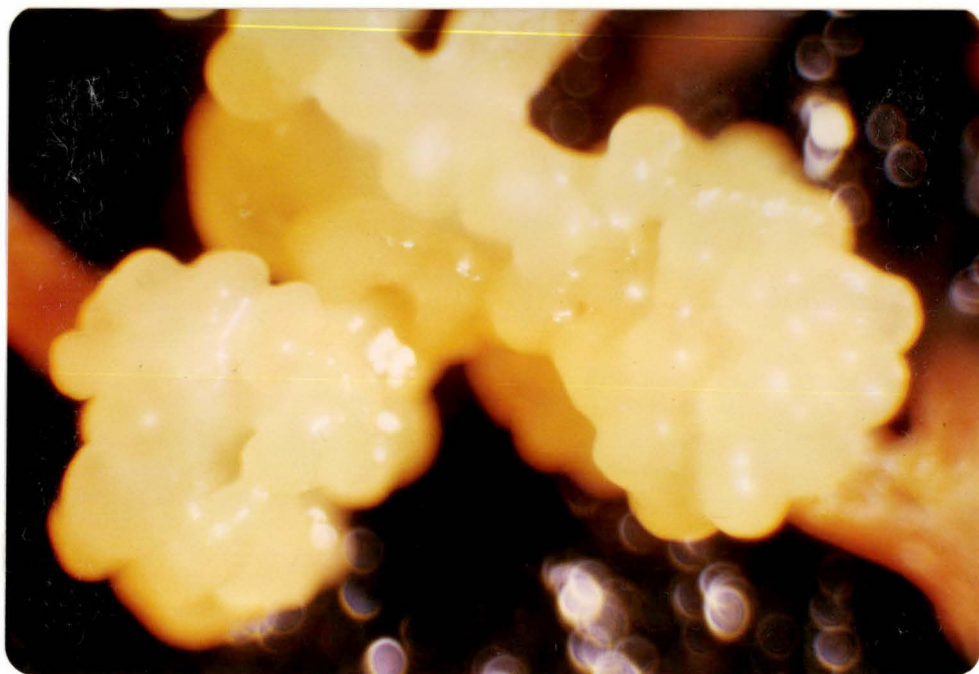


Figure 6. Normal development, 11 hours postillumination; 35x.

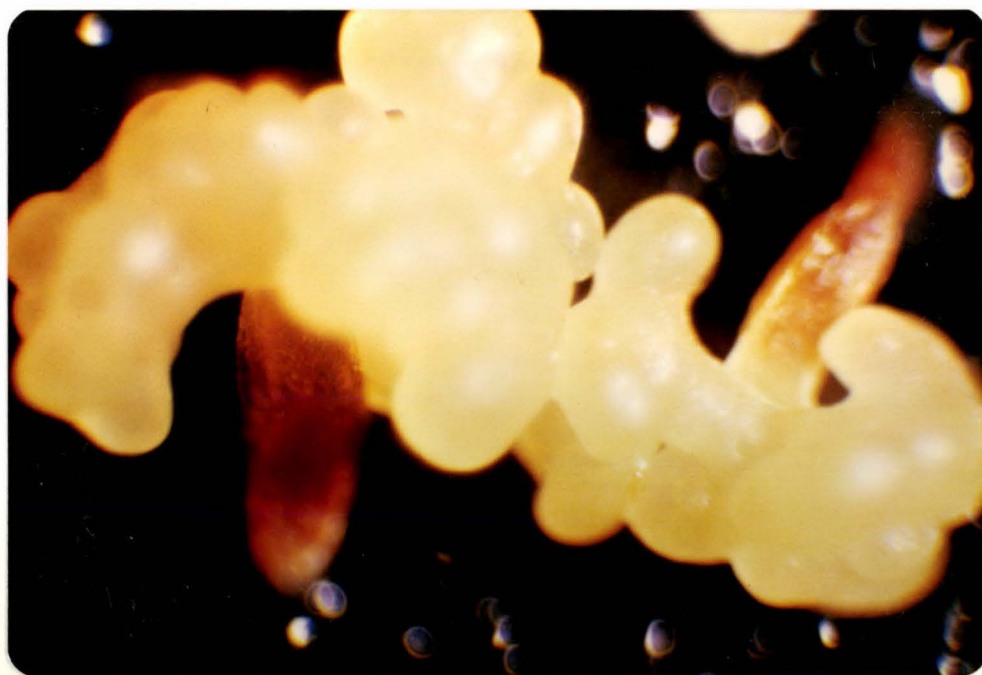


Figure 7. Normal development, 15 hours postillumination; 35x.

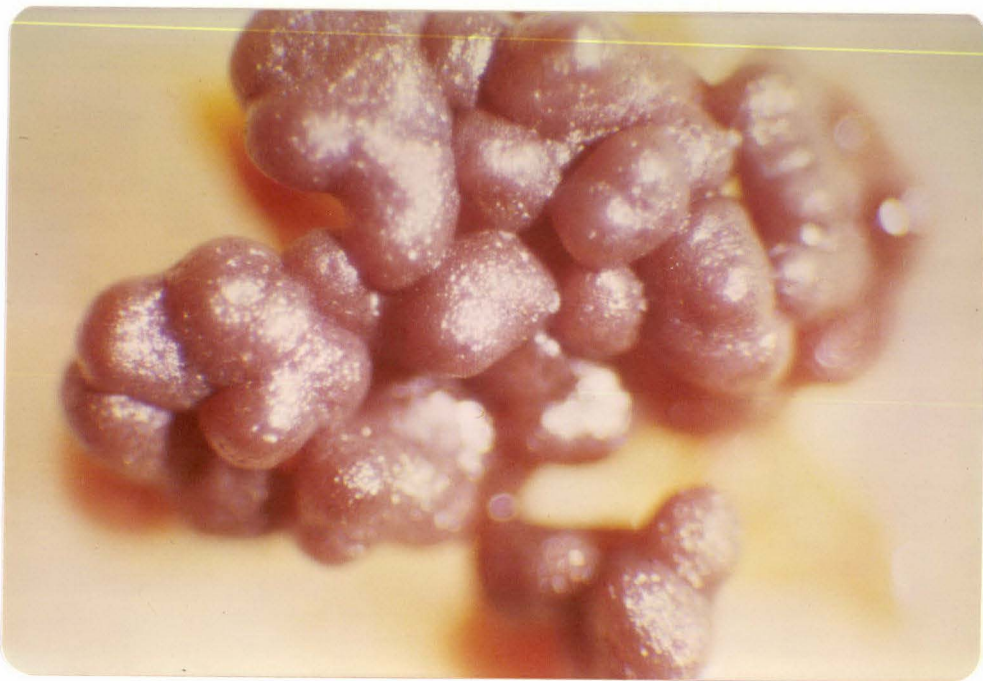


Figure 8. Normal development, 17 hours postillumination; 35 \times .



Figure 9. Normal development, 19 hours postillumination; 35 \times .

nodules have begun to develop. By 7 hours postillumination (Fig. 4), the nodules have begun to elevate and expand, and by 9 hours postillumination (Fig. 5), differentiation into separate stalks and heads has begun. This differentiation is complete by 11 hours postillumination (Fig. 6) when the sporangial heads have developed the lobed appearance of mature sporangia and the stalks have become hardened. Little change in morphology is evident at 15 hours postillumination (Fig. 7) but by 17 hours postillumination (Fig. 8), the sporangial heads have begun to darken. By 19 hours postillumination (Fig. 9), this darkening process, which is considered to be the melanization of the spore walls, is complete. No further morphological changes occur after 19 hours postillumination and the sporangia are morphologically fully mature at this time.

The timing of events in the developmental sequence from plasmodial strands at the end of the period of illumination to mature sporangia is highly consistent for separate experiments, under the experimental conditions described. Results from four separate experiments indicate that the process of melanization, which is the most clearcut change to occur during sporangial development, begins in each experiment between 15 and 17 hours postillumination.

Light microscopy of thick sections from fully mature sporangia (Fig. 10) indicates that the contents of the sporangial head have cleaved entirely into spores. In the scanning electron microscope (Figs. 11 and 12) the thin peridium surrounding the mass of spores and the characteristic spines on the spores are evident.

The ultrastructural changes that accompany normal sporangial development are shown in Figures 13 - 24. At 5 hours postillumination (Fig. 13), the still syncytial plasmodium contains typical interphase nuclei. At 11 hours postillumination (Fig. 14), cleavage of the contents of the sporangial head into separate units has begun with the formation of a cleavage furrow. The cleavage process continues through to 13 hours postillumination (Fig. 15) when some nuclear degeneration is also apparent. Cleavage is complete by 15 hours postillumination (Fig. 16), at which stage irregularly-shaped masses of cytoplasm (protospores)

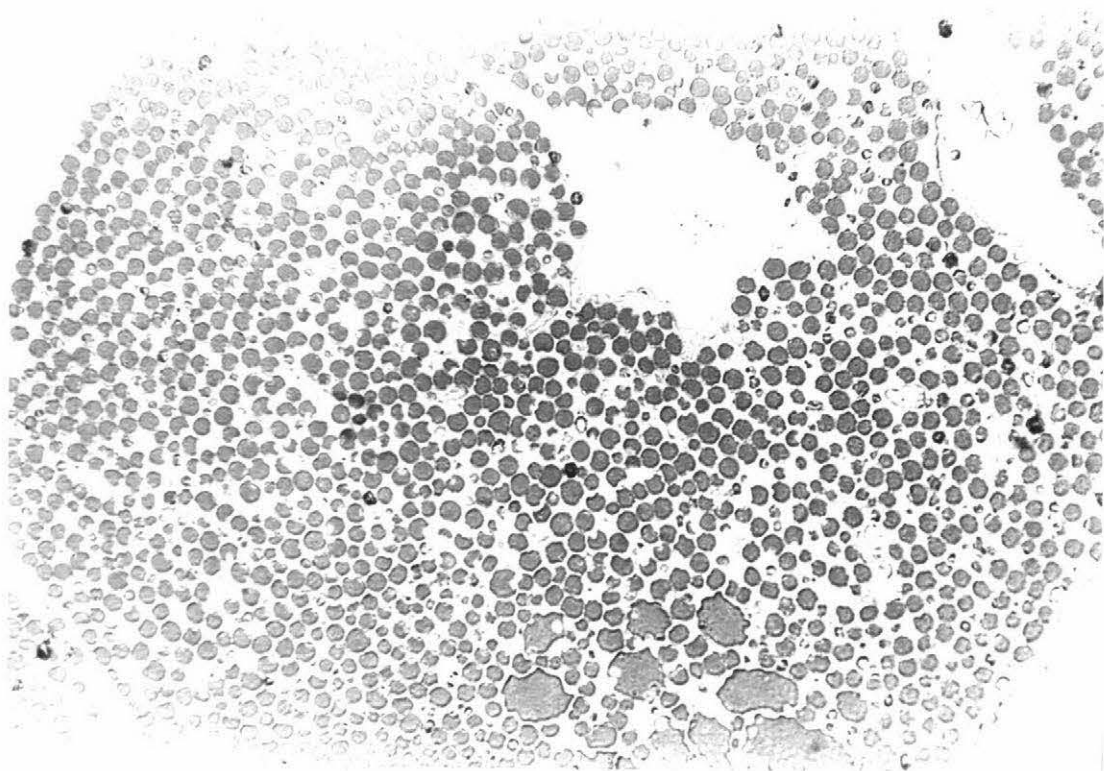


Figure 10. Normal development, fully mature sporangium; 275x.

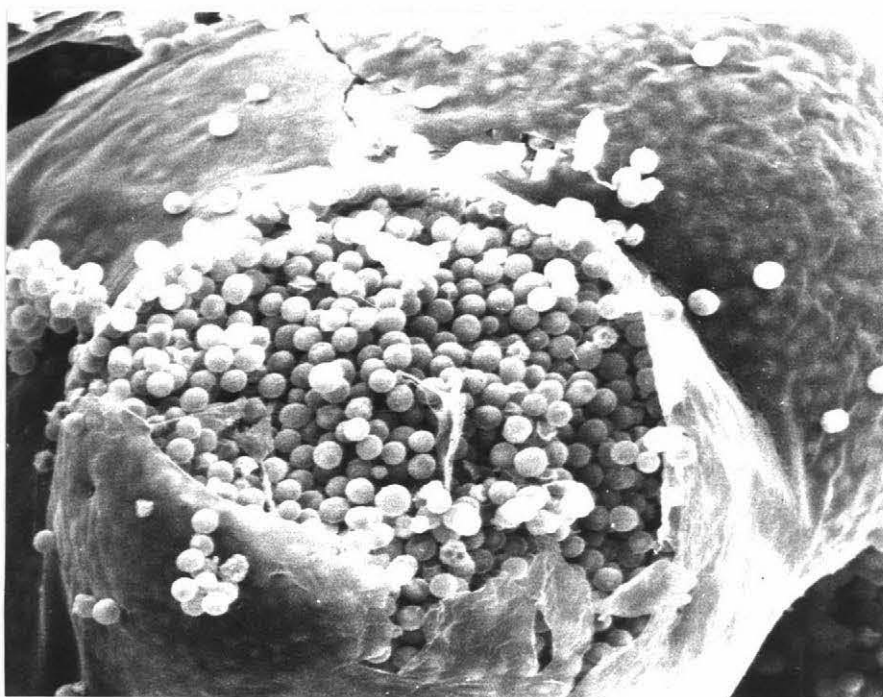


Figure 11. Normal development, mature sporangium; 400 \times .

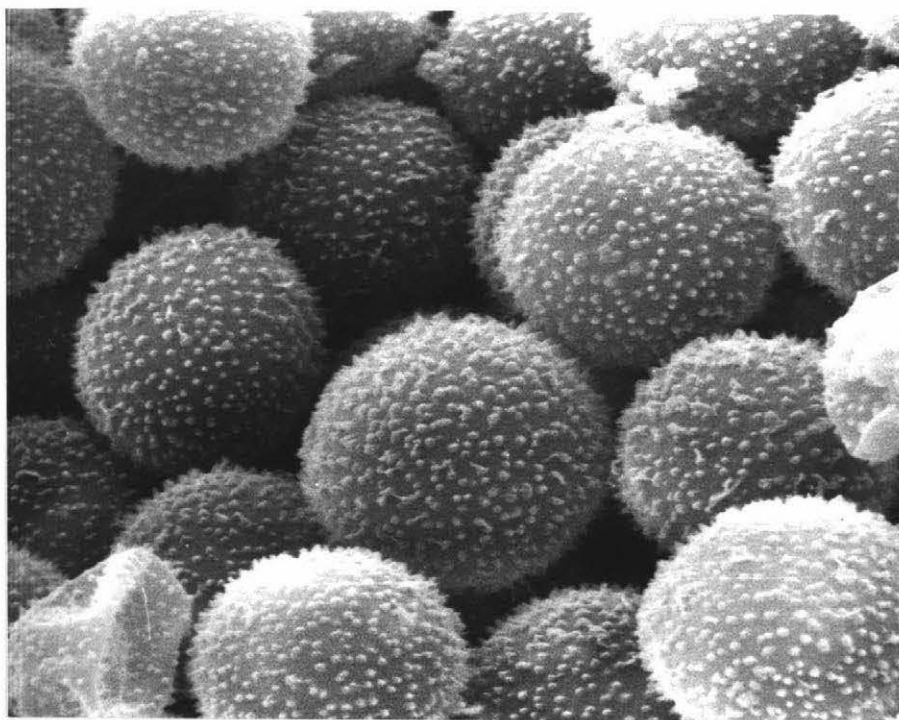


Figure 12. Normal development, mature spores; 4000 \times .

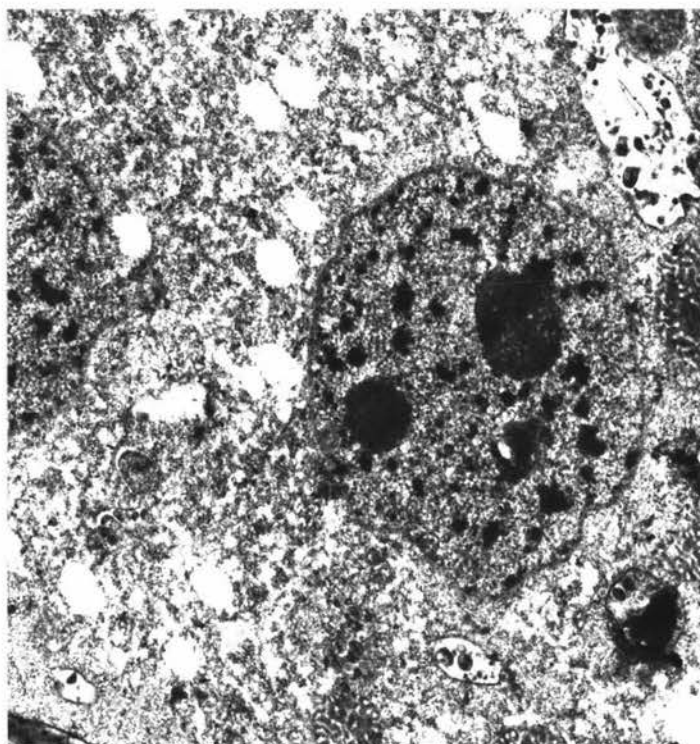


Figure 13. Normal development, 5 hours postillumination;
13,600 \times .

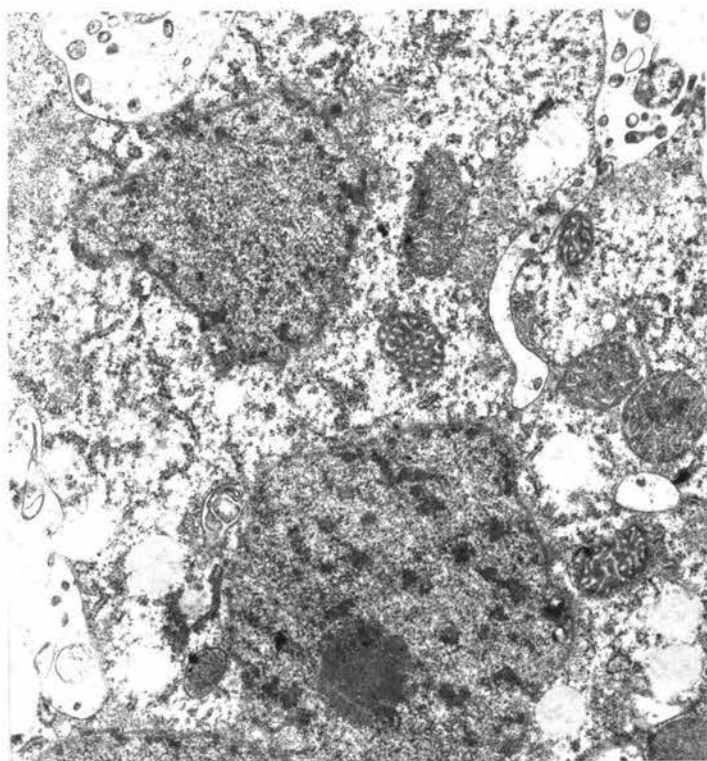


Figure 14. Normal development, 11 hours postillumination;
13,600 \times .

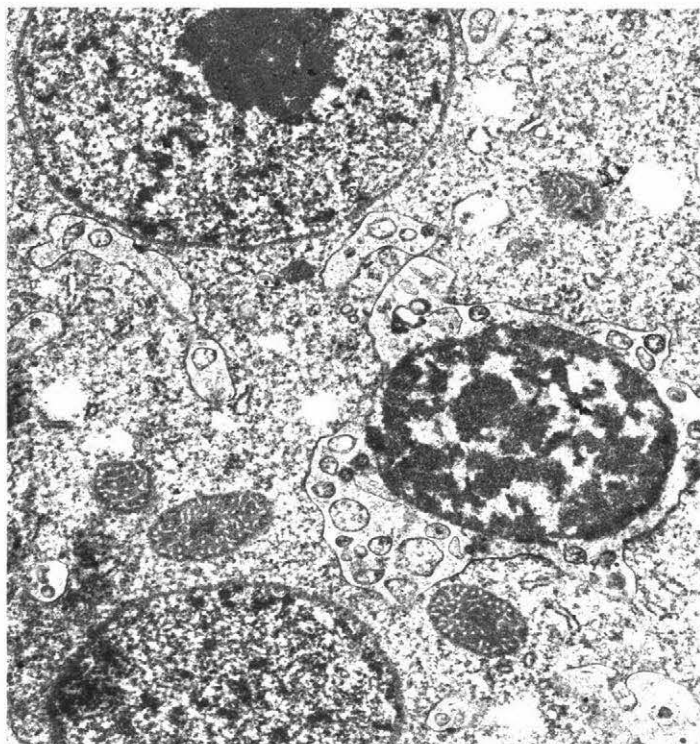


Figure 15. Normal development, 13 hours postillumination;
13,600 \times .

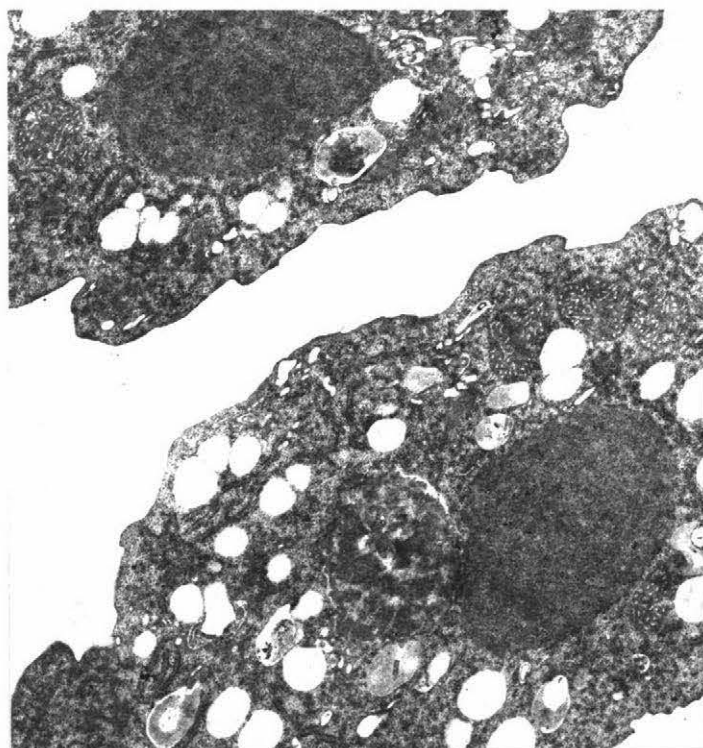


Figure 16. Normal development, 15 hours postillumination;
13,600 \times .

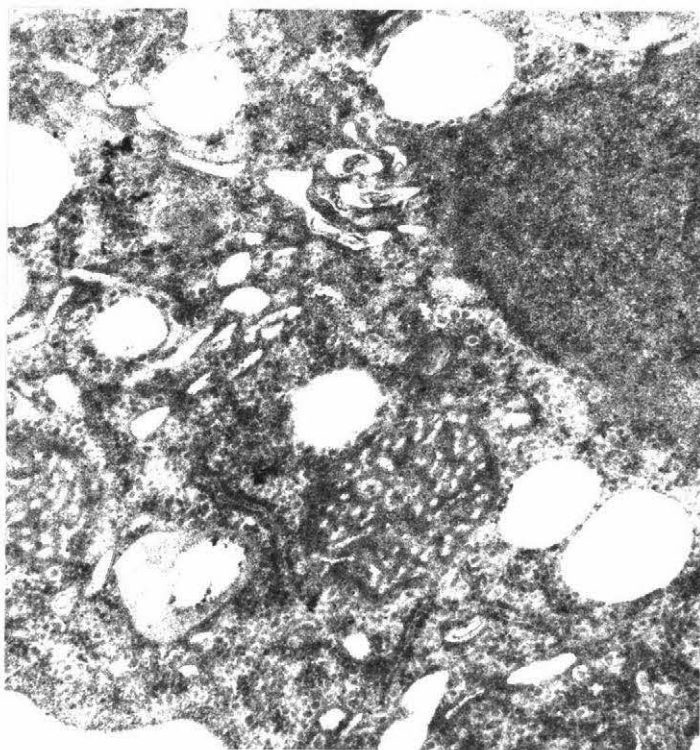


Figure 17. Normal development, 15 hours postillumination;
47,500 \times .

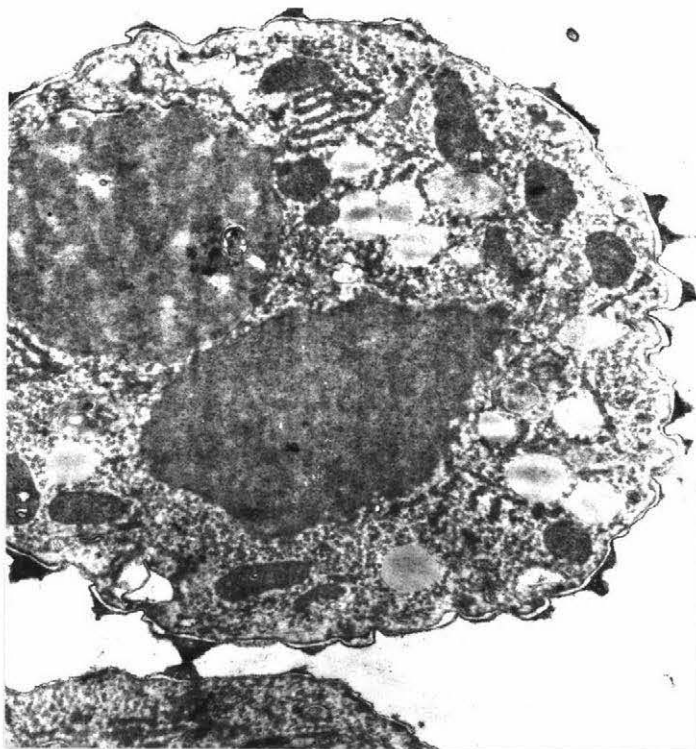


Figure 18. Normal development, 17 hours postillumination;
13,600 \times .

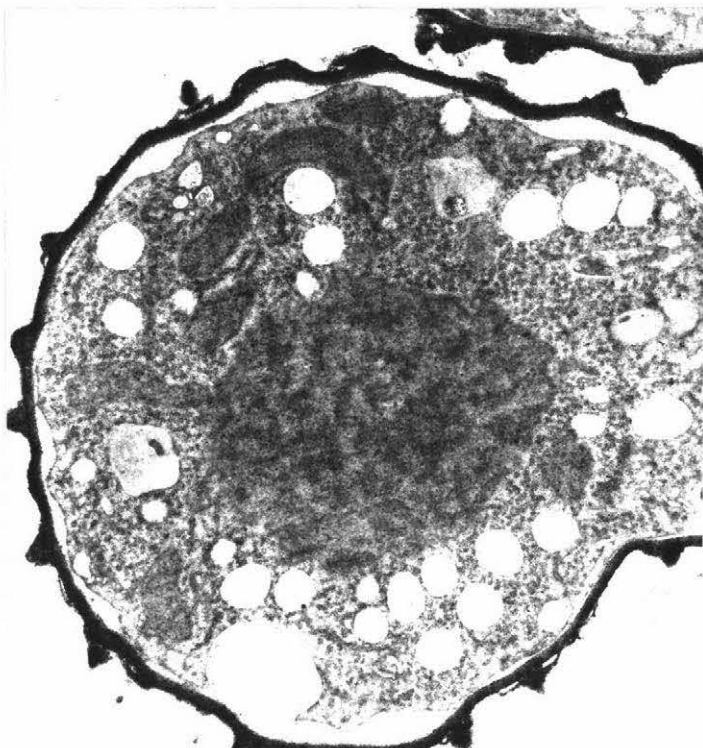


Figure 19. Normal development, 19 hours postillumination;
13,600 \times .

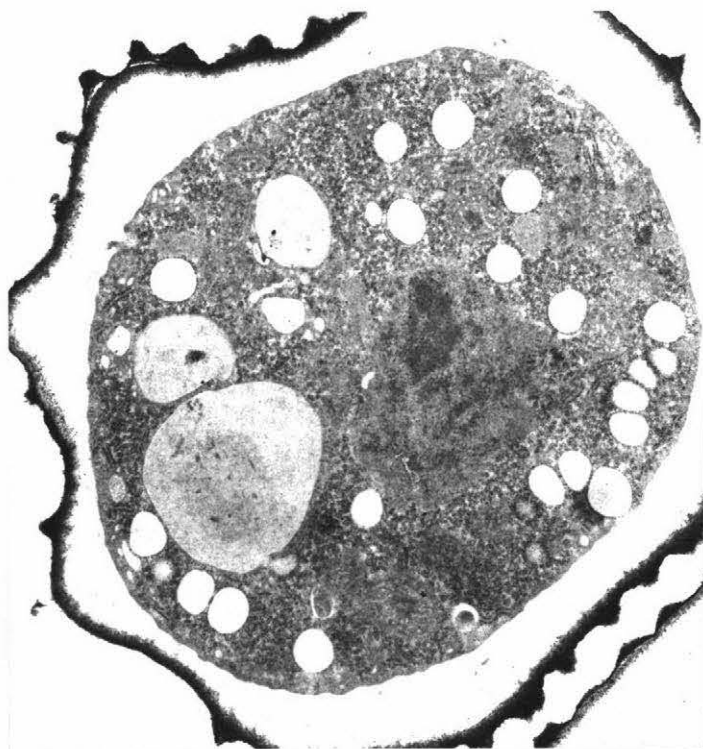


Figure 20. Normal development, 24 hours postillumination;
13,600 \times .

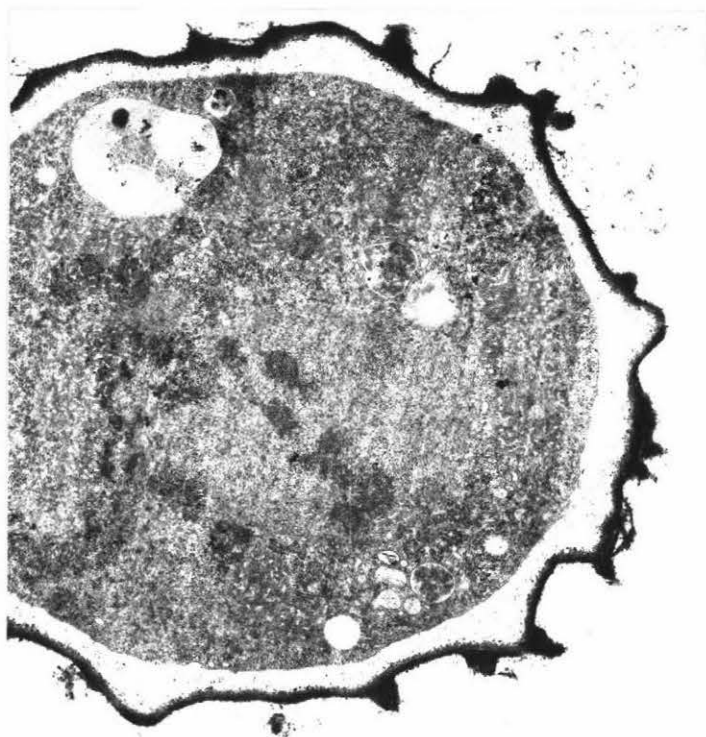


Figure 21. Normal development, 48 hours postillumination;
13,600 \times .

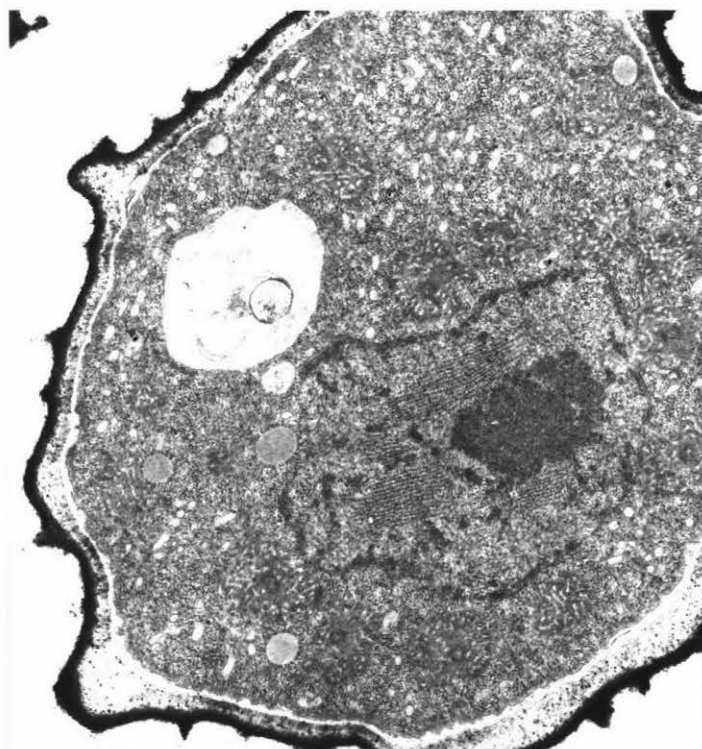


Figure 22. Normal development, 96 hours postillumination;
13,600 \times .

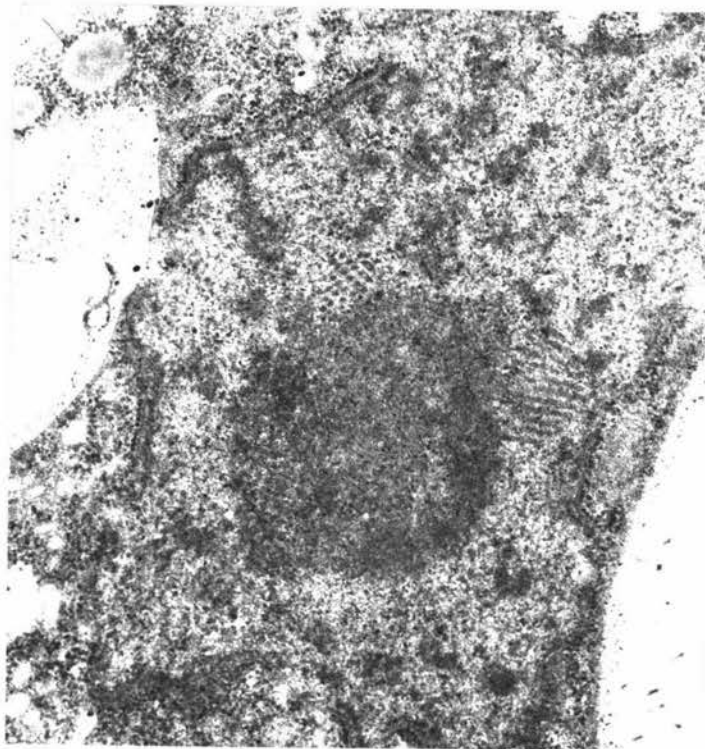


Figure 23. Normal development, 96 hours postillumination;
35,700 \times .

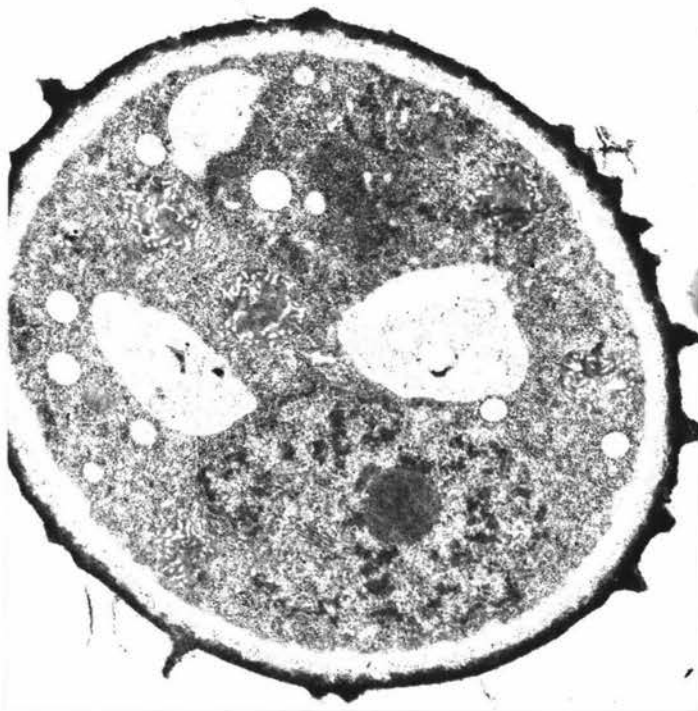


Figure 24. Normal development, 7 days postillumination;
13,600 \times .

have formed within the sporangial head. The cleavage process is not perfectly synchronous throughout each sporangial head - at 11 hours postillumination cleavage is not very extensive while at 13 hours postillumination protospores in various stages of development are apparent. The nuclei of protospores at 15 hours postillumination (Fig. 16) have lost the typical interphase appearance of earlier stages of development and this probably reflects the onset of mitosis at about this time. Rough endoplasmic reticulum (Goodman and Rusch, 1970) also first becomes apparent at this stage of development (Figs. 16 and 17). At 17 hours postillumination (Fig. 18), the protospores have begun to round up and to produce spore wall and spine material. The formation of the spore wall and spines is complete at 19 hours postillumination (Fig. 19). From these electron micrographs and others not presented, the mature spore wall appears to be composed of two distinct layers. The inner layer is relatively electron transparent and is fibrous in appearance whereas the outer layer is electron opaque and is granular in appearance. At 24 hours postillumination (Fig. 20), the spore nucleus has resumed the typical interphase appearance. At 48 hours postillumination (Fig. 21), the nucleus can be seen to be undergoing further changes which probably reflect the onset of meiosis. Between 24 and 48 hours postillumination there is a considerable decrease in the number of vacuoles within the spore but otherwise little change occurs within the mature spore. At both 96 hours and 7 days postillumination (Figs. 22, 23, and 24), little additional change occurs apart from the appearance of bundles of microtubules (Laane *et al.*, 1976) within otherwise typical, interphase nuclei.

3.1.3 Heat shocks during early development (0, 2, 4, and 6 hours postillumination).

When sporulating plasmodia are heat shocked early in development i.e. at 0, 2, 4, and 6 hours postillumination, mature sporangia are subsequently produced that are almost indistinguishable from those produced by non-heat shocked plasmodia (Figs. 25, 26, 28, and 30). However soon after heat shocks at 4 and 6 hours postillumination (Figs. 27 and

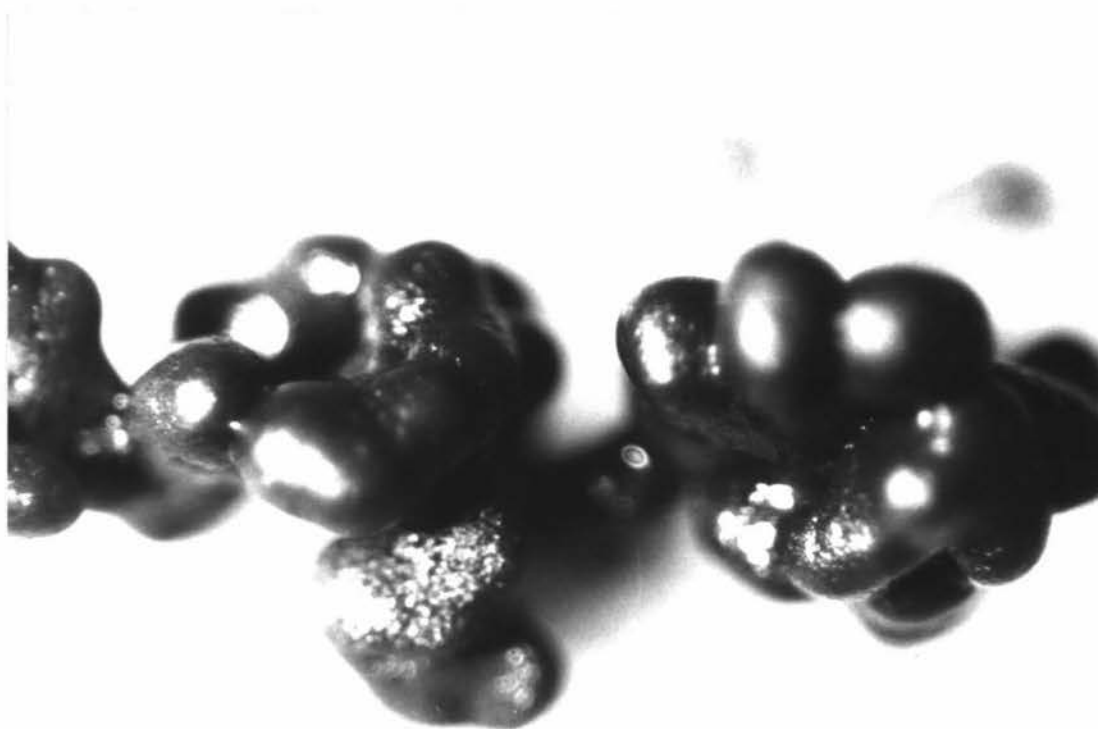


Figure 25. Development following 0 hours postillumination heat shock, 19 hours postillumination; 38 \times .

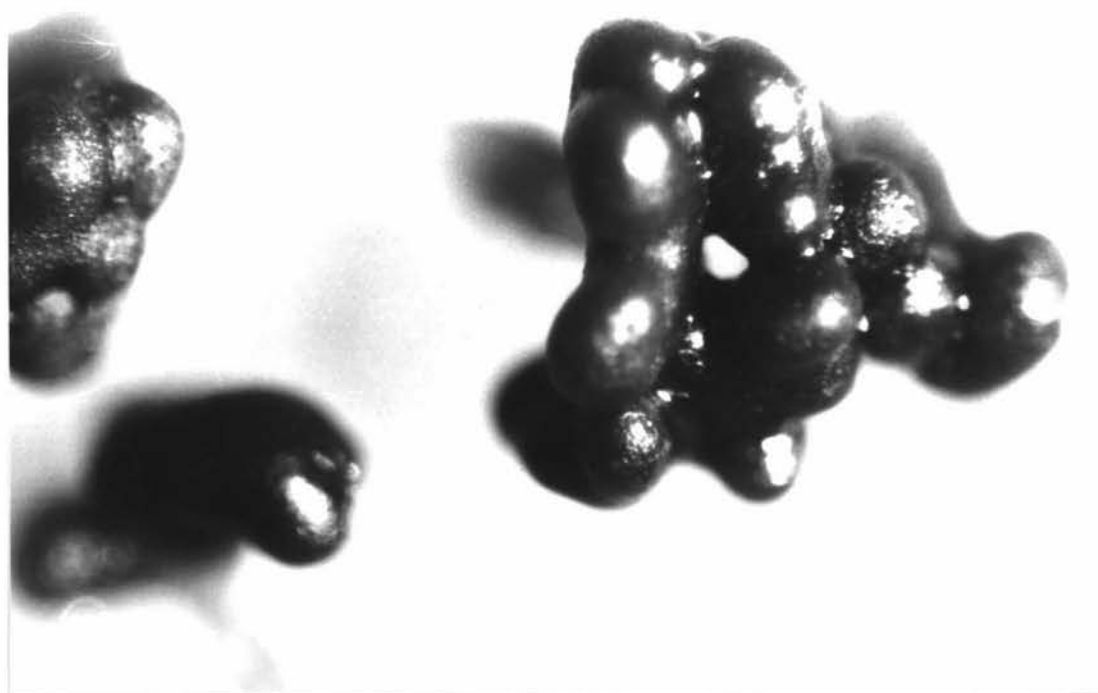


Figure 26. Development following 2 hours postillumination heat shock, 19 hours postillumination; 38 \times .

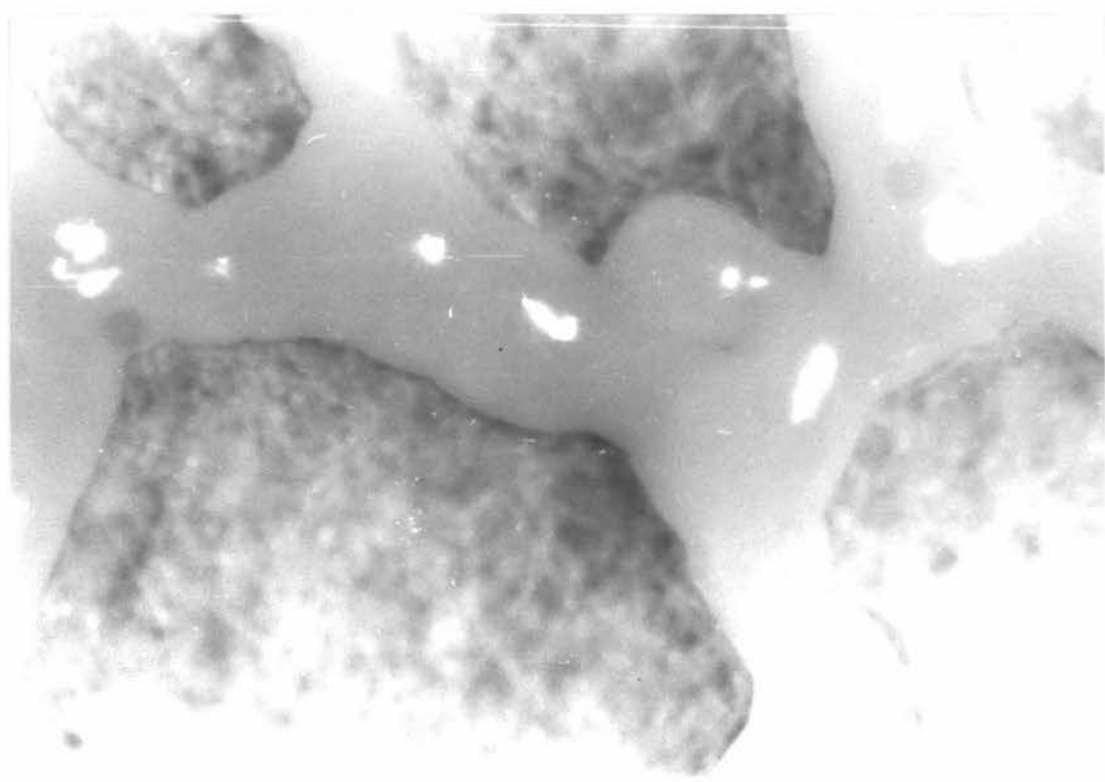


Figure 27. Development following 4 hours postillumination heat shock, 5 hours postillumination; 38x.



Figure 28. Development following 4 hours postillumination heat shock, 19 hours postillumination; 38x.

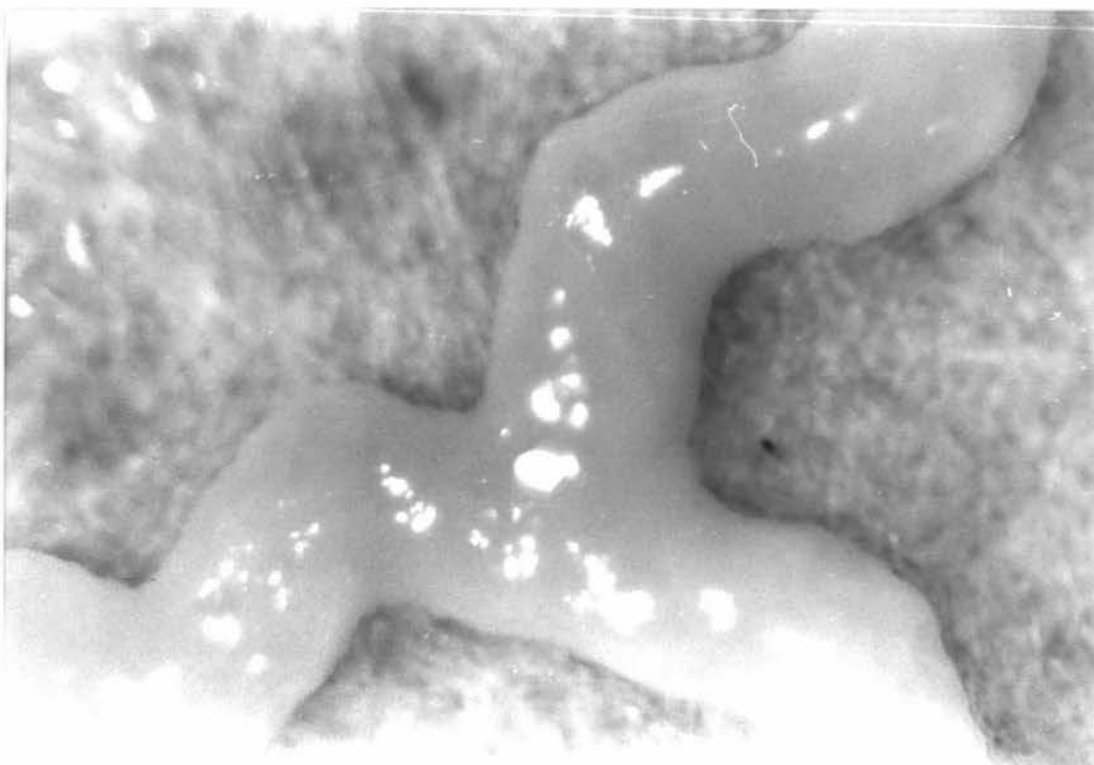


Figure 29. Development following 6 hours postillumination heat shock, 7 hours postillumination; 38 \times .

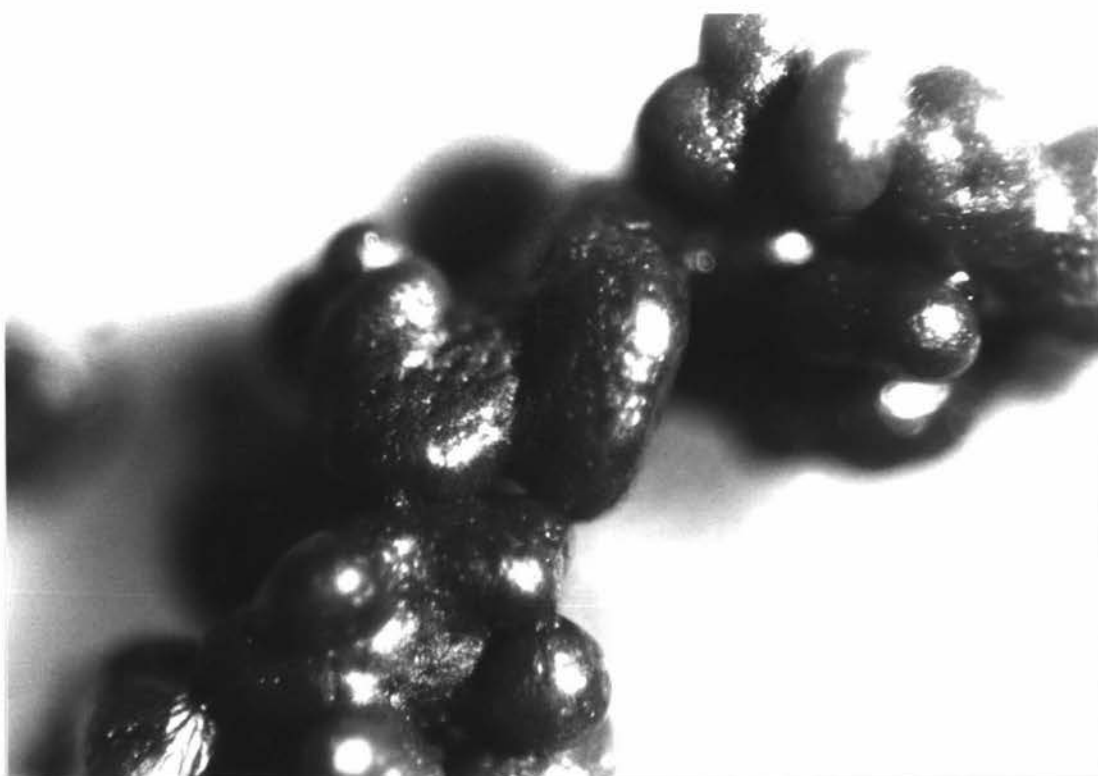


Figure 30. Development following 6 hours postillumination heat shock, 19 hours postillumination; 38 \times .

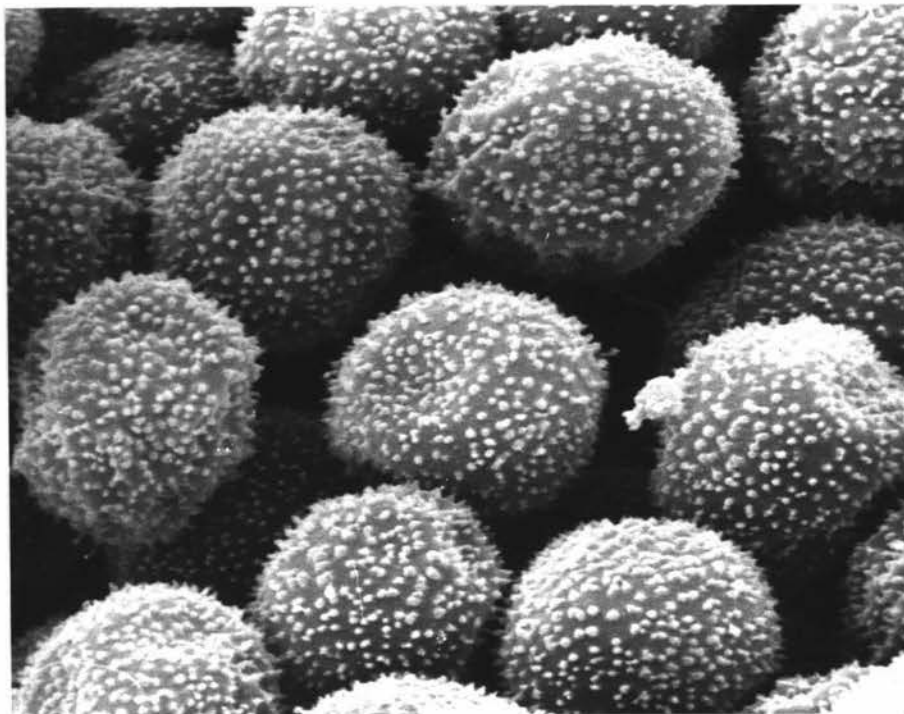


Figure 31. Development following 0 hours postillumination heat shock, mature spores; 4000 \times .

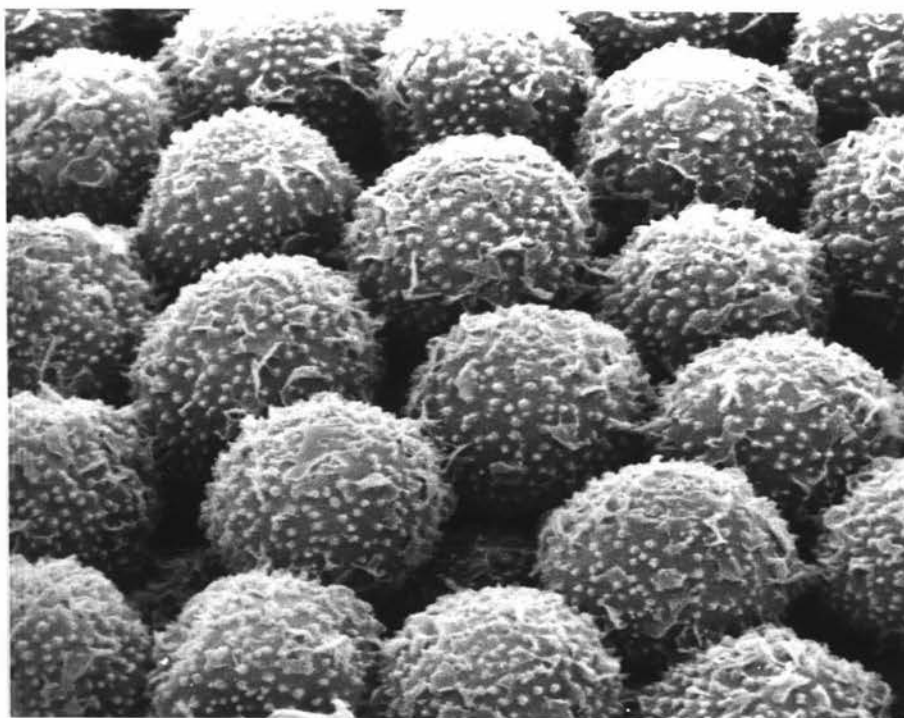


Figure 32. Development following 2 hours postillumination heat shock, mature spores; 4000 \times .

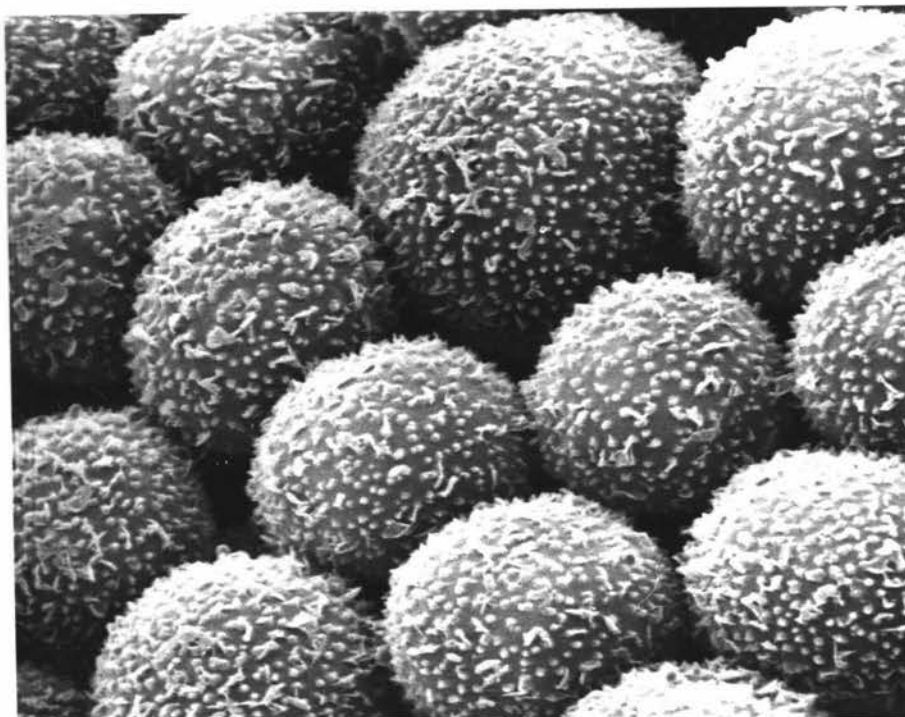


Figure 33. Development following 4 hours postillumination heat shock, mature spores; 4000 \times .

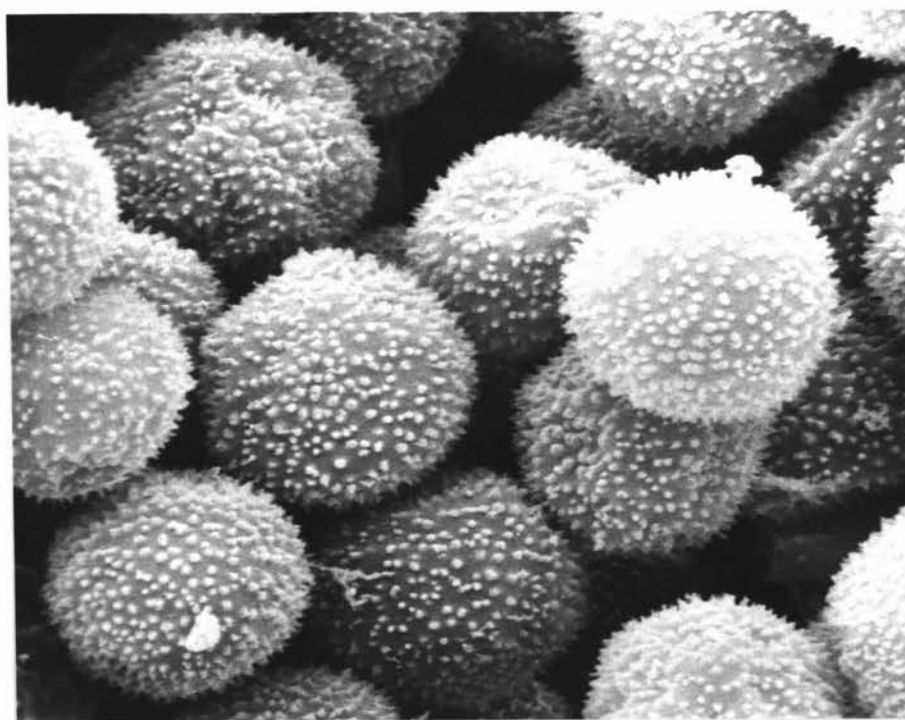


Figure 34. Development following 6 hours postillumination heat shock, mature spores; 4000 \times .

28), the plasmodial strands are somewhat thicker than those of non-heat shocked plasmodia (Figs. 3 and 4) and appear to lack the nodules typical of these stages of development. In addition, the strands of heat shocked plasmodia at both of these times are orange rather than the yellow of non-heat shocked plasmodia (not shown). The only apparent effects on subsequent development of the heat shocks early in development are smaller sporangia in the case of 6 hours postillumination heat shock and a developmental delay in all cases. This developmental delay is most clearly seen when the times at which melanization occurs are compared. Thus at 17 hours postillumination, when melanization is complete in non-heat shocked plasmodia, there is only partial melanization in plasmodia heat shocked at 0 hours postillumination and no melanization in plasmodia heat shocked at 2, 4, and 6 hours postillumination. In all cases, melanization is complete at 19 hours postillumination.

In the scanning electron microscope (Figs 30 - 33), the mature spores produced subsequent to heat shocks early in development are indistinguishable from those of non-heat shocked plasmodia (Fig. 12).

3.1.4 Heat shocks at 8 hours postillumination.

Half an hour after a heat shock begun at 8 hours postillumination, sporangia at an early stage of development appear to have collapsed (Fig. 35). At 19 hours postillumination (Fig. 36), no subsequent development has occurred and this developmental arrest continues until at least 48 hours postillumination. From 48 hours onwards, some plasmodia do recommence the sporulation process and proceed to produce sporangia containing mature spores which, in the scanning electron microscope (Figs. 37 and 38), are indistinguishable from those produced by non-heat shocked plasmodia (Figs. 11 and 12). However individual plasmodia recommence sporulation at different times rather than exhibiting the parallel development typical of control plasmodia. The recovery process itself involves the production of new plasmodial strands from which new sporangia develop rather than the continuation of the original sporulation process.

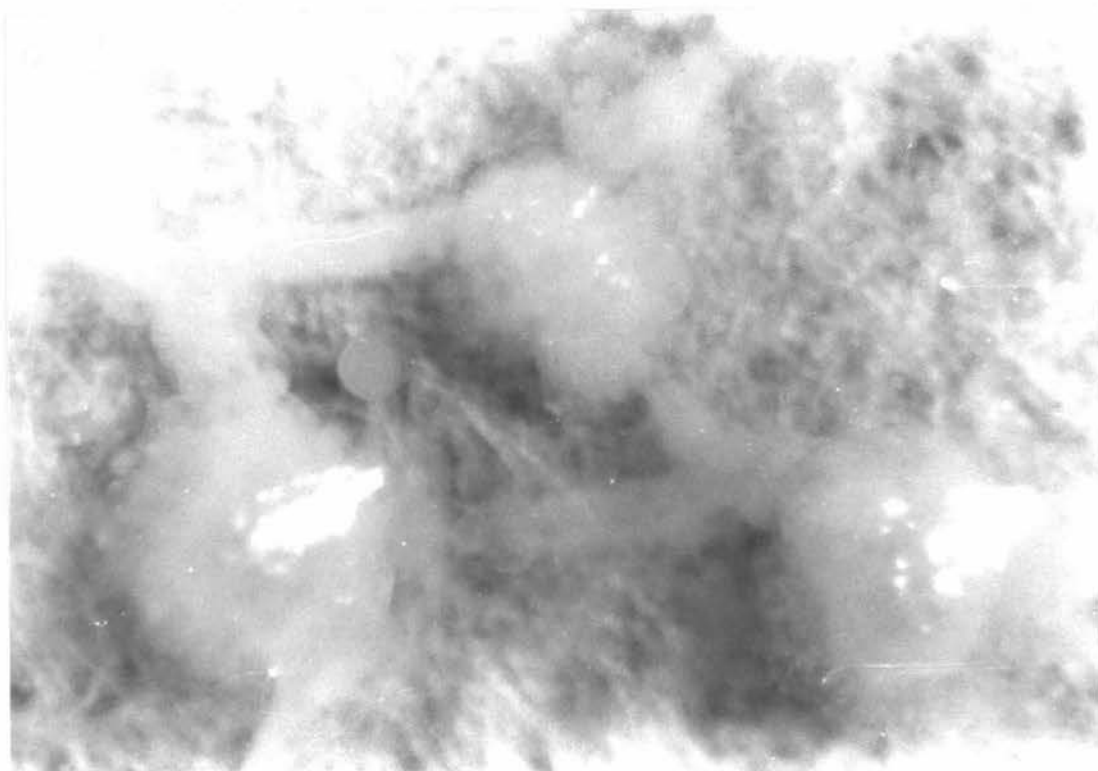


Figure 35. Development following 8 hours postillumination heat shock, 9 hours postillumination; 38 \times .

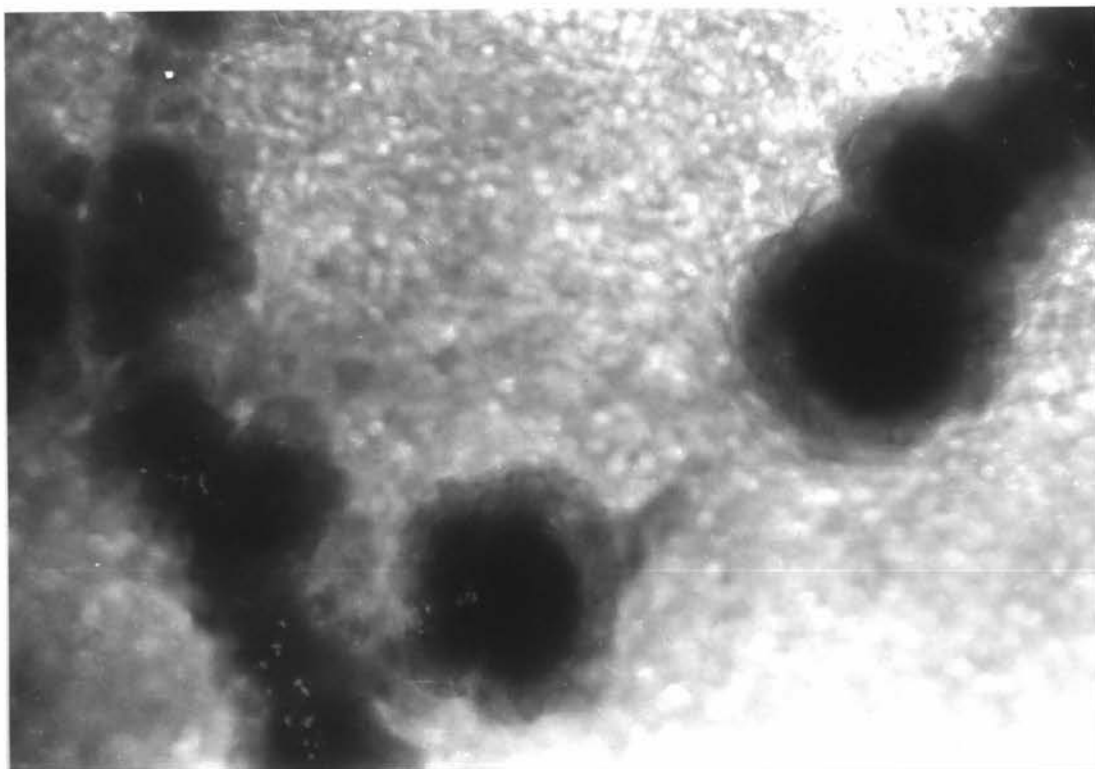


Figure 36. Development following 8 hours postillumination heat shock, 19 hours postillumination; transmitted illumination; 38 \times .

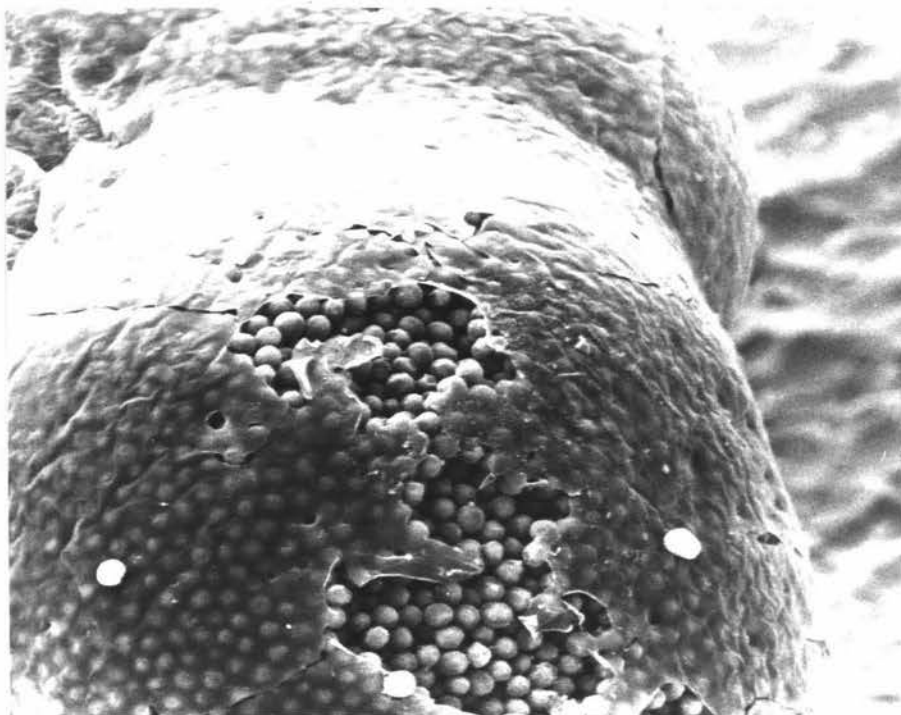


Figure 37. Development following 8 hours postillumination heat shock, 7 days postillumination; 400 \times .



Figure 38. Development following 8 hours postillumination heat shock, 7 days postillumination; 4000 \times .

The ultrastructural changes induced by a heat shock at 8 hours postillumination can be seen in Figures 39 - 46. Half an hour after the heat shock (Fig. 39), the nucleus contains more diffuse chromocentres and a crystal-like structure resembling bundles of microtubules but with larger tubular components, hence referred to as a macrotubular structure (Del Castillo *et al.*, 1978). These nuclear abnormalities are apparent at 11 hours postillumination (Fig. 40) at which time there also appears to be a general degeneration of cytoplasmic material and abnormal vacuoles and cristae within mitochondria (Figs. 40 and 41). At 17 hours postillumination (Fig. 42) and at 17 hours postillumination (Fig. 43), the general degeneration of cytoplasmic material continues and at the latter time, the nucleus also appears to be degenerating (Fig. 44). This degeneration continues to be apparent at both 24 and 48 hours postillumination. However at 96 hours postillumination (Fig. 45), there appears to be some recovery with the formation of areas of normal cytoplasm containing normal nuclei and mitochondria. At 7 days postillumination (Fig. 46), spores have been produced that, apart from the unusual, large vacuole within the cytoplasm, are very similar to normal spores.

In another experiment involving a heat shock at 8 hours postillumination, the initial effect on the developing sporangia is similar to that described above (Fig. 47) but at 13 hours postillumination (Fig. 48), some subsequent development has occurred. At 17 hours postillumination (Fig. 49), the developing sporangia have begun to melanize but are grossly abnormal. No further development occurs after 24 hours postillumination (Fig. 50) at which time the sporangia lack normal stalks and lobes. In addition, the plasmodial strands are still quite prominent, being thickened and pigmented compared with non-heat shocked plasmodia. In the scanning electron microscope (Fig. 51), spores from these sporangia also appear abnormal, lacking the characteristic spines and the uniform size typical of normal spores.

The typical response of plasmodia heat shocked at 8 hours postillumination is developmental arrest for at least 48 hours followed by non-synchronous development. However it

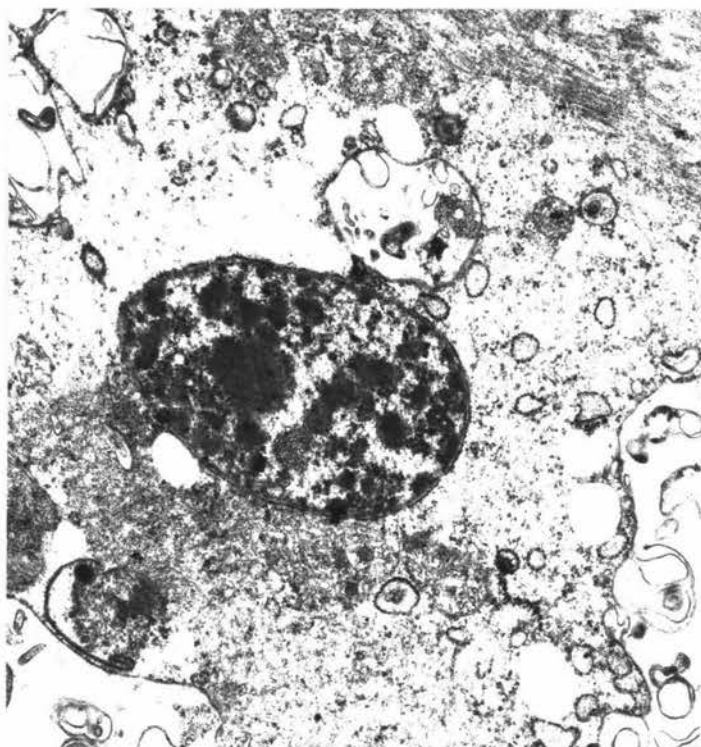


Figure 39. Development following 8 hours postillumination heat shock, 9 hours postillumination; 13,600 \times .

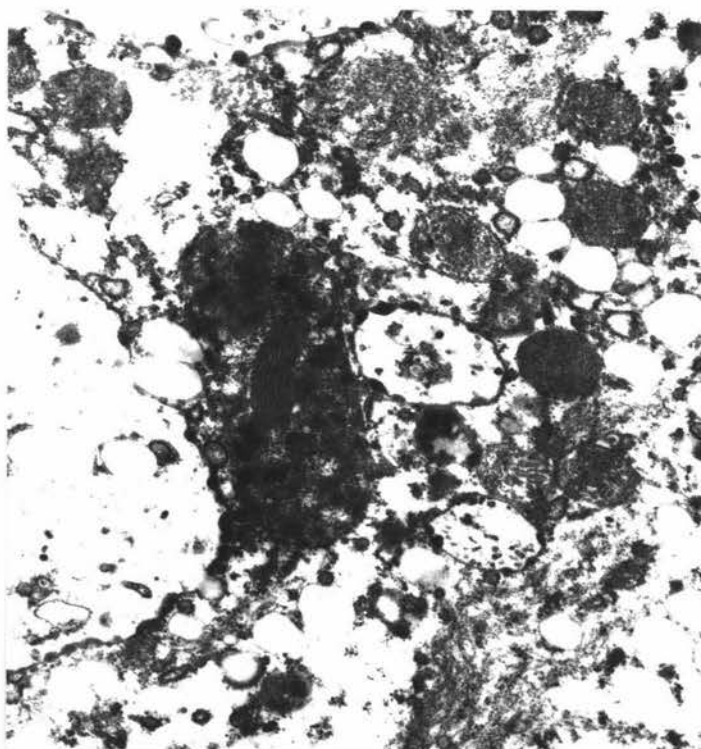


Figure 40. Development following 8 hours postillumination heat shock, 11 hours postillumination; 13,600 \times .

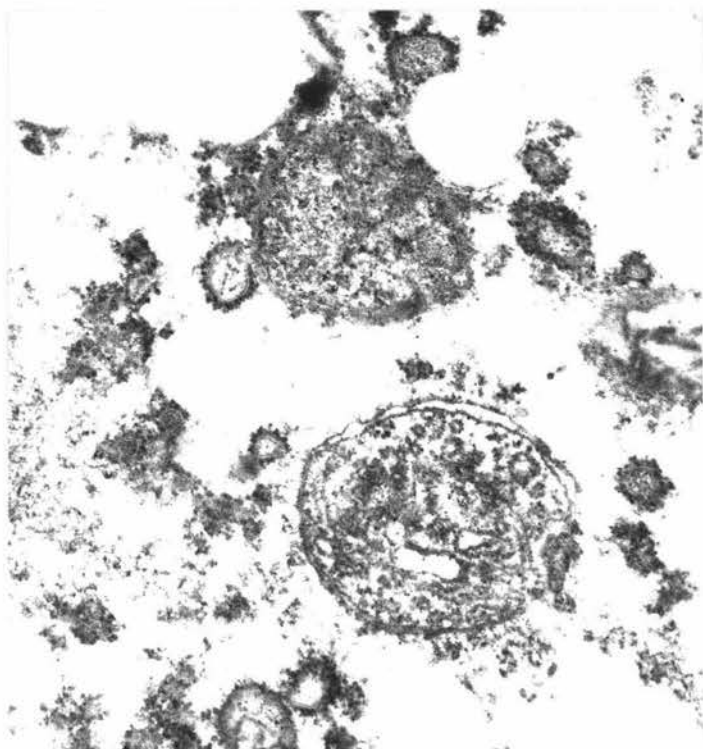


Figure 41. Development following 8 hours postillumination heat shock, 11 hours postillumination; 47,400 \times .

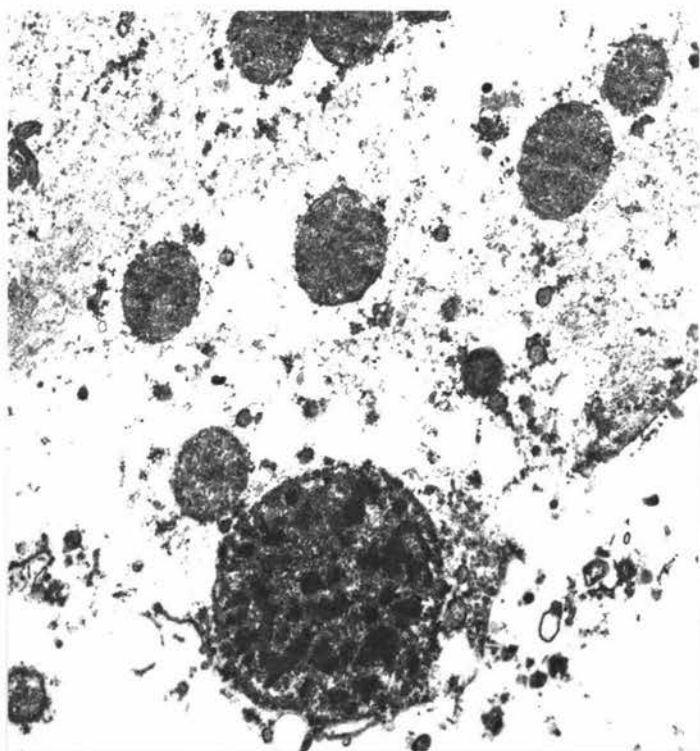


Figure 42. Development following 8 hours postillumination heat shock, 15 hours postillumination; 13,600 \times .

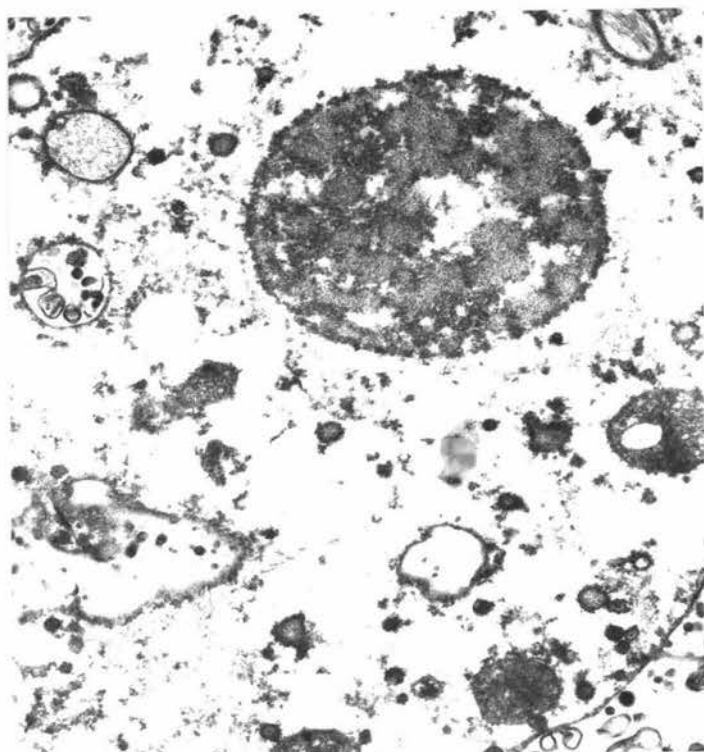


Figure 43. Development following 8 hours postillumination heat shock, 17 hours postillumination; 13,600 \times .

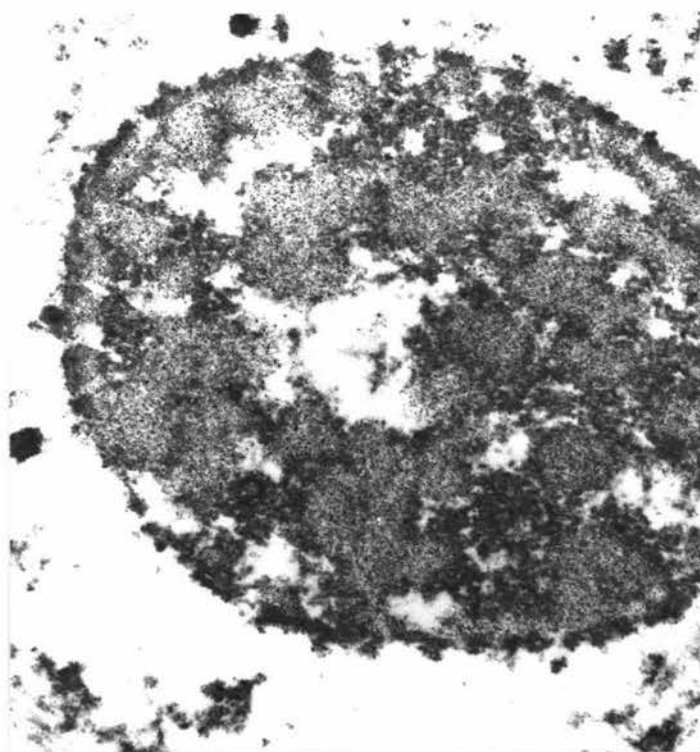


Figure 44. Development following 8 hours postillumination heat shock, 17 hours postillumination; 35,700 \times .

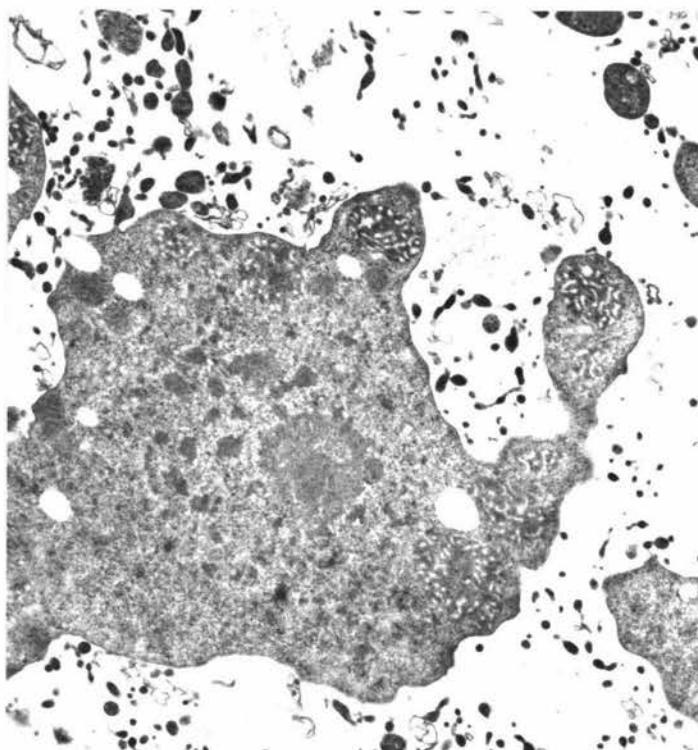


Figure 45. Development following 8 hours postilluminaton heat shock, 96 hours postilluminaton; 13,600 \times .

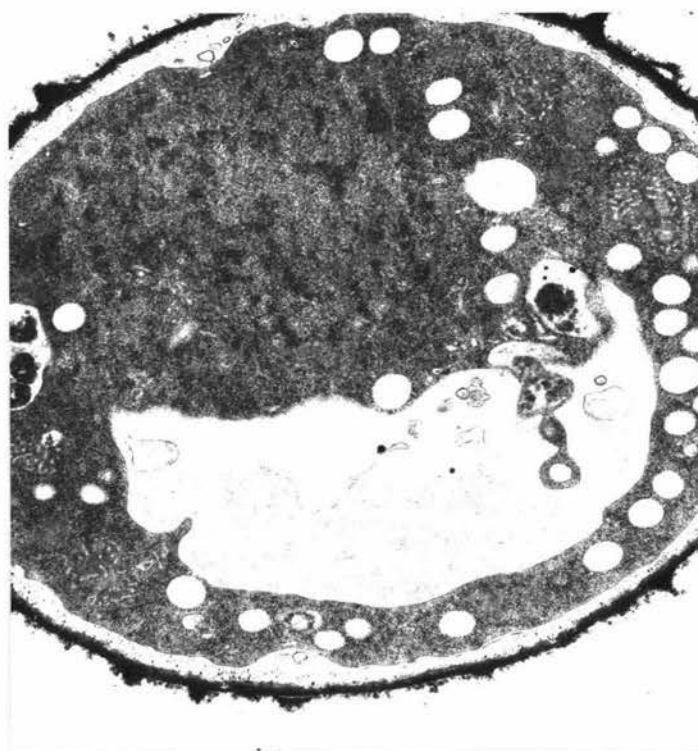


Figure 46. Development following 8 hours postilluminaton heat shock, 7 days postilluminaton; 13,600 \times .

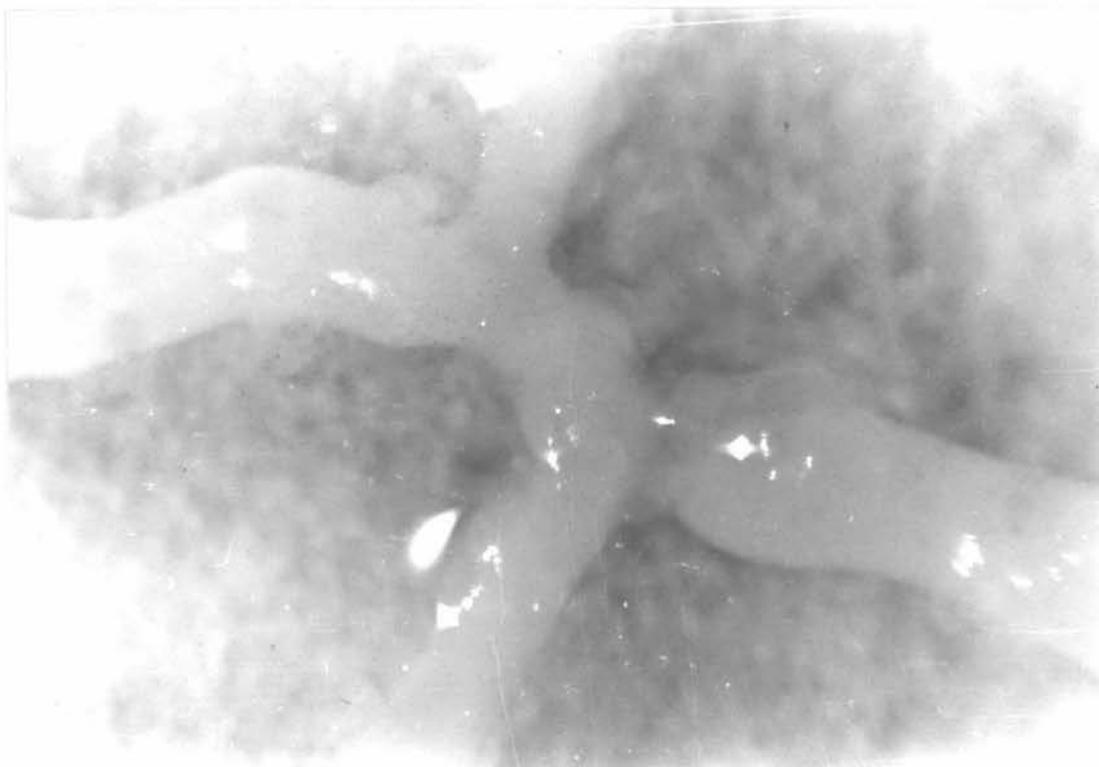


Figure 47. Development following 8 hours postillumination heat shock, 9 hours postillumination; 38 \times .

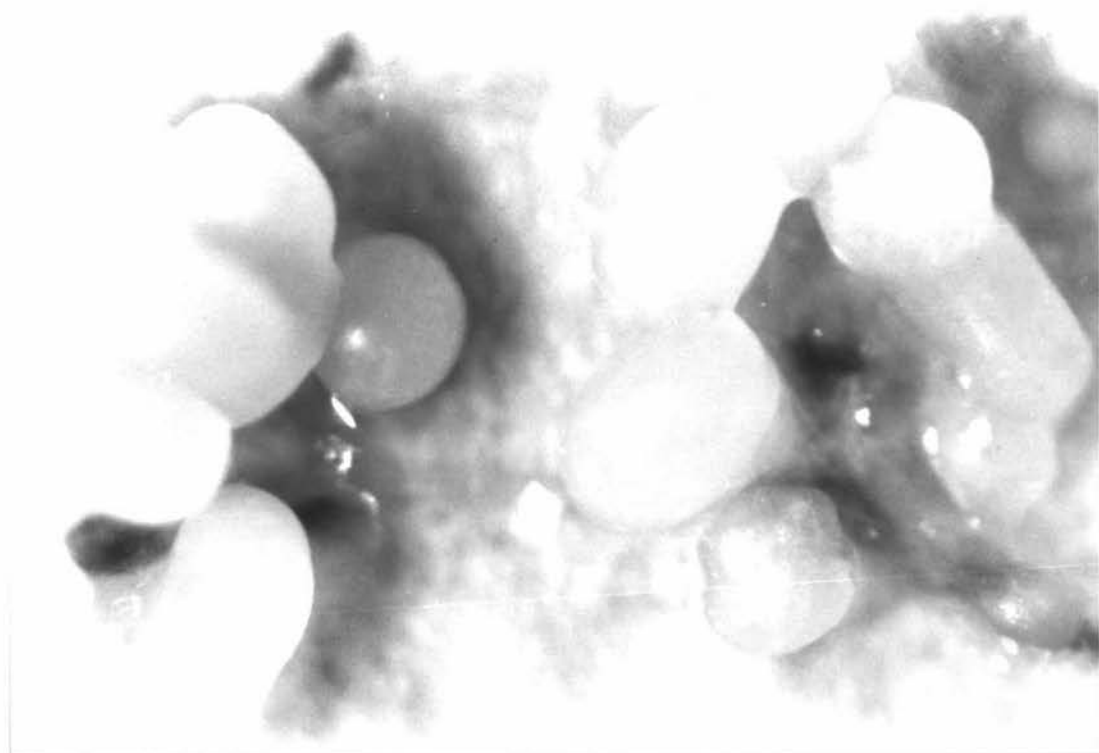


Figure 48. Development following 8 hours postillumination heat shock, 13 hours postillumination; 38 \times .

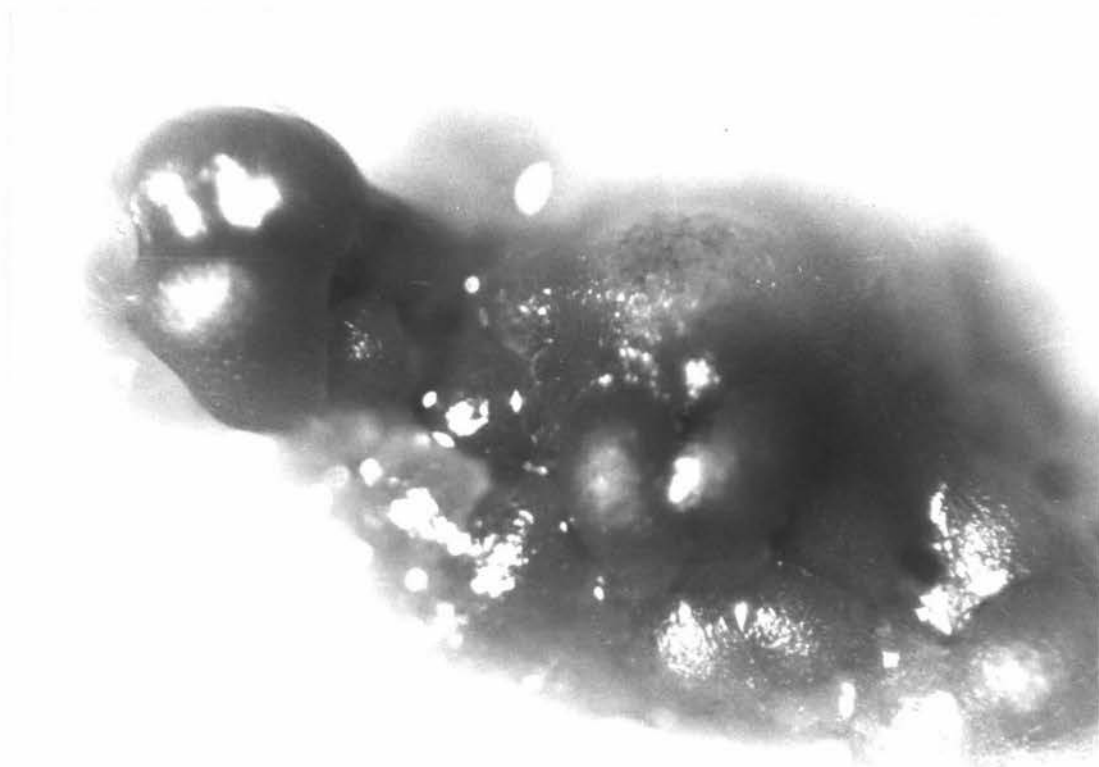


Figure 49. Development following 8 hours postillumination heat shock, 17 hours postillumination; 38x.

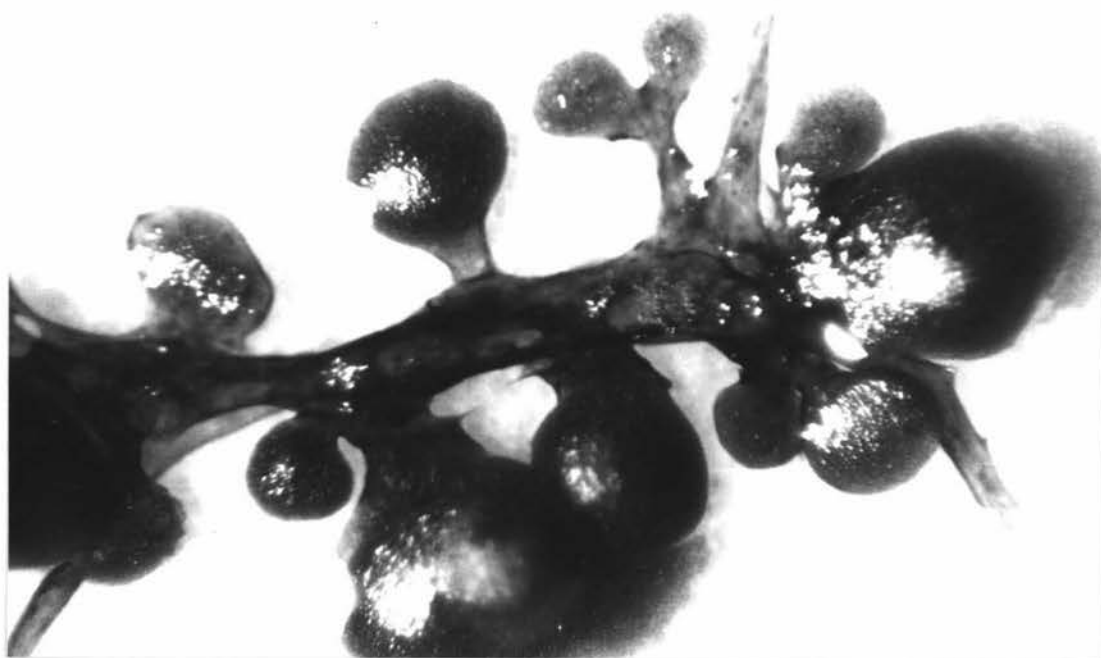


Figure 50. Development following 8 hours postillumination heat shock, 24 hours postillumination; 38x.

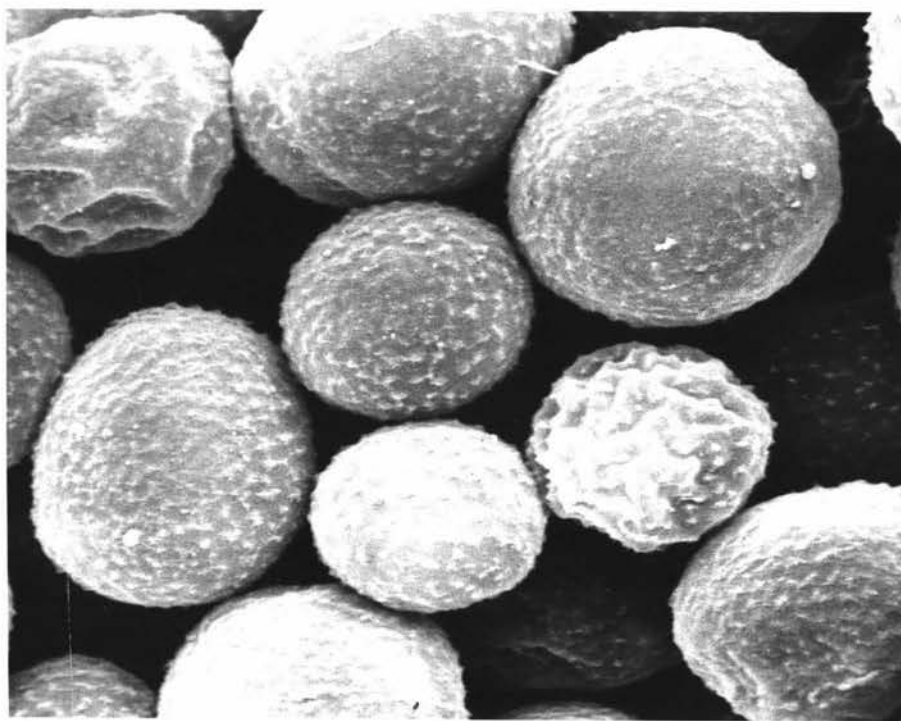


Figure 51. Development following 8 hours postillumination heat shock, 96 hours postillumination; 4000 \times .

is apparent that plasmodia at 8 hours postillumination are at a particularly sensitive stage of development. Thus undetected variations in the rates of development of plasmodia in different experiments, or slight variations in the heat shock temperature, may account for the atypical response described for one heat shock at 8 hours postillumination.

3.1.5 Heat shocks at 10 hours postillumination.

The typical effects of a heat shock begun at 10 hours postillumination on the morphology of subsequent sporangia can be seen in Figures 52 and 53. Half an hour after the heat shock (Fig. 52) sporangial heads have assumed a spherical appearance rather than the lobed appearance of control sporangia at this stage of development. Little change occurs in the appearance of these sporangia up to 19 hours postillumination (Fig. 53) apart from the slight darkening of sporangial heads. However the sporangia remain light brown in colour and do not become completely black as do fully developed, control sporangia. Since the pigmentation process has not been studied chemically, the colouration which does occur in these sporangia may represent either partial melanization or quite a different process such as breakdown of sporangial material. No further change occurs in the morphology of these sporangia upon continued incubation.

In the scanning electron microscope (Figs. 54 and 55), sporangia produced subsequent to a 10 hours postillumination heat shock can be seen to lack spores completely. Instead, a network of threads, which may represent the capillitium reported to occur in many species of Myxomycetes (Alexopoulos and Mims, 1979), and a large number of mitochondrion-sized granules are apparent.

The ultrastructural changes induced by a heat shock at 10 hours postillumination can be seen in Figures 56 - 59. At 11 hours postillumination (Fig. 56), many vacuoles are apparent within the cytoplasm and the chromocentres of the nucleus appear more diffuse than those of non-heat shocked plasmodia. Mitochondria also appear abnormal, containing a number of small vacuoles. At 13 hours postillumination

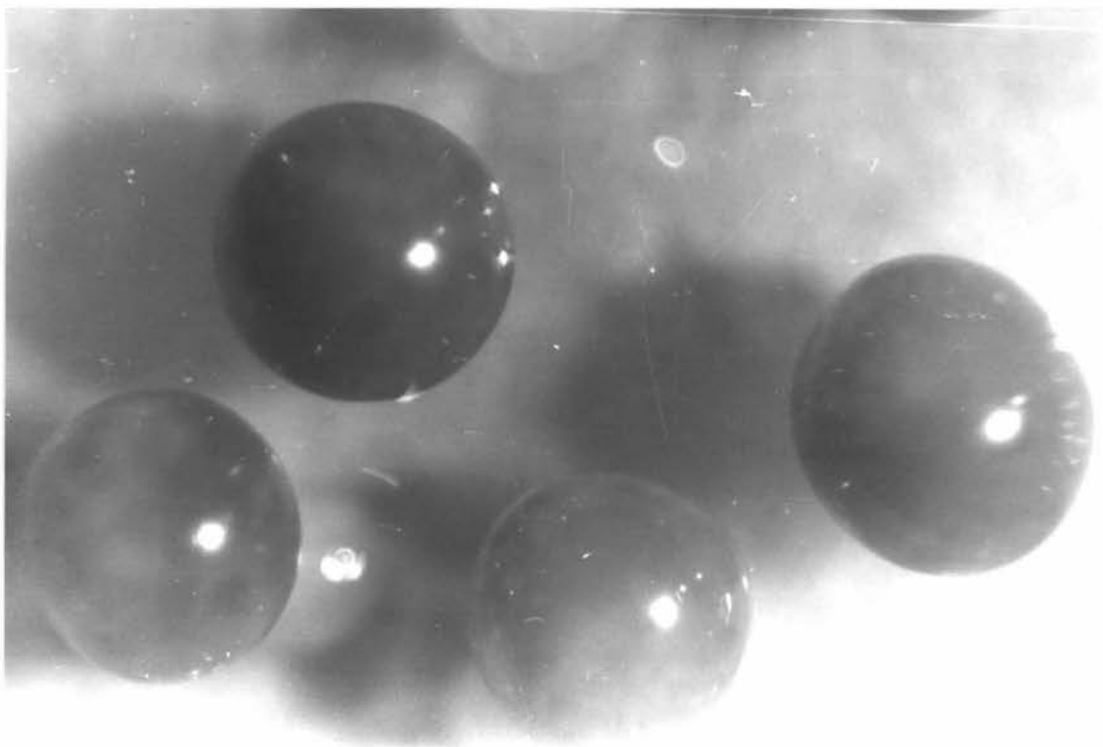


Figure 52. Development following 10 hours postillumination heat shock, 11 hours postillumination; 38 \times .

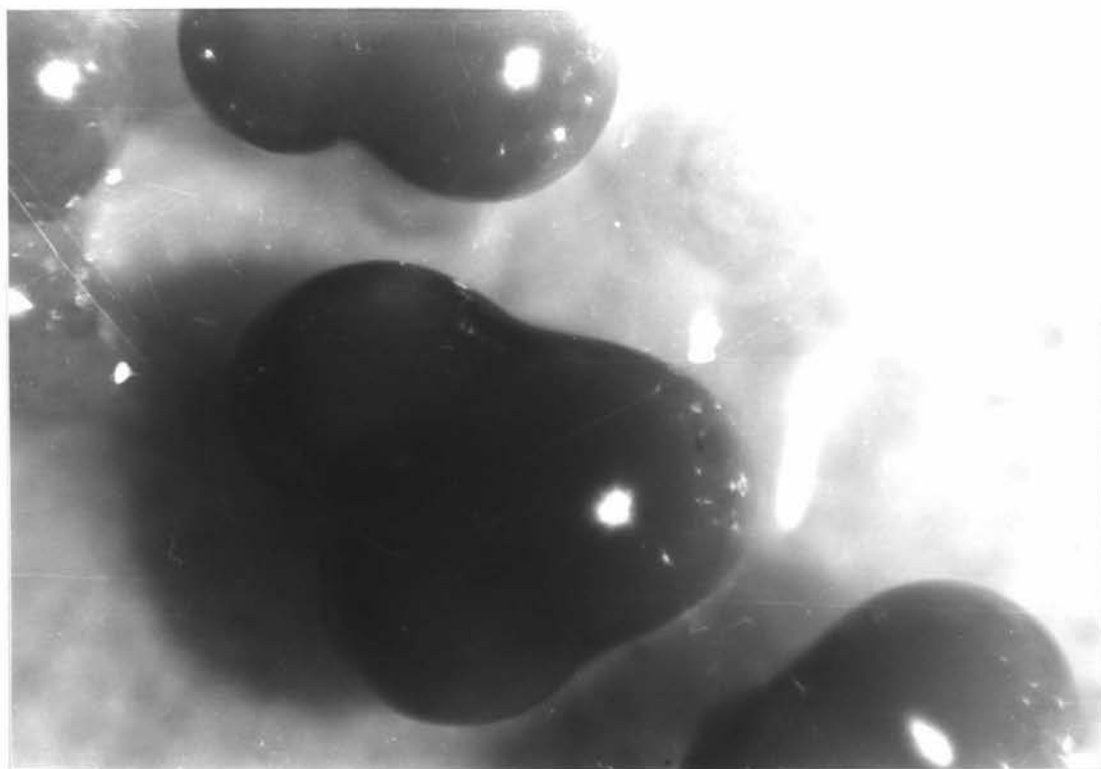


Figure 53. Development following 10 hours postillumination heat shock, 19 hours postillumination; 38 \times .

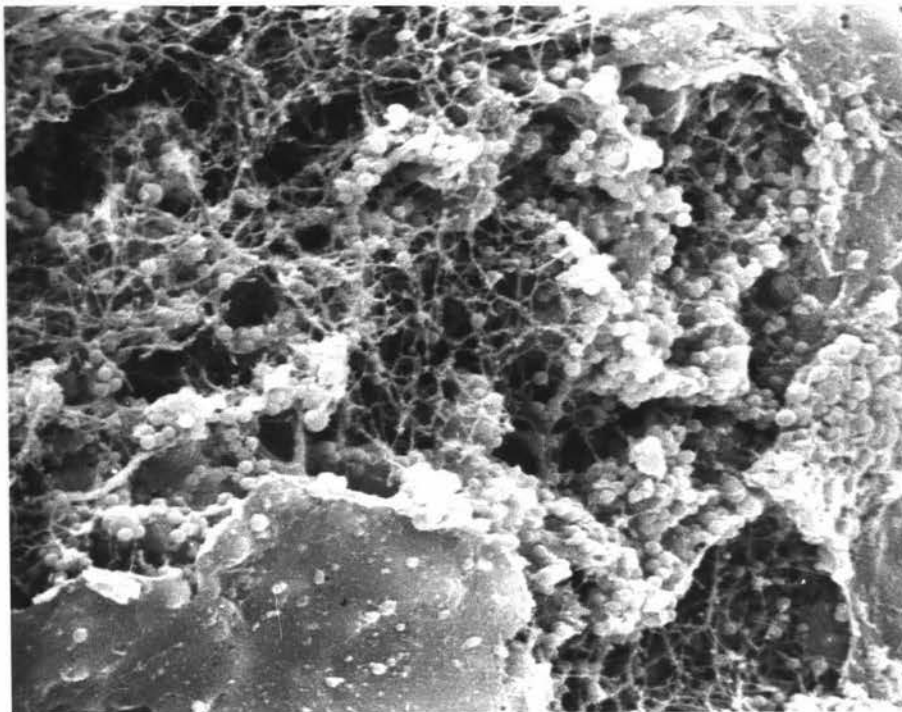


Figure 54. Development following 10 hours postillumination heat shock, 96 hours postillumination; 4000x.

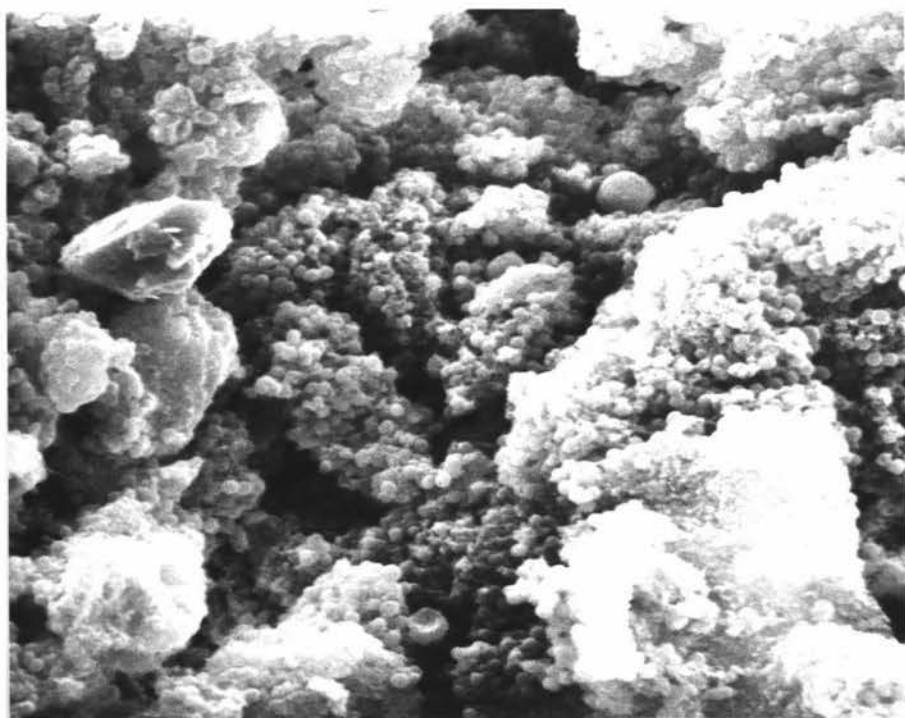


Figure 55. Development following 10 hours postillumination heat shock, 7 days postillumination; 4000x.

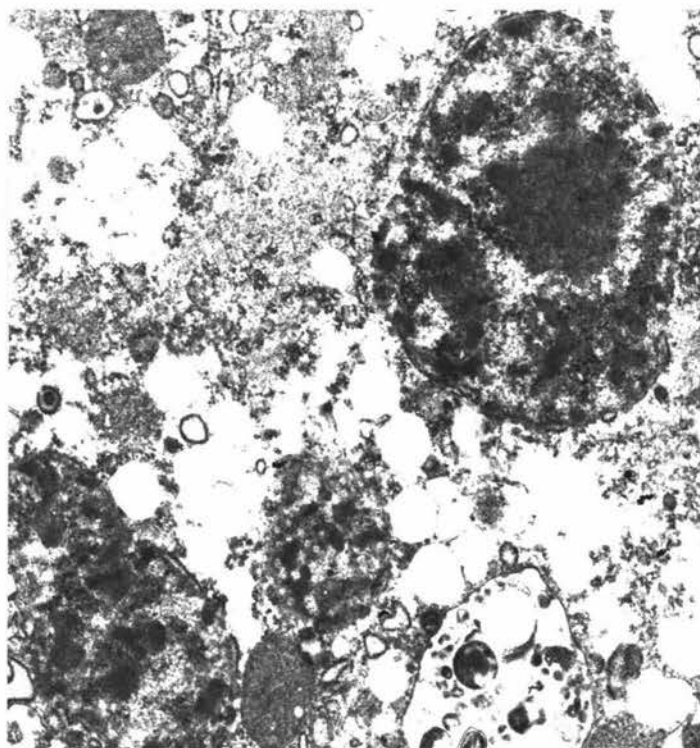


Figure 56. Development following 10 hours postillumination heat shock, 11 hours postillumination; 13,600 \times .

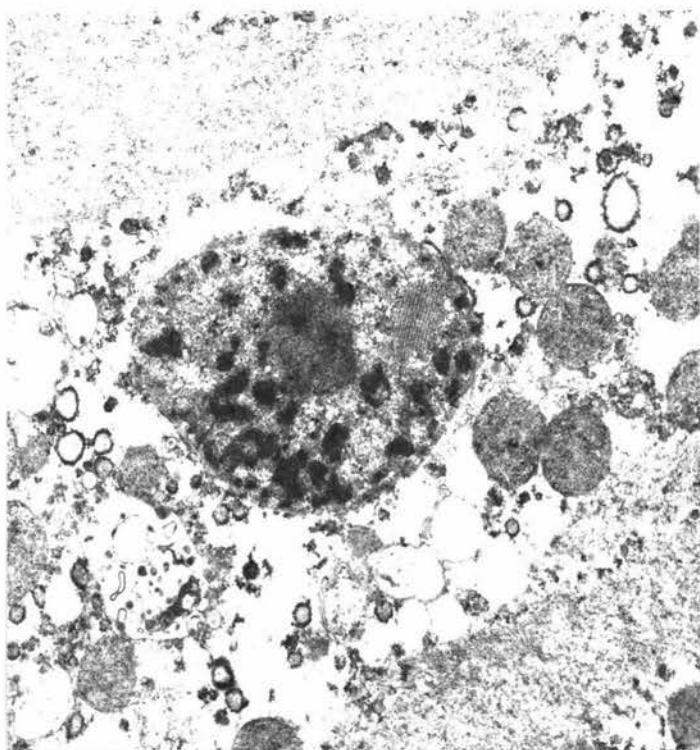


Figure 57. Development following 10 hours postillumination heat shock, 13 hours postillumination; 13,600 \times .

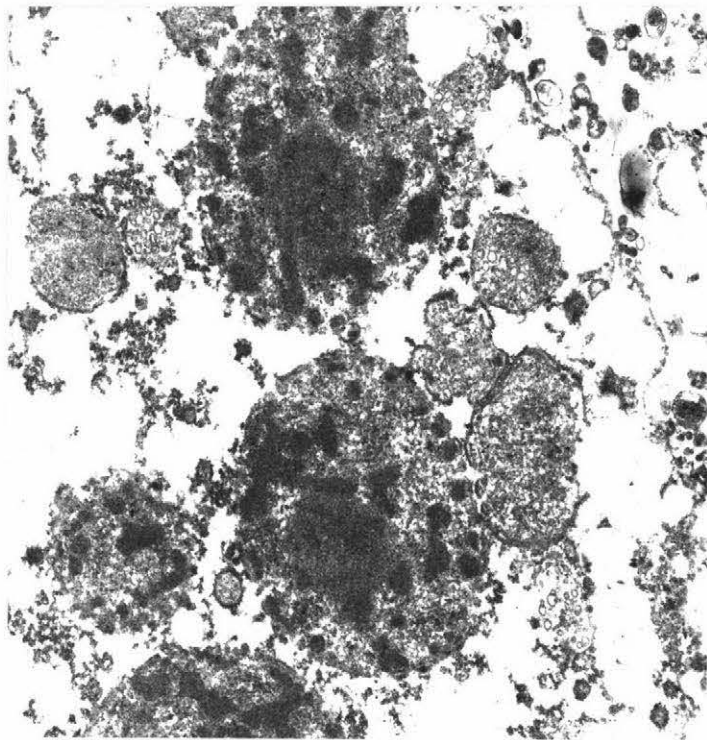


Figure 58. Development following 10 hours postillumination heat shock, 24 hours postillumination; 13,600 \times .

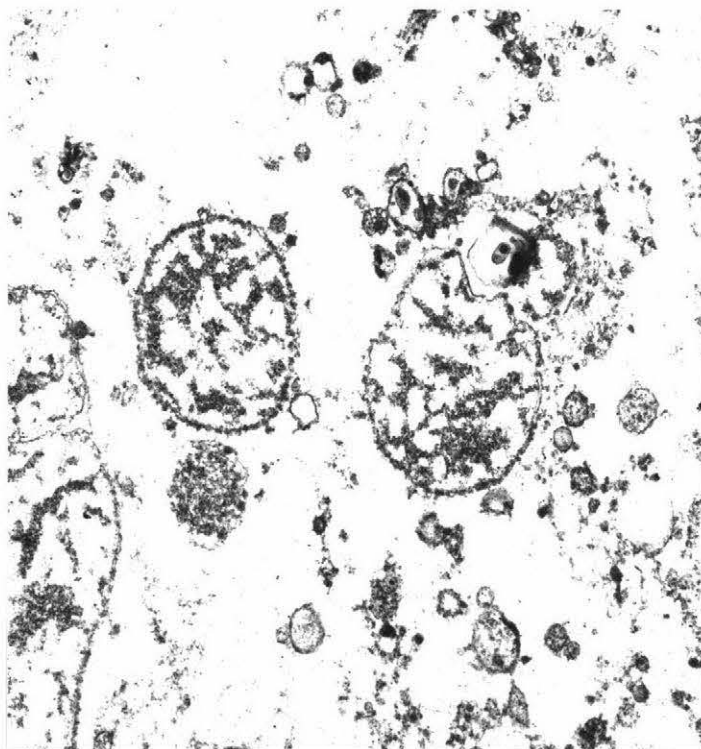


Figure 59. Development following 10 hours postillumination heat shock, 96 hours postillumination; 13,600 \times .

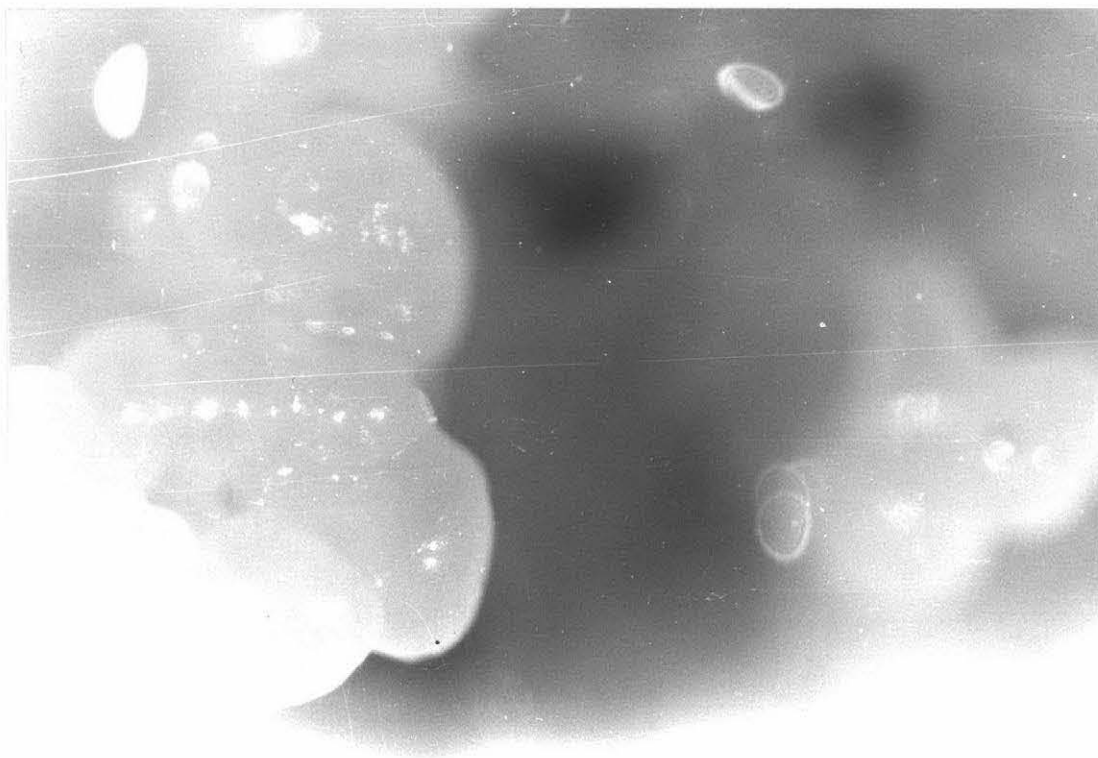


Figure 60. Development following 12 hours postillumination heat shock, 13 hours postillumination; 38x.

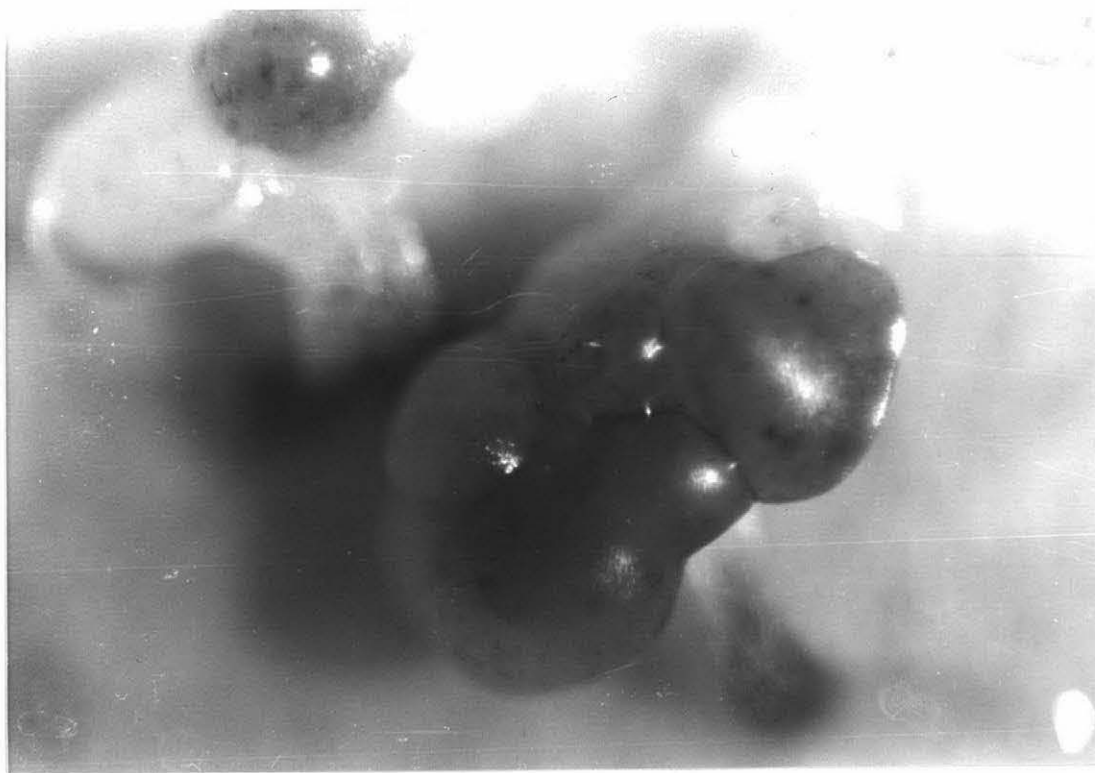


Figure 61. Development following 12 hours postillumination heat shock, 17 hours postillumination; 38x.

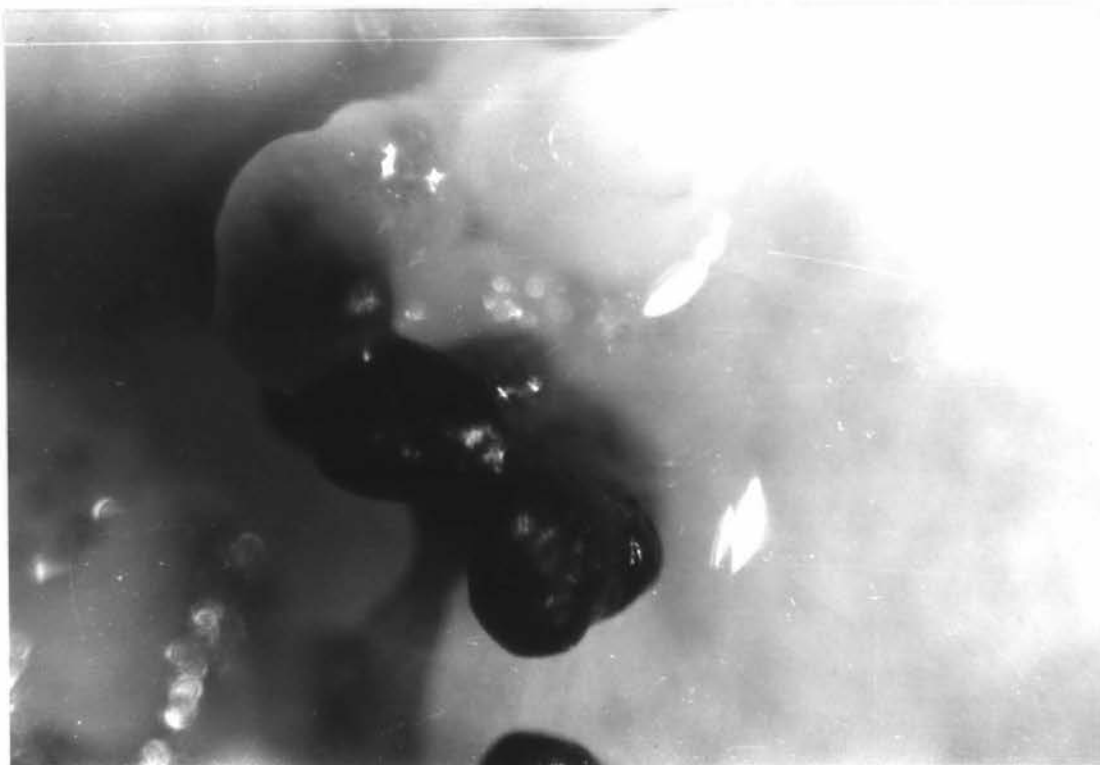


Figure 62. Development following 12 hours postilluminaton
heat shock, 19 hours postilluminaton; 38 \times .

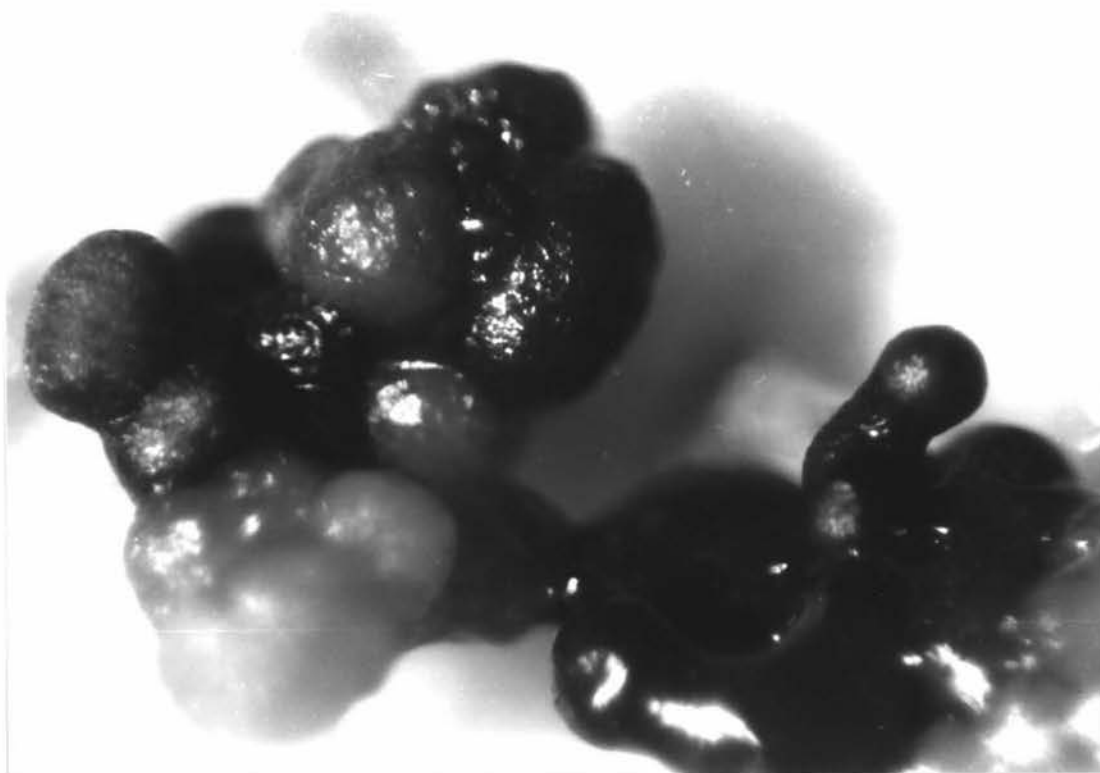


Figure 63. Development following 12 hours postilluminaton
heat shock, 24 hours postilluminaton; 38 \times .

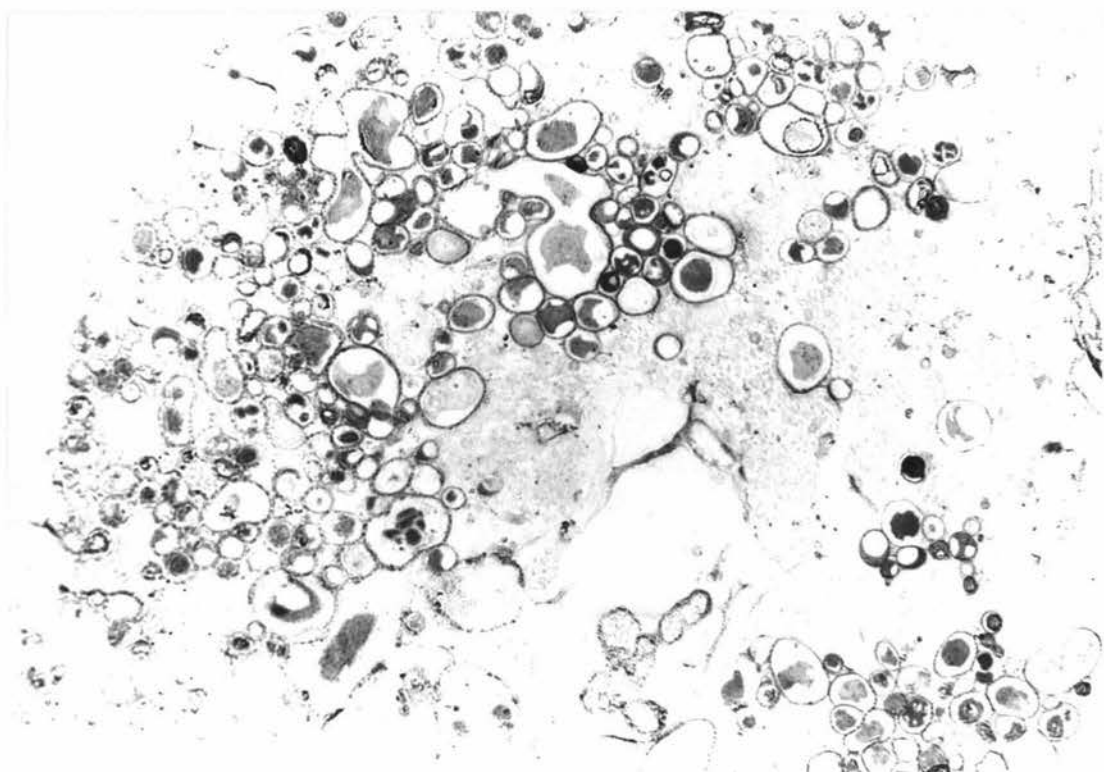


Figure 64. Development following 12 hours postillumination heat shock, melanized sporangium; 440 \times .

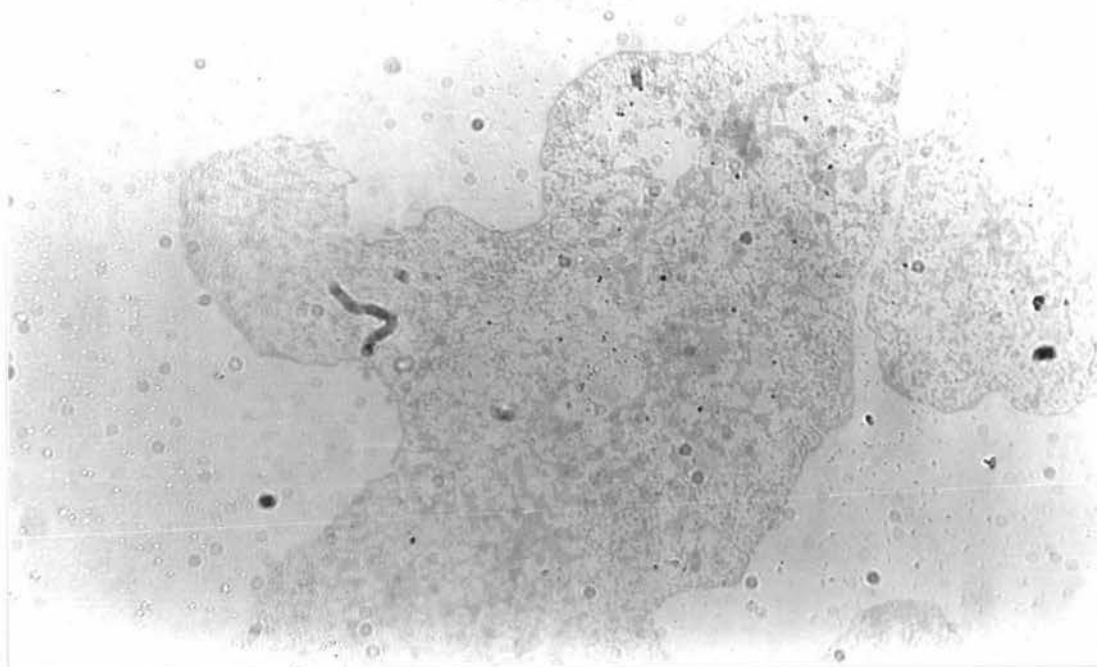


Figure 65. Development following 12 hours postillumination heat shock, non-melanized sporangium; 440 \times .

to involve the complete cleavage of the cytoplasm within the sporangial head as it does in normal development (Fig. 10). In addition, those spores that are formed within melanized sporangia are abnormal in both shape and size (Fig. 64). Clearly, the process of melanization and hence the colour of mature sporangia is dependant upon a degree of cleavage and spore formation, albeit abnormal, within the sporangium. That some sporangia as well as areas of other sporangia do not cleave into spores and therefore do not appear black, probably reflects the non-synchronous nature of this aspect of development.

In the scanning electron microscope (Fig. 66), it can again be seen that those spores that are produced subsequent to a heat shock at 12 hours postillumination vary greatly in both size and shape. The walls of these spores are often broken, a feature not seen in preparations of normal spores, and lack the characteristic spines. Intracellular organelles, including nuclei and mitochondria, are clearly visible. It is assumed that in this case, heat shock has interfered with that part of the developmental process concerned with deposition of spore wall material and consequently these spores are more fragile than normal spores. In an area of a sporangium apparently not containing spores (Fig. 67), many mitochondrion-sized granules and other material are clearly visible. These granules have not been identified but they do resemble what are assumed to be mitochondria within spores (Fig. 66).

The ultrastructural changes induced by a heat shock at 12 hours postillumination can be seen in Figures 68 - 77. At 13 hours postillumination (Fig. 68), there is little apparent difference between these plasmodia and plasmodia undergoing normal development. However intramitochondrial vacuoles and slightly electron-dense vacuoles within the cytoplasm are abnormal features. At 15 hours postillumination (Fig. 69), there is little evidence of cleavage and the nuclei are abnormal in a number of respects: many breaks within the nuclear membrane, diffuse chromocentres, and intranuclear vacuoles. Other electron micrographs of these sporangia (not shown) show some nuclei with more extensive vacuolation and the presence of intranuclear



Figure 66. Development following 12 hours postillumination heat shock, 7 days postillumination; 4000 \times .

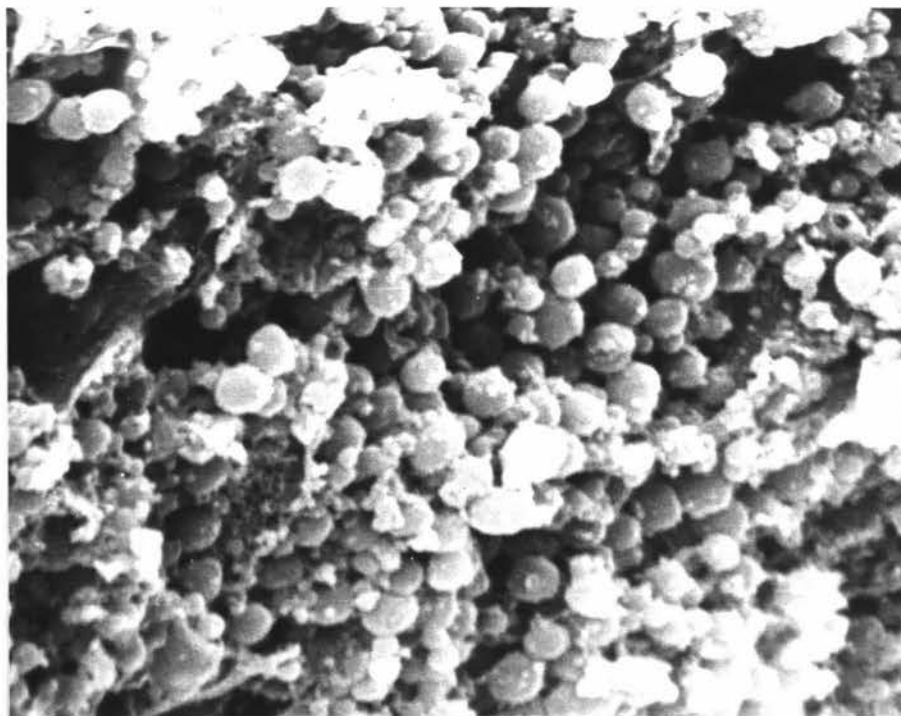


Figure 67. Development following 12 hours postillumination heat shock, 7 days postillumination; 4000 \times .

macrotubular structures. Mitochondria also appear abnormal (Fig. 70), being rounded and containing numerous vacuoles and abnormal cristae. At 17 hours postillumination (Fig. 71), some cleavage is apparent and at 19 hours postillumination there are areas on the thin section where spores are evident (Fig. 72) as well as areas on the same section where no spores are evident (Fig. 73). Those spores that are produced at this time contain apparently normal organelles but are larger and of a more irregular shape than normal spores. In those areas where spores are not produced, both nuclei and mitochondria exhibit abnormalities similar to those seen at 15 hours postillumination suggesting that development has been arrested in these areas. At 24 hours postillumination (Fig. 74) the spores are abnormally large and the spore wall is somewhat thinner than normal. The spines are also much less prominent than normal. In non-spore producing areas at the same time (Fig. 75), degeneration of cellular organelles and cytoplasmic material is evident, as are what are presumed to be areas of cleavage, perhaps indicating an abortive cleavage. Little further change occurs in spores up to 96 hours postillumination (Fig. 76) and they still contain a number of abnormal features. These include the presence of many small vacuoles within the cytoplasm, an irregular shape and size, thin spore walls which lack prominent spines, and the large vacuole between the spore wall and the cytoplasm. The thin spore wall and lack of prominent spines are in agreement with the evidence obtained in the scanning electron microscope. Those areas of the sporangial cytoplasm that do not contain spores are undergoing extensive degeneration at 96 hours postillumination (Fig. 77). Thus the early separation into spore producing and non-spore producing areas remains; those areas of the sporangial cytoplasm that have failed to cleave into spores early in development appear not to recover this capacity. Again, this probably reflects variations in developmental state within the sporangial cytoplasm at the time of the heat shock.

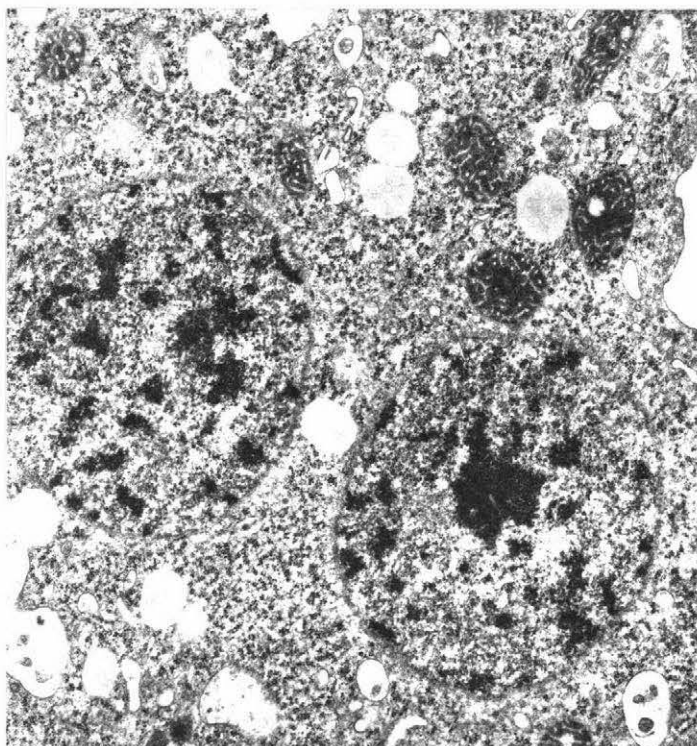


Figure 68. Development following 12 hours postillumination heat shock, 13 hours postillumination; 13,600 \times .

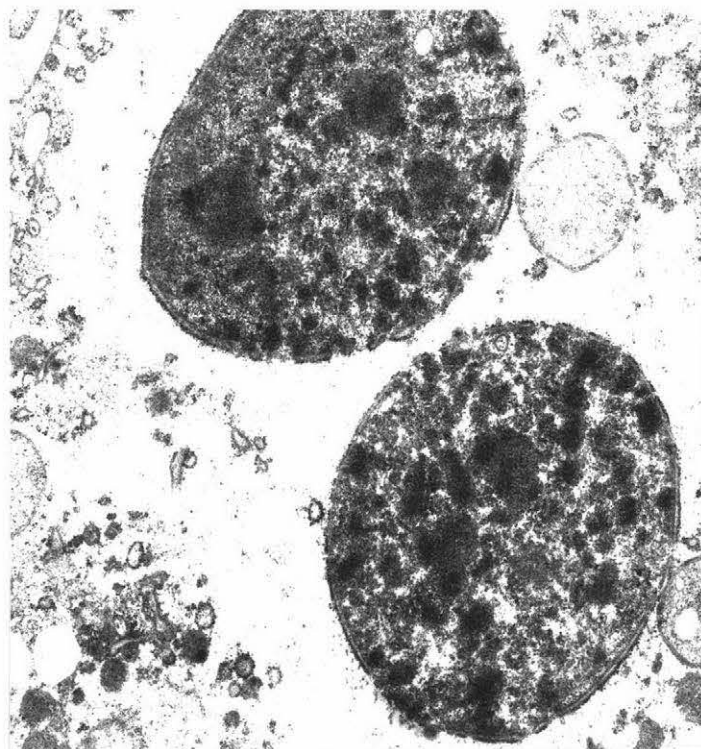


Figure 69. Development following 12 hours postillumination heat shock, 15 hours postillumination; 13,600 \times .

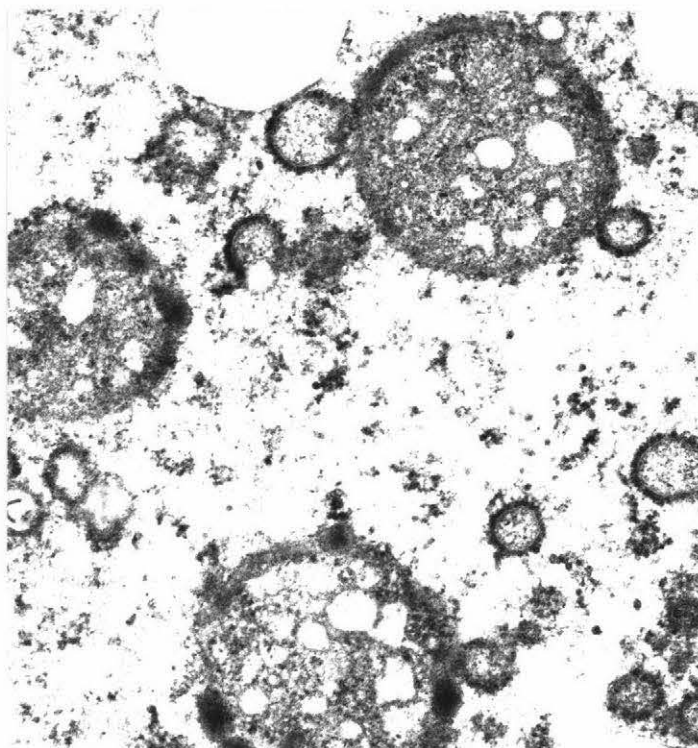


Figure 70. Development following 12 hours postillumination heat shock, 15 hours postillumination; 47,400 \times .

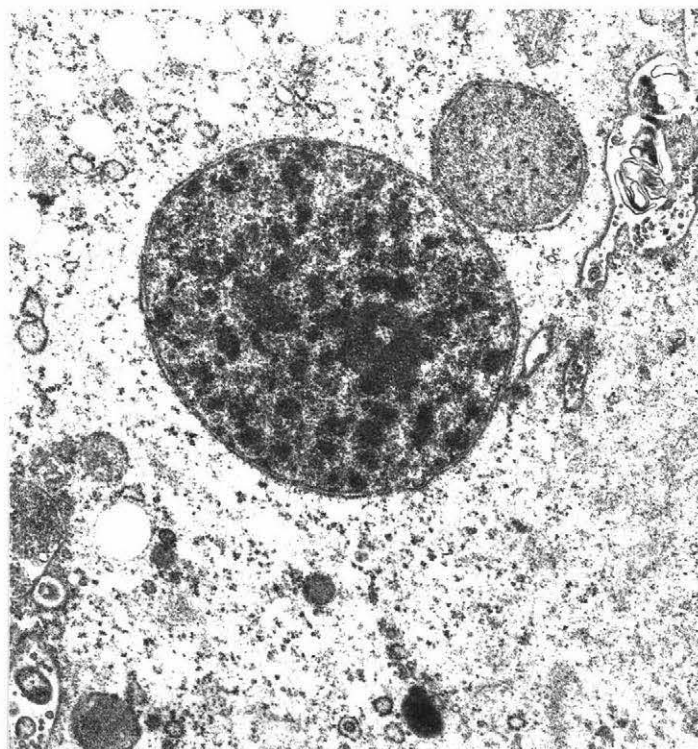


Figure 71. Development following 12 hours postillumination heat shock, 17 hours postillumination; 13,600 \times .

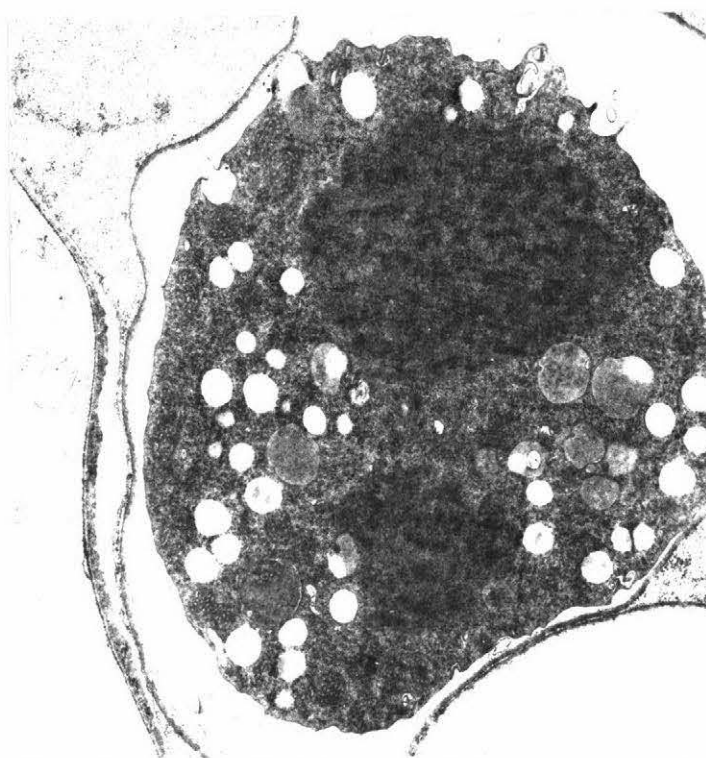


Figure 72. Development following 12 hours postillumination heat shock, 19 hours postillumination; 10,500 \times .

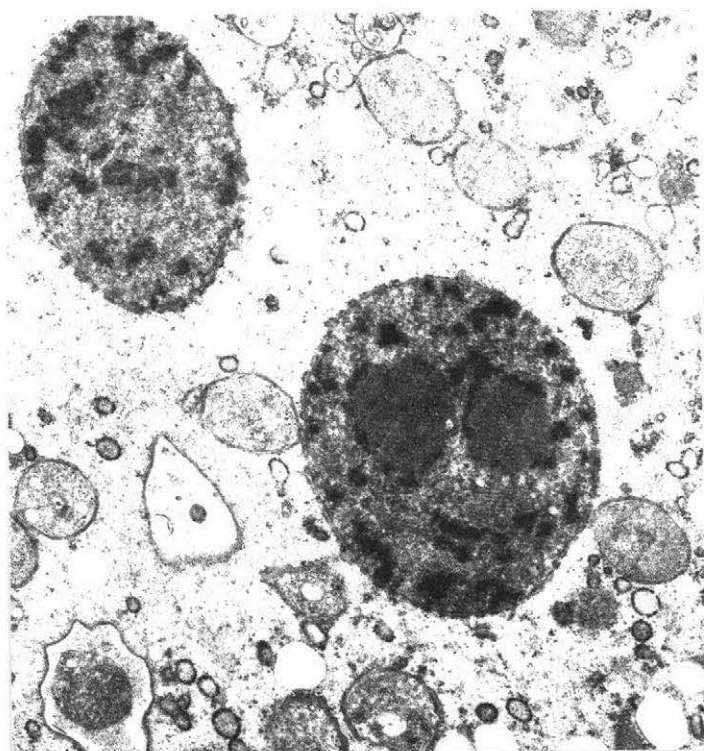


Figure 73. Development following 12 hours postillumination heat shock, 19 hours postillumination; 13,600 \times .

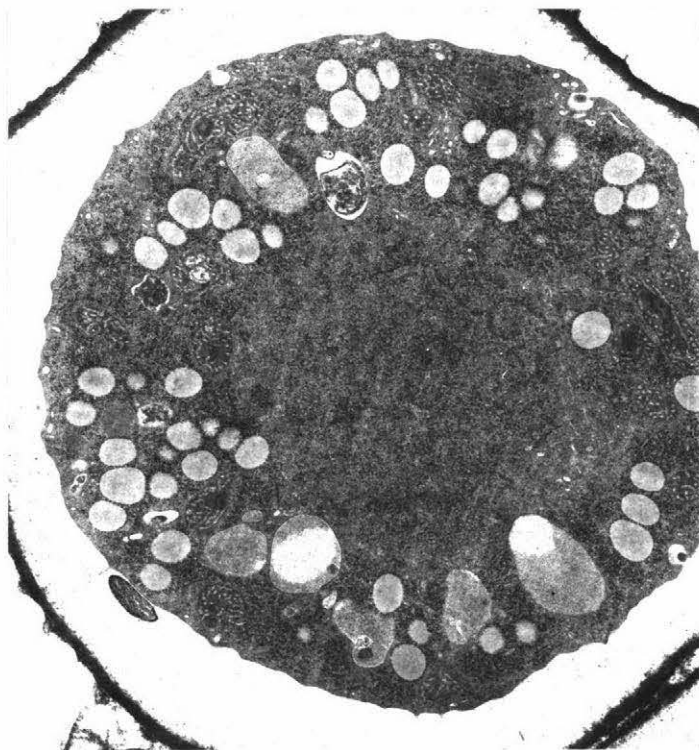


Figure 74. Development following 12 hours postillumination heat shock, 24 hours postillumination; 10,500 \times .

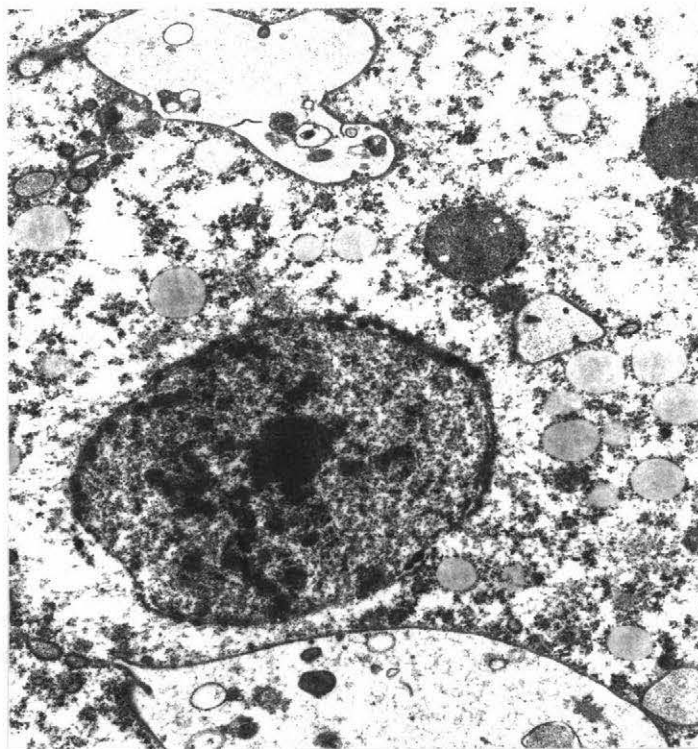


Figure 75. Development following 12 hours postillumination heat shock, 24 hours postillumination; 13,600 \times .



Figure 76. Development following 12 hours postillumination heat shock, 96 hours postillumination; 13,600 \times .

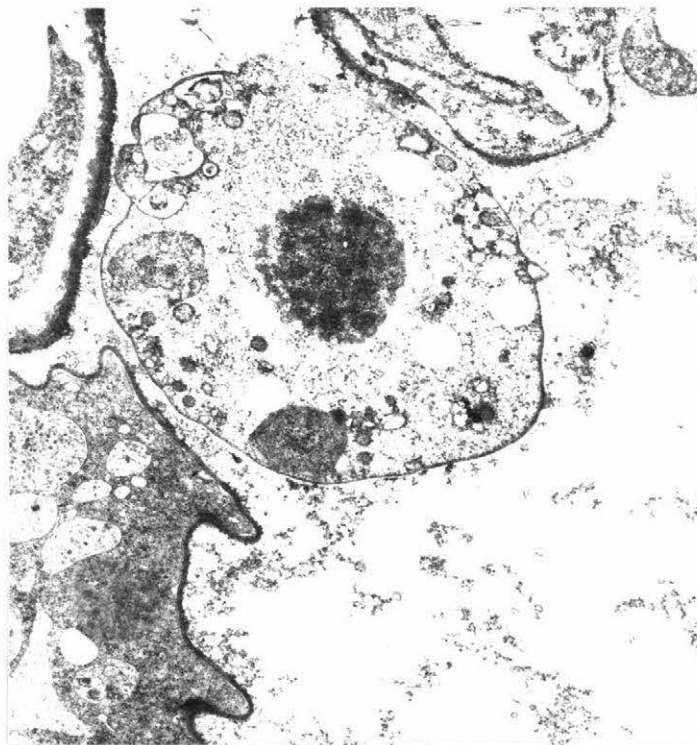


Figure 77. Development following 12 hours postillumination heat shock, 96 hours postillumination; 13,600 \times .

3.1.7 The effect of heat shock on the synthesis of polyphenol oxidase.

The synthesis of melanin from its precursors is catalyzed by the enzyme polyphenol oxidase. In *Physarum*, the evidence suggests that the deposition of melanin in the spore wall begins sometime between 13 and 15 hours post-illumination. Therefore it is likely that the genes necessary for melanin synthesis are transcribed shortly before this and the actual time of transcription could be determined by heat shock treatment. Thus a heat shock at a critical time would repress the transcription of these genes and, consequently, the mature spores would melanize abnormally if at all. Melanization being such a clearcut event during development, any abnormality in this process would be clearly visible.

L,3,4-dihydroxyphenylalanine (L-DOPA) acts as a substrate for polyphenol oxidase and is oxidized by it to DOPA quinone. The quinone undergoes spontaneous ring closure and reaction with water to yield indole-5,6-quinone, which then polymerizes to form a complex pigmented product. Osmium tetroxide reacts with the latter, producing an insoluble electron-dense precipitate and it is this reaction upon which the electron microscopic localization of the enzyme depends.

The appearance of histochemical activity for polyphenol oxidase during normal development and during development subsequent to a heat shock at 12 hours postillumination can be seen in Figures 78 - 83. At 13 hours postillumination during normal development (Fig. 78), when cleavage is almost complete, no enzyme activity is apparent. However at 15 hours postillumination (Fig. 79), when cleavage is complete, a number of vacuoles containing electron-dense material are apparent. These vacuoles are still apparent at 19 hours postillumination (Fig. 80) when the spore wall as well as the spines on the spore wall have also become electron-dense. In the control at this time which was incubated without the substrate for the enzyme (Fig. 81), the vacuoles, spore walls, and spore wall spines are all considerably less electron-dense. However little difference is apparent in controls incubated with an inhibitor of the enzyme (not

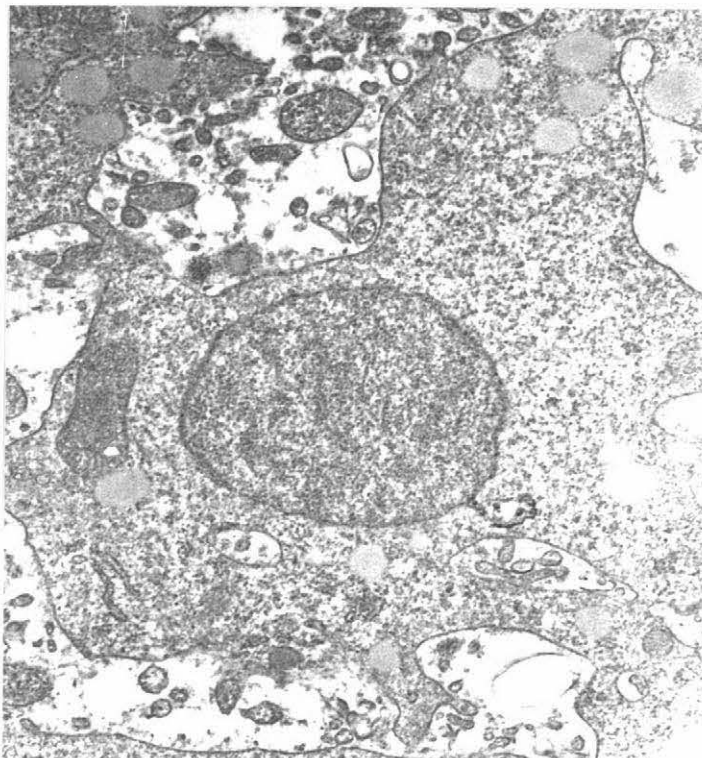


Figure 78. Normal development, polyphenol oxidase activity at 13 hours postillumination; 13,600 \times .

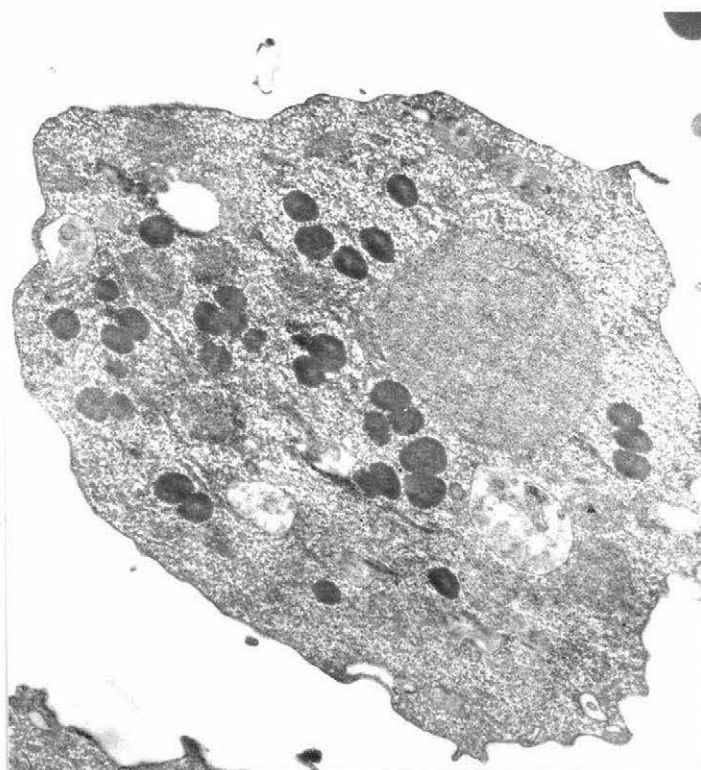


Figure 79. Normal development, polyphenol oxidase activity at 15 hours postillumination; 13,600 \times .

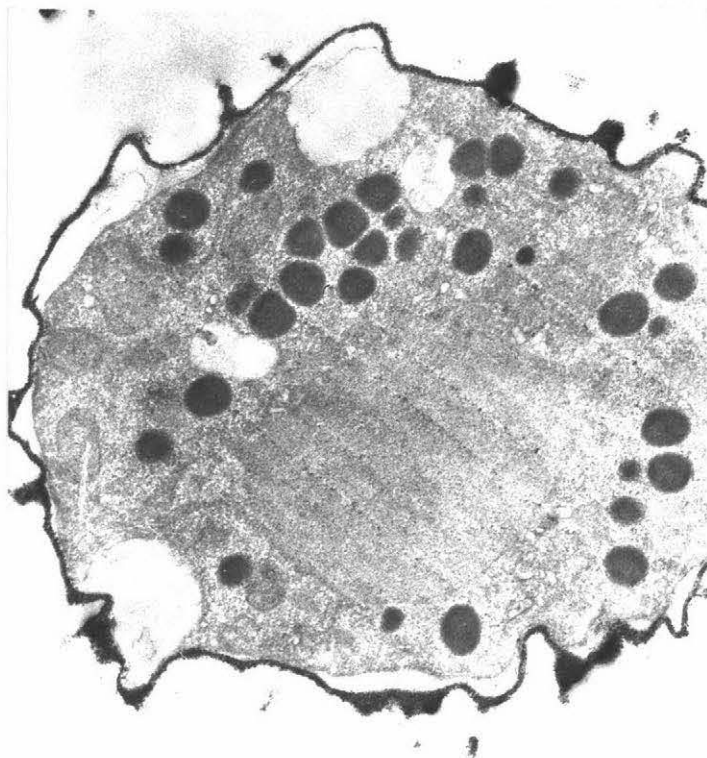


Figure 80. Normal development, polyphenol oxidase activity at 19 hours postillumination; 13,600 \times .

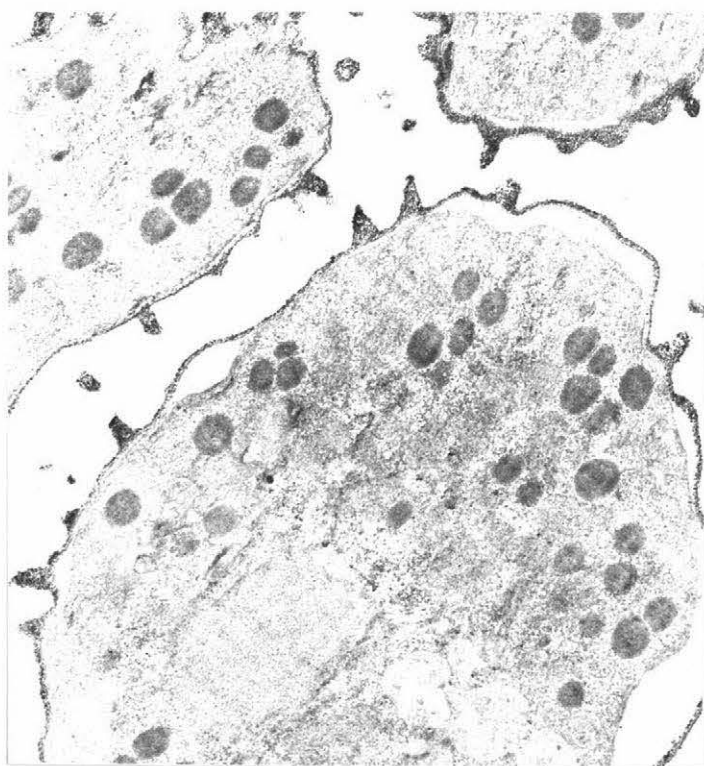


Figure 81. Normal development, polyphenol oxidase control lacking substrate, 19 hours postillumination; 13,600 \times .

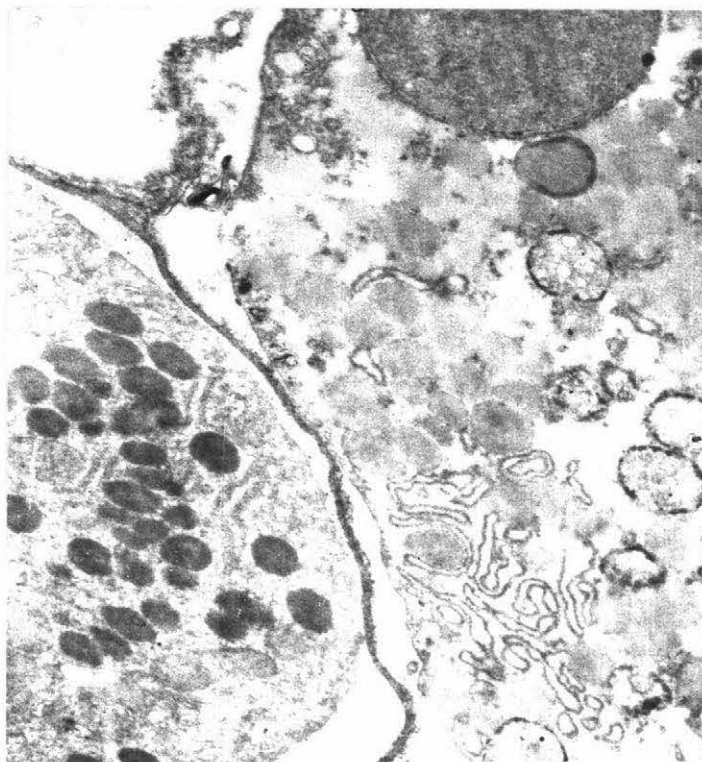


Figure 82. Development following 12 hours postillumination heat shock, polyphenol oxidase activity at 19 hours postillumination; 13,600 \times .

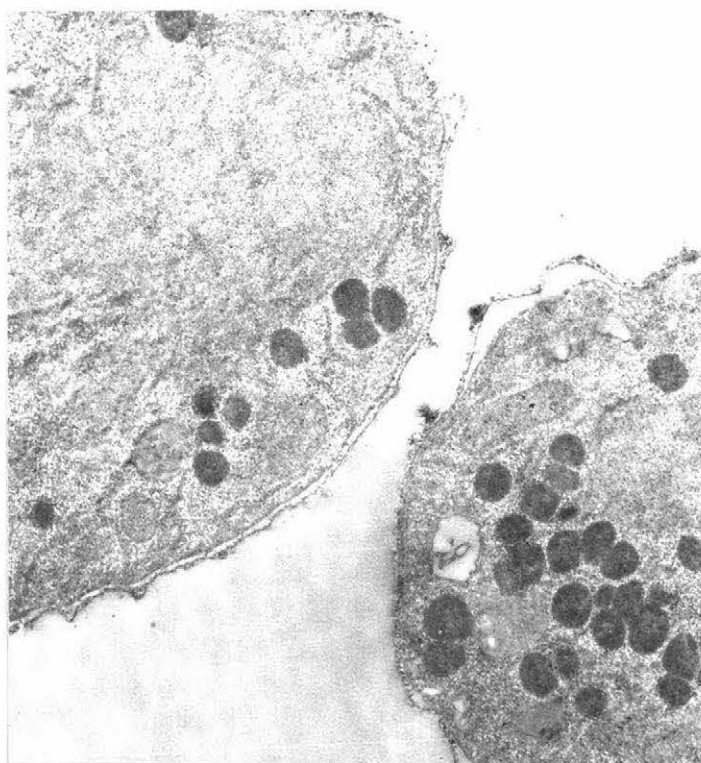


Figure 83. Development following 12 hours postillumination heat shock, polyphenol oxidase control lacking substrate, 19 hours postillumination; 13,600 \times .

shown).

At 19 hours postillumination and subsequent to a heat shock at 12 hours postillumination (Fig. 82), a distribution of electron-dense material similar to that seen in normal spores is apparent. However in this case the spore wall is not as electron-dense and no electron-dense material is evident in those areas of the sporangium which have failed to cleave into spores. Again, the control incubated without the substrate is considerably less electron-dense in the equivalent areas (Fig. 83) but little difference is apparent in the enzyme inhibited control (not shown).

If it is assumed that the electron-dense material does in fact represent sites of polyphenol oxidase activity, then this enzyme first becomes apparent in the normal developmental sequence shortly before melanization. Furthermore, the enzyme is closely associated with the spore wall and its activity here probably reflects its role in spore wall formation. Subsequent to a heat shock at 12 hours postillumination, enzyme activity is still associated with the cytoplasmic vacuoles but much less so with the spore wall. However it is possible that the lower activity in the spore wall is a result of abnormal spore wall formation and not a cause of it.

3.1.8 The effects on subsequent development of a 12 hour heat treatment at 32° prior to illumination.

The maximum growth temperature for *Physarum* is 30 - 31°C (Wilkins, personal communication) and to determine whether a temperature slightly above this is capable of inducing developmental abnormalities similar to those described above, starved plasmodia were incubated for 12 hours at 32°C during the starvation period. The timing of the heat treatment, 48 - 60 hours after plating out, coincides with the second mitosis after fusion of the microplasmodia, an event which is assumed to be particularly sensitive to elevated temperature.

The fully mature sporangia produced subsequent to a 32°C heat treatment (Fig. 84) exhibit gross abnormalities, lacking sporangial stalks and the normal lobed appearance. Complete melanization appears to have occurred but the ultrastructure

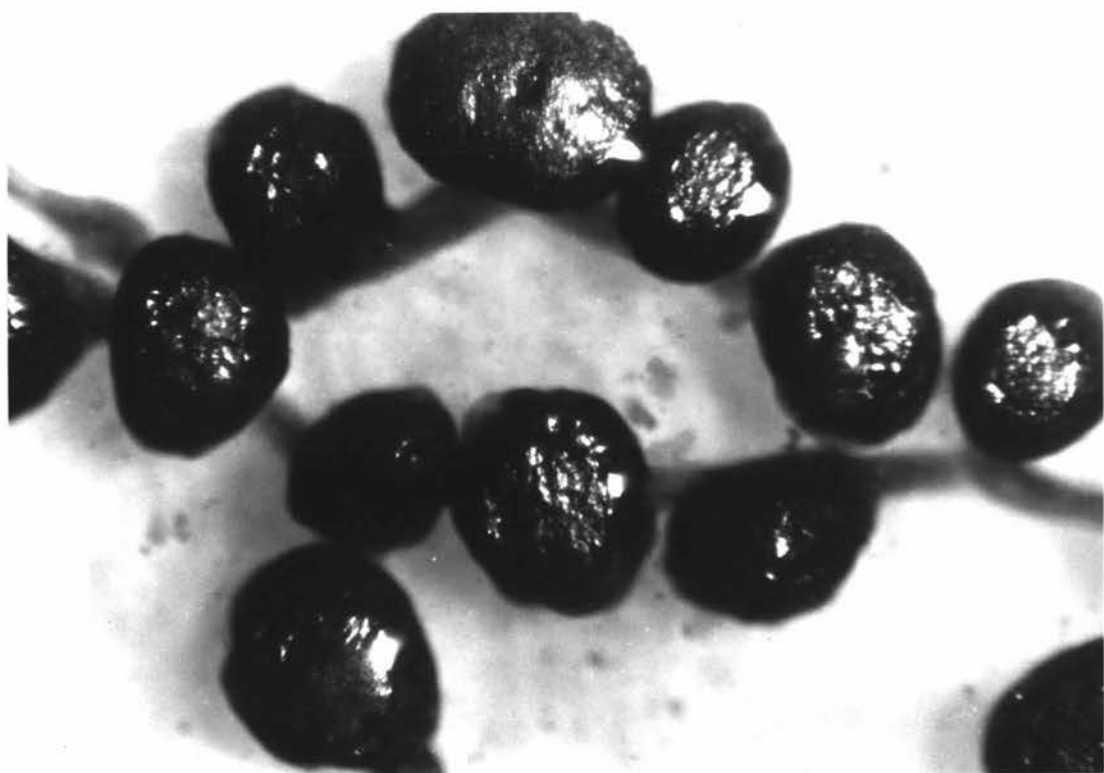


Figure 84. Development following incubation at 32°C from 48 to 60 hours after plating out microplasmodia; 38×.

of these sporangia was not determined.

3.1.9 The effects of heat shock on sporulation and germination frequencies.

For many experiments aimed at determining the developmental effects of heat shocks at various stages of development, the actual sporulation frequencies of the plasmodia heat shocked at each time point were not recorded. However, based on personal observation, almost 100% of those plasmodia heat shocked at each time point subsequently behaved as described above. In addition, non-heat shocked plasmodia often sporulated with a frequency of slightly less than 100%. These observations are supported by the results obtained from one experiment aimed at determining the actual values (Table 1).

The germination frequencies of mature spores were only rarely determined. Instead, the scanning electron microscope was used to establish whether the spores produced subsequent to heat shock were normal or not. However, where germination frequencies were determined (Table 2), it can be seen that those spores produced subsequent to heat shocks were not adversely affected in their germination capacity.

3.1.10 Summary of the major features of *Physarum* development and the effects of heat shock on development.

A summary of the major features of the developmental sequence from plasmodia to mature sporangia is given in Figure 85a. The first indication of sporangial development is the appearance, at approximately 5 hours postillumination, of nodules on the plasmodial strands. These nodules elevate and enlarge to form pillars which then begin to differentiate into stalks and heads at approximately 7 hours postillumination. From 9 to 11 hours postillumination, the stalks become hardened and the heads further differentiate into lobules. Within the lobules, cytoplasmic cleavage occurs in a nonsynchronous manner to form protospores from approximately 11 to 15 hours postillumination. The protospores subsequently round up, and develop spines and other melanized spore wall material, so that at 19 hours postillumination the sporangia and spores are morphologically fully developed. Within the

Treatment	hours postillumination		
	24	48	72
Control	10/10	10/10	10/10
4 hours pi heat shock	8/10	8/10	8/10
6 hours pi heat shock	10/10	10/10	10/10
8 hours pi heat shock	0/10	0/10	0/10
10 hours pi heat shock	9/10*	9/10*	9/10*
12 hours pi heat shock	9/10*	9/10*	9/10*

* abnormal sporangia typical of heat shocks at these times.
(pi) postillumination.

Table I. Sporulation frequencies of control and heat shocked plasmodia.

Treatment	Germination frequency (%)
Control	0.7
0 hours pi heat shock	0.6
12 hours pi heat shock	1.6

(pi) postillumination.

Table II. Germination frequencies of spores produced by control and heat shocked plasmodia.

spores, mitosis occurs during the period from 15 to 19 hours postillumination and a subsequent meiosis occurs at around 48 hours postillumination. Since spores do not reach their full germination capacity until they are at least 3 to 5 days old, it is assumed that a further maturation process occurs within the fully formed spores.

A summary of the developmental effects induced by heat shocks at different stages of development is given in Figure 85b. Heat shocks administered early in development, i.e. up to approximately 6 hours postillumination, induce a slight delay in the subsequent steps in the differentiation process but do not affect the morphology of the resulting sporangia and spores. The frequency of sporulation of such plasmodia is not significantly different from that of control plasmodia although the sporangia produced subsequent to a 6 hours postillumination heat shock are somewhat smaller. The germination frequency of spores produced subsequent to a heat shock at 0 hours postillumination indicates that they are as viable as control spores. In the scanning electron microscope, spores produced subsequent to heat shocks at 0, 2, 4, and 6 hours postillumination are structurally normal.

When plasmodia are heat shocked at either 7 or 8 hours postillumination, they usually undergo no further differentiation until at least 48 hours postillumination. The only changes that occur during this time are a darkening of the plasmodial strands and their diffuse appearance. Occasionally some development does occur soon after a heat shock at 8 hours postillumination but the sporangia and spores so produced are grossly abnormal. However this is an atypical response to heat shock at this stage of development. Those heat shocked plasmodia that recommence sporulation after 48 hours, do so in a nonsynchronous manner and only following the formation of new plasmodial strands. Thus, in this case, the sporulation process is renewed rather than continued and leads to the production of sporangia and spores that are indistinguishable from those of control plasmodia. It is probable that the original developmental process is arrested by the heat shock and subsequent development can only occur after a period of regeneration. At the ultrastructural level, abnormalities appear in the

cytoplasm, mitochondria, and nuclei of the plasmodia soon after the heat shock. Subsequently, a general degenerative process appears to begin. For new plasmodial growth to occur after 48 hours postillumination, some areas of the cytoplasm must either recover or escape from this process.

When plasmodia are heat shocked late in development, i.e. at 10 and 12 hours postillumination, only abnormal sporangia are produced and little, if any, subsequent development occurs. Apparently the option to renew development, still available at 8 hours postillumination, has been lost at these later stages of development. At both 10 and 12 hours postillumination, sporangial development is well advanced and consequently heat shocks at these times cannot prevent the production of sporangia. However all those plasmodia that have reached the normal stage of development at the time of these heat shocks subsequently produce typical abnormal sporangia. Following a heat shock at 10 hours postillumination, at which time sporangia have begun to form lobules, the sporangia develop no further. Although the sporangial heads do appear to subsequently darken, their appearance is light brown rather than black and is probably due to the breakdown of cytoplasmic material, seen to occur in thin sections of these sporangia, rather than the production of melanin. Indeed, there is evidence to suggest that melanization is dependant on the development of spore walls, an event which does not occur subsequent to a heat shock at 10 hours postillumination. When plasmodia are heat shocked at 12 hours postillumination however, the sporangia have completed the formation of lobules and these have begun to cleave into protospores. In this case, it appears that those areas of the sporangium that have cleaved into protospores at the time of the heat shock are protected to some extent from the heat shock and some further development can occur. Thus partial melanization of the sporangium is seen to occur in association with spore wall formation. The spores that are produced subsequent to a heat shock at 12 hours postillumination appear to be somewhat more viable than control spores and this may be due to the thinner spore walls of these spores and hence their easier germination. Apart from the abnormal spore walls, these spores appear normal.

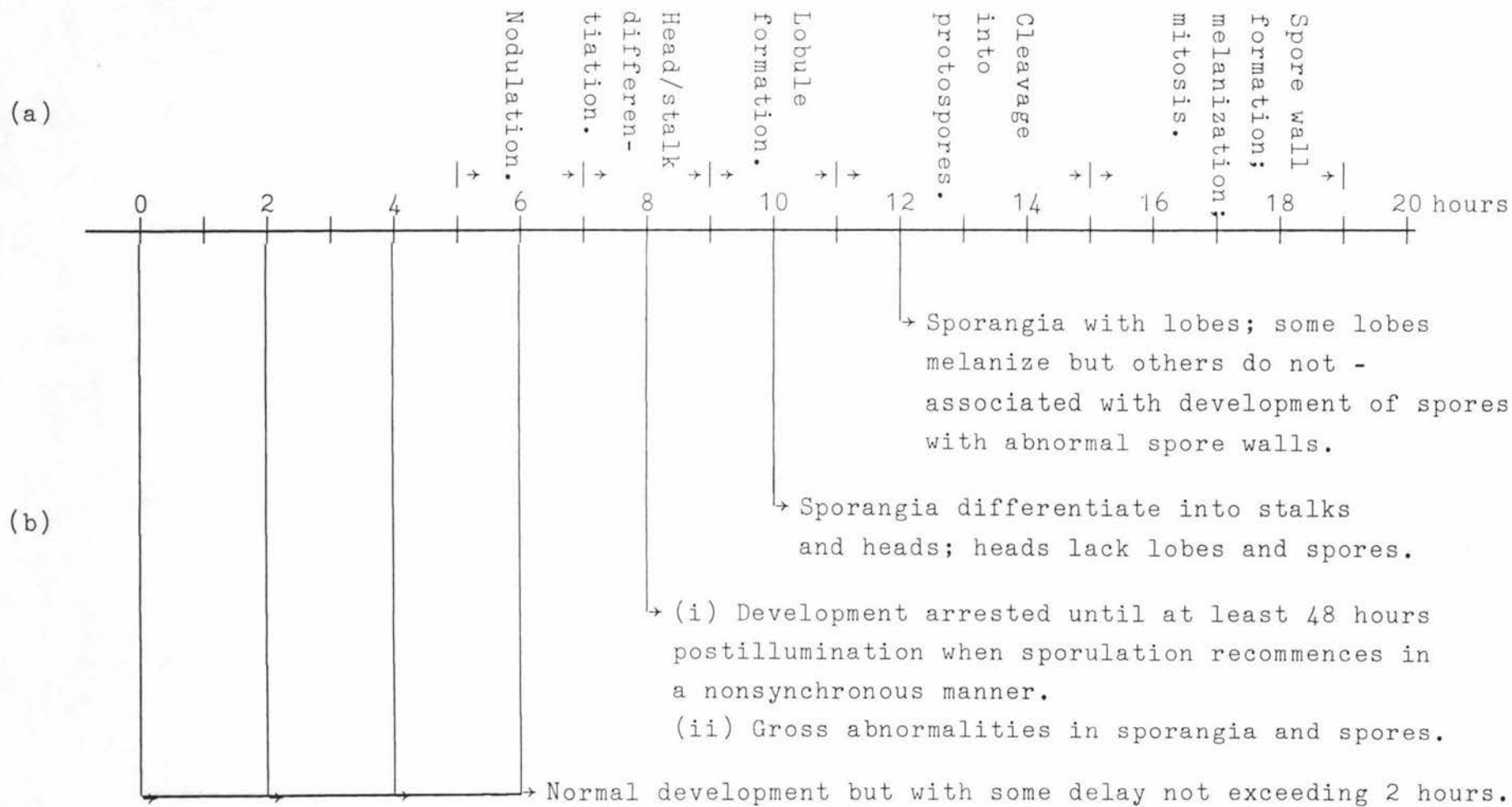


Figure 85. (a) Normal development of plasmodia following 72 hours of starvation and 4 hours of illumination. (b) Development following heat shocks at specified stages of development.

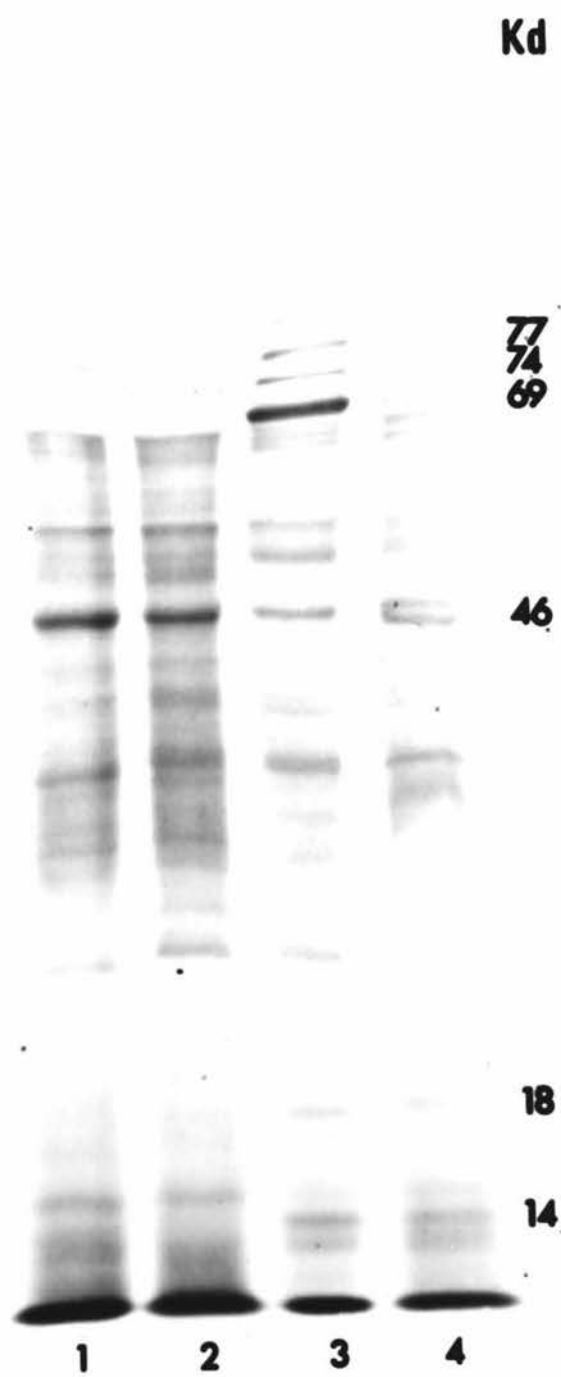
3.2 Effects of heat shock on protein synthesis.

3.2.1 Heat shocks during development.

To establish whether developing *Physarum* plasmodia synthesize heat shock proteins, and if so, whether the synthesis of these proteins is influenced by the developmental state of the plasmodia, plasmodia were simultaneously heat shocked and labeled at 0 hours post-illumination and again at 7 hours postillumination (Fig. 86). At both 0 and 7 hours postillumination, control plasmodia synthesize a variety of proteins as indicated by the large number of bands in the respective tracks. The predominant protein at both stages of development has an approximate molecular weight of 46,000 daltons. *Physarum* actin, a major protein product during normal plasmodial growth, has a molecular weight of 45,000 daltons (Jockush *et al.*, 1971) and therefore the predominant protein reported here is referred to as the actin band. On shifting to 37°C, a marked change occurs in the patterns of proteins synthesized by plasmodia. At both 0 and 7 hours postillumination, the control protein bands are generally less intense with a change in the actin band most pronounced. Clearly the synthesis of actin at the heat shock temperature is considerably reduced. However, as well as the changes that occur in the synthesis of those proteins normally synthesized at 22°C, a number of new protein bands appear in plasmodia heat shocked at 0 hours postillumination. These new proteins have approximate molecular weights of 77,000, 74,000, 69,000, 18,000, and 14,000 daltons. Since the induction by heat shock of these new proteins resembles the heat shock response of *Drosophila*, they are referred to as heat shock proteins. Of these heat shock proteins, the 69,000 dalton protein band predominates. A similar set of proteins is also synthesized by plasmodia when heat shocked at 7 hours postillumination, however in this case the 69,000 dalton protein band no longer predominates. In addition, it is difficult to distinguish the 74,000 and 77,000 dalton protein bands from control bands at a similar molecular weight.

Although the pattern of normal protein synthesis is somewhat suppressed at the heat shock temperature and especially the synthesis of actin, many control bands are

Figure 86. Effects of 37°C heat shocks on protein synthesis of developing plasmodia at 0 hours and 7 hours postillumination. Plasmodia were simultaneously heat shocked and labeled with 5µCi (¹⁴C) amino acid mix per plate for 1 hour. Track 1 - control plasmodia labeled at 22°C at 0 hours postillumination; track 2 - control plasmodia labeled at 22°C at 7 hours postillumination; track 3 - heat shocked plasmodia labeled at 37°C at 0 hours postillumination; track 4 - heat shocked plasmodia labeled at 37°C at 7 hours postillumination. Gel electrophoresis was carried out with a total of 815 counts per track and the fluorogram was developed after 72 days.



still visible in both 0 and 7 hours postillumination heat shock tracks. This indicates that the proteins normally synthesized at 22°C continue to be synthesized after the plasmodia are shifted to the heat shock temperature. To establish whether these proteins are actually being synthesized at the elevated temperature or are synthesized in the period it takes for the plasmodia to equilibrate at the heat shock temperature, the plasmodia were allowed to equilibrate at the heat shock temperature for 30 minutes before being labeled for 1 hour (Fig. 87). At 0 hours postillumination, plasmodia normally synthesize a large number of proteins and again the actin band predominates. In this experiment, actin has an approximate molecular weight of 44,000 daltons. However when plasmodia at 0 hours postillumination are heat shocked at 37°C, the intensity of all control bands, and especially the actin band, are greatly reduced while a number of bands not appearing at 22°C become apparent. These new bands, representing heat shock proteins, have approximate molecular weights of 78,000, 75,000, 73,000, 70,000, 17,000, and 14,000 daltons, with the 70,000 dalton protein band being most predominant. At 10 hours postillumination, when plasmodia are in quite a different developmental state, the overall synthesis of proteins at 22°C and the synthesis of actin in particular appears to be reduced relative to 0 hours postillumination. Clearly the patterns of protein synthesis at these two times are quite different, reflecting the altered requirements at different stages of development. However when plasmodia at 10 hours postillumination are heat shocked, a set of new protein bands with molecular weights very similar to those synthesized by plasmodia heat shocked at 37°C at 0 hours postillumination, becomes apparent. An additional protein band with an approximate molecular weight of 85,000 daltons can also be identified in the 10 hours postillumination heat shock track. In contrast to the fate of normal protein synthesis during a heat shock at 0 hours postillumination, most normal protein bands are apparent when plasmodia are heat shocked at 10 hours postillumination in addition to the heat shock protein bands.

Thus in the case of plasmodia heat shocked at 37° at 0

Figure 87. Effects of 32°C and 37°C heat shocks on protein synthesis of developing plasmodia at 0 hours and 10 hours postillumination. Plasmodia were simultaneously heat shocked and labeled with 20 μ Ci (³⁵S) methionine per plate for 1 hour following a 30 minute pretreatment at the new temperature. Track 1 - control plasmodia labeled at 22°C at 0 hours postillumination; track 2 - heat shocked plasmodia labeled at 32°C at 0 hours postillumination; track 3 - heat shocked plasmodia labeled at 37°C at 0 hours postillumination; track 4 - control plasmodia labeled at 22°C at 10 hours postillumination; track 5 - heat shocked plasmodia labeled at 37°C at 10 hours postillumination. Gel electrophoresis was carried out with a total of 1230 counts per track and the fluorogram was developed after 90 days.

hours postillumination, a pretreatment at the elevated temperature before the addition of label effectively reduces the labeling of normal proteins while heat shock proteins are still labeled. Therefore it can be assumed that the heat shock effectively reduces the synthesis of normal proteins. However in the case of plasmodia heat shocked at 10 hours postillumination, the pretreatment does not appear to be effective in reducing the extent to which normal proteins are labeled at the elevated temperature although this may be associated with the low level of normal protein synthesis at this stage of development.

For the experiment relating to Figure 87, the actual level of incorporation of radioactive precursors into plasmodial protein for each treatment was determined (Table 3). It can be seen from these results that the synthesis of all proteins is reduced by approximately 75% by a heat shock at 37°C at 0 hours postillumination. Since heat shock proteins are strongly synthesized at this stage of development, this decrease in total protein synthesis is a reflection of the reduction in synthesis of normal proteins at the elevated temperature. However in the case of a heat shock at 10 hours postillumination, the synthesis of normal proteins is at a low level at 22°C and little change occurs in the total protein synthesis at the elevated temperature. While there is little change in total protein synthesis at 10 hours postillumination, it is clear from the fluorogram that heat shock proteins are synthesized at this time and that the level of heat shock protein synthesis is very similar to that at 0 hours postillumination. The level of total protein synthesis at the heat shock temperature (37°C) is also very similar and therefore it cannot be assumed that heat shock at 10 hours postillumination fails to repress the synthesis of normal proteins.

3.2.2 The response to different heat shock temperatures.

To establish whether the synthesis of heat shock proteins and the parallel repression of normal protein synthesis could be induced by a heat shock at a temperature lower than 37°C, plasmodia were heat shocked at 32°C as well as 37°C at 0 hours postillumination (Fig. 88). Again, control

<u>Treatment</u>	<u>ug protein/ plasmodium ($\times 10^3$)</u>	<u>counts/ plasmodium ($\times 10^4$)</u>	<u>counts/μg plasmodial protein</u>
22°C control at 0 hours pi	4.1	27	66
32°C heat shock at 0 hours pi	3.9	56	140
37°C heat shock at 0 hours pi	2.1	3.7	18
22°C control at 10 hours pi	3.3	5.2	16
37°C heat shock at 10 hours pi	2.2	3.9	18

(pi) postillumination.

Table III. Incorporation of labeled precursors into plasmodial protein at 22°C, 32°C, and 37°C during development (c.f. Fig. 87).

plasmodia at this stage of development synthesize a variety of proteins, with the predominant protein being actin of approximately 45,000 daltons for this experiment. Upon transfer to 32°C, plasmodia continue to synthesize normal proteins, as indicated by the relative intensities of the bands in each track, at a similar level as control plasmodia with the exception of actin which appears to be synthesized at a slightly reduced level. However in addition to these normal proteins, plasmodia at 32° synthesize a set of heat shock proteins with approximate molecular weights of 86,000, 78,000, 75,000, 73,000, 69,000, and 18,000 daltons. Upon transfer to 37°C, the synthesis of normal proteins and especially that of actin is reduced while the same set of heat shock proteins as synthesized by 32°C heat shocked plasmodia is synthesized. An additional protein band at 82,000 daltons can also be identified. At both heat shock temperatures, the 69,000 dalton heat shock protein predominates. Following a prolonged exposure of an identical fluorogram (Fig. 89), the large number of normal protein bands at 22°C are considerably more distinct and the effect of a 37°C heat shock on these bands is more evident. The heat shock proteins synthesized at 37°C are also more distinct and an additional heat shock protein band at approximately 14,000 daltons appears. However a number of normal proteins do incorporate label at 37°C indicating that the synthesis of these proteins is not entirely repressed at the elevated temperature.

A similar result for the effect of a 32°C heat shock on protein synthesis was obtained in a separate experiment (Fig. 87). In addition to the heat shock proteins identified above for this fluorogram, the synthesis of a new protein with an approximate molecular weight of 62,000 daltons was detected in this experiment. In this case the total level of incorporation of labeled precursors into plasmodial protein was also determined (Table 3). As can be seen from these results, total protein synthesis at 32°C is approximately twice that at 22°C for plasmodia at 0 hours postillumination. To what extent this increase in total protein synthesis is due to increased synthesis of normal proteins or the synthesis of new heat shock proteins is unclear.

Figure 88. Effects of 32°C and 37°C heat shocks on protein synthesis of developing plasmodia at 0 hours post-illumination. Plasmodia were simultaneously heat shocked and labeled with 20µCi (³⁵S) methionine per plate for 1 hour following a 30 minute pretreatment at the new temperature. Track 1 - control plasmodia labeled at 22°C; track 2 - heat shocked plasmodia labeled at 32°C; track 3 - heat shocked plasmodia labeled at 37°C. Gel electrophoresis was carried out with a total of 7700 counts per track and the fluorogram was developed after 21 days.

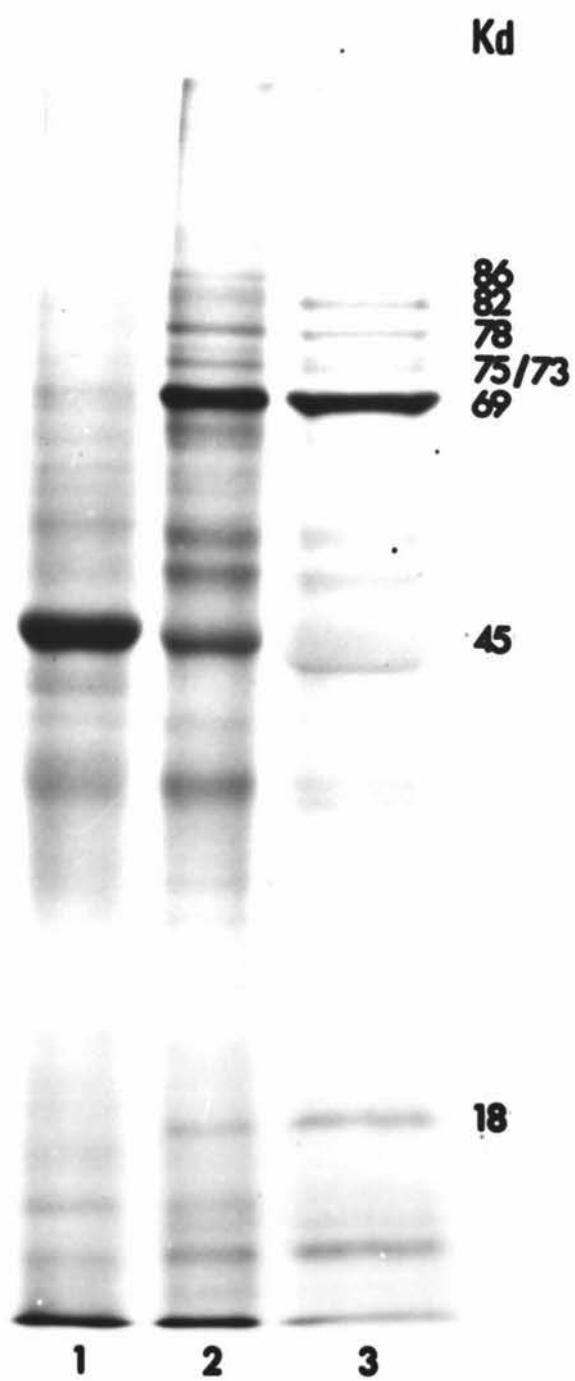
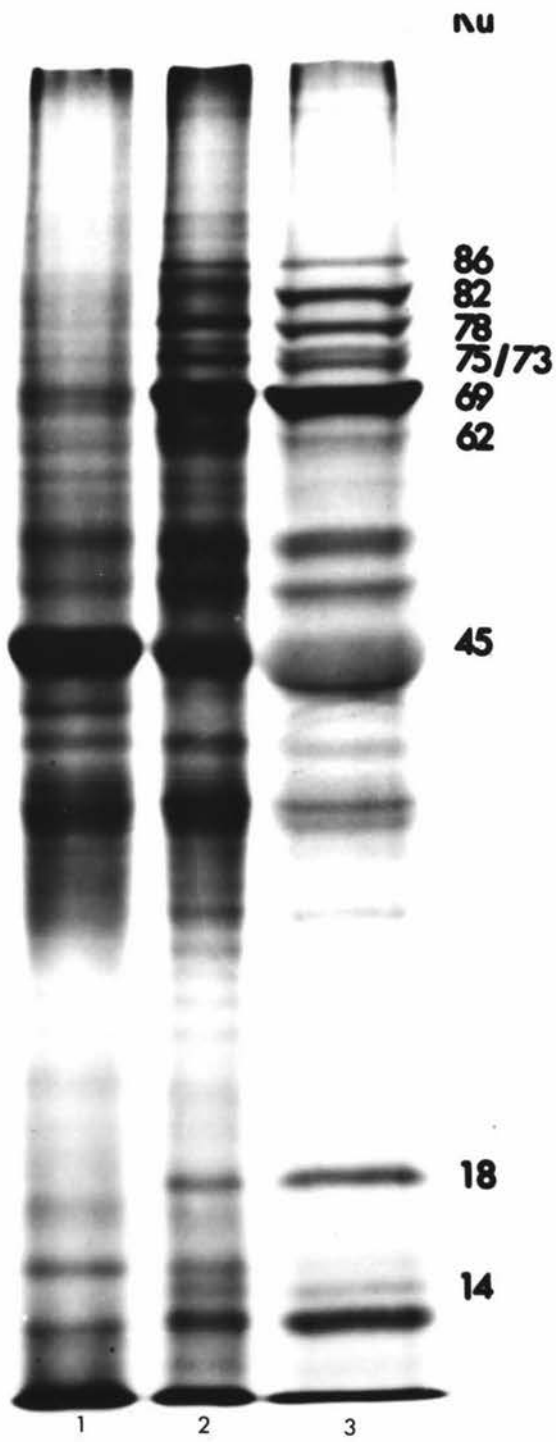


Figure 89. Effects of 32°C and 37°C heat shocks on protein synthesis of developing plasmodia at 0 hours post-illumination. Details as for Figure 88 but developed after 34 days.

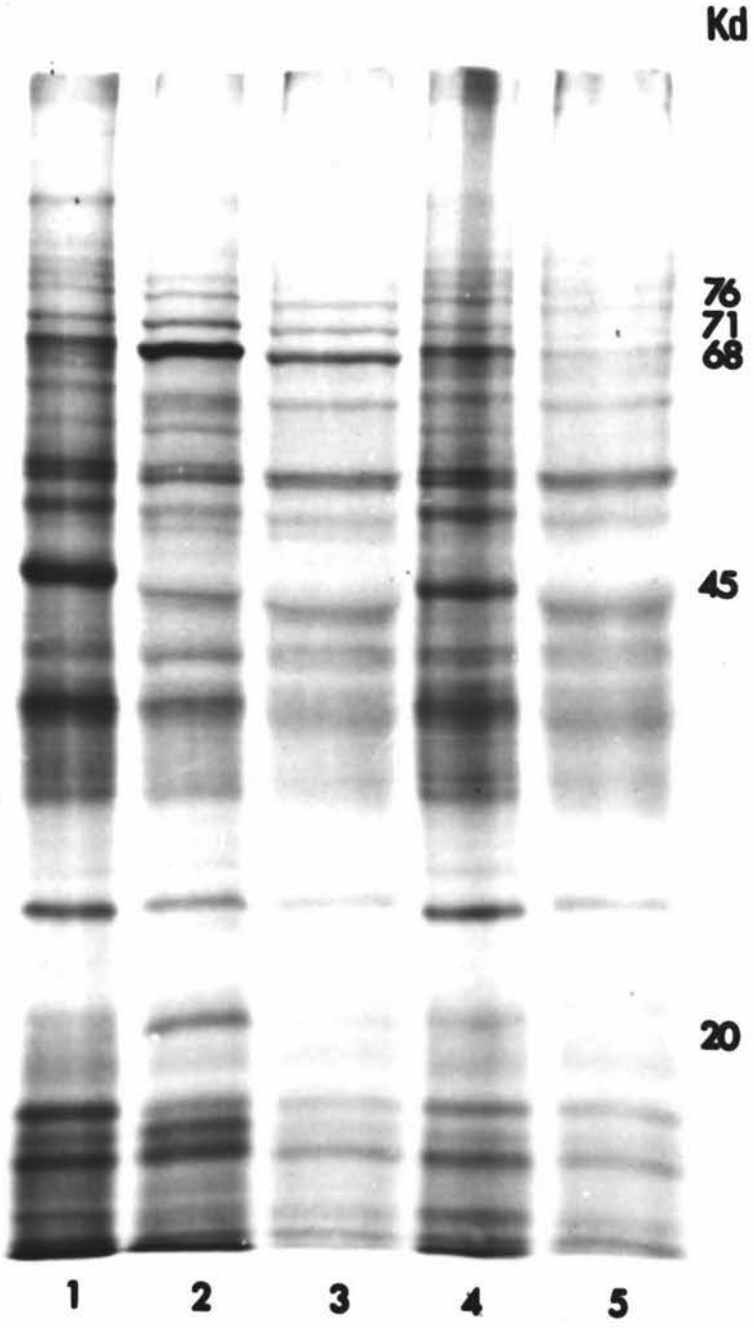


3.2.3 Protein synthesis patterns of developing plasmodia on return to 22°C following heat shock.

To determine the longer term effects of a 37°C heat shock on subsequent protein synthesis at 22°C, plasmodia at 2 hours postillumination were heat shocked for 30 minutes and then returned to 22°C (time 0 hours). Control plasmodia and heat shocked plasmodia were labeled from 0 - 1 hours and from 2 - 3 hours, and heat shocked plasmodia alone were labeled from 1 - 2 hours following the return to 22°C (Figure 90). Control plasmodia in both period synthesize a variety of proteins typical of early development with the synthesis of actin, which in this case has an approximate molecular weight of 45,000 daltons, predominating. An unusual feature of both of the control tracks is the appearance of a band at 68,000 daltons, a protein usually associated with heat shock proteins. It is possible that this protein represents a stress protein, the synthesis of which is induced by the two shifts of control plasmodia to fresh SM at 22°C. From 0 - 1 hours after the heat shock, heat shocked plasmodia synthesize control proteins at a slightly reduced level in addition to synthesizing a set of heat shock proteins with molecular weights of 76,000, 71,000, 68,000, and 20,000 daltons. Again the 68,000 dalton heat shock protein predominates. From 1 - 2 hours, the synthesis of control proteins appears to be greatly reduced while the synthesis of most heat shock proteins appears to be somewhat reduced also. Thus the 20,000 dalton heat shock protein band does not appear in this track. From 2 - 3 hours, the synthesis of both control and heat shock proteins appears to be considerably reduced.

For the experiment relating to Figure 90, the actual level of incorporation of radioactive precursors into plasmodial protein for each period following heat shock was determined (Table 4). Control plasmodia from 0 - 1 hours have a rate of total protein synthesis similar to that of non-heat shocked plasmodia at 0 hours postillumination (Table 3). However heat shocked plasmodia during the same period have a much reduced rate of total protein synthesis, similar to that of plasmodia labeled during heat shock. From 1 - 2 hours following the heat shock, the rate of total protein synthesis is reduced even further, reflecting the apparent low level of

Figure 90. Effects of 37°C heat shock on subsequent protein synthesis at 22°C. Plasmodia at 2 hours post-illumination were heat shocked for 30 minutes at 37°C, returned to 22°C (time 0 hours), and subsequently labeled with 7.5µCi (¹⁴C) amino acid mix per plate for 1 hour. Track 1 - control plasmodia labeled from 0 - 1 hour; track 2 - heat shocked plasmodia labeled from 0 - 1 hours; track 3 - heat shocked plasmodia labeled from 1 - 2 hours; track 4 - control plasmodia labeled from 2 - 3 hours; track 5 - heat shocked plasmodia labeled from 2 - 3 hours. Gel electrophoresis was carried out with a total of 1400 counts per track and the fluorogram was developed after 46 days.



<u>Treatment</u>	<u>ug protein/ plasmodium ($\times 10^3$)</u>	<u>counts/ plasmodium ($\times 10^4$)</u>	<u>counts/μg plasmodial protein</u>
Control 0 - 1 hours	2.9	21	77
Heat shocked 0 - 1 hours	3.3	3.8	12
Heat shocked 1 - 2 hours	3.1	0.46	1.5
Control 2 - 3 hours	3.0	10	33
Heat shocked 2 - 3 hours	2.8	0.82	2.9

Table IV. Incorporation of labeled precursors into plasmodial protein following a 30 minute heat shock (c.f. Fig. 90).

synthesis of both control and heat shock proteins in the corresponding fluorogram. From 2 - 3 hours following the heat shock, control plasmodial protein synthesis is approximately 50% less than during the period from 0 - 1 hours, while the rate of protein synthesis of heat shocked plasmodia during this period remains low.

Clearly heat shock proteins are synthesized during the first hour at 22°C following heat shock but their synthesis subsequently diminishes to a very low level by the end of the third hour. The synthesis of control proteins is considerably reduced immediately after the heat shock and their synthesis remains at a low level until at least the end of the third hour. Over the same period, the synthesis of proteins by non-heat shocked plasmodia also decreases but to a far less extent. Thus, while heat shock effectively represses the synthesis of normal proteins and induces the synthesis of heat shock proteins, no recovery in normal protein synthesis is evident before the end of three hours following the heat shock, although heat shock protein synthesis does decay to negligible levels by this time.

From the earlier experiments involving the effects of heat shock on plasmodial development described above, it was assumed that a 30 minute, 37°C heat shock at 2 hours post-illumination would not adversely affect the subsequent development of plasmodia used in this experiment. Thus the patterns of protein synthesis of plasmodia at various times following the heat shock should reflect the recovery of developmental capacity. To confirm this assumption, four control and eleven heat shocked, non-labeled plasmodia were returned to 22°C and examined at 24 hours postillumination, at which time sporulation should have been complete. All four control plasmodia had sporulated at this time but none of the heat shocked plasmodia had done so. Therefore in this experiment, a heat shock at 2 hours postillumination induced an atypical developmental arrest and consequently the changes in protein synthesis following heat shock reported here reflect the repression of normal protein synthesis and the decay of heat shock protein synthesis following the heat shock but not necessarily the resumption of protein synthesis associated with resumption of normal development. All heat

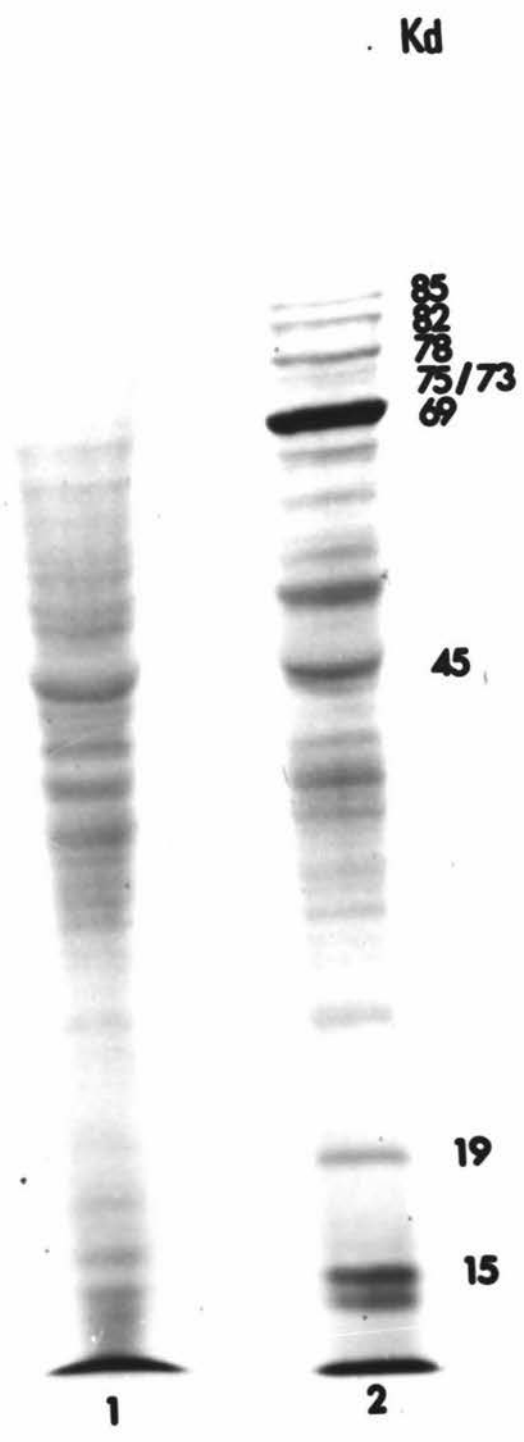
shocked plasmodia did resume normal sporulation at approximately 60 hours postillumination. The causes of this atypical result are unknown.

3.2.4 Heat shock during plasmodial growth.

In the presence of sufficient nutrients, *Physarum* plasmodia continue to grow but do not undergo sporulation. However when the supply of nutrients is exhausted, growth ceases and the developmental sequence leading to mature spores occurs. In these two different states, different genes must be expressed and thus different patterns of normal protein synthesis would be expected. To establish whether growing plasmodia have the same protein synthetic response to heat shock as sporulating plasmodia, in spite of the assumed difference in normal protein synthesis, they were heat shocked under the same conditions (Fig. 91). Control plasmodia maintained at 22°C synthesize a wide variety of proteins with an overall pattern resembling that of sporulating plasmodia. The most notable difference, bearing in mind that only relatively major protein products are being detected by this method, is the decreased rate of synthesis of actin which, in this experiment, has a molecular weight of approximately 45,000 daltons. When growing plasmodia are heat shocked at 37°C, they appear to synthesize control proteins at a level similar to that of plasmodia maintained at 22°C. In addition, heat shocked growing plasmodia synthesize the set of heat shock proteins typical of sporulating plasmodia with approximate molecular weights of 84,000, 82,000, 77,000, 69,000, 19,000, and 15,000 daltons. Again the 69,000 dalton heat shock protein predominates. Another novel protein with an approximate molecular weight of 51,000 daltons is also synthesized by heat shocked growing plasmodia. This protein band is only occasionally evident in tracks from heat shocked, sporulating plasmodia (Fig. 87, track 5).

When the results of the actual levels of radioactive precursors incorporated into protein of control and heat shocked growing plasmodia are examined (Table 5), it can be seen that heat shock increases the rate of total protein synthesis by approximately 100%. This clearly reflects the simultaneous synthesis of both control and heat shock

Figure 91. Effects of 37°C heat shock on protein synthesis of growing plasmodia. Growing plasmodia were simultaneously heat shocked and labeled with 7.5 μ Ci (14 C) amino acid mix per plate for 1 hour following a 30 minute pretreatment at the new temperature. Track 1 - control plasmodia labeled at 22°C; track 2 - heat shocked plasmodia labeled at 37°C. Gel electrophoresis was carried out with a total of 4400 counts per track and the fluorogram was developed after 26 days.



<u>Treatment</u>	<u>ug protein/ plasmodium ($\times 10^3$)</u>	<u>counts/ plasmodium ($\times 10^4$)</u>	<u>counts/μg plasmodial protein</u>
22 C control	3.4	5.7	17
37 C heat shock	2.5	9.5	38

Table V. Incorporation of labeled precursors into protein of growing plasmodia at 22°C and 37°C (c.f. Fig. 91).

proteins at the elevated temperature. Clearly heat shock of growing plasmodia does not lead to a repression of normal protein synthesis as it does for sporulating plasmodia.

3.2.5 Summary of *Physarum* heat shock responses.

A summary of the protein synthetic responses of sporulating plasmodia at different stages of development and of plasmodia undergoing active growth, to heat shock, is given in Table 6. In interpreting these results, it is necessary to be aware of the errors involved in calculating the molecular weight values. Errors are introduced through the calculated R_f values for the molecular weight standards as well as the R_f values of the unknown protein bands. Additional errors are introduced in the fitting of a line through the four protein standard molecular weight/ R_f points. Hence the molecular weight values given in Table 6 can only be considered approximate. While the actual errors have not been calculated, the variation in the molecular weight for actin illustrates the errors involved. Therefore it is probable that just one set of heat shock proteins is induced in heat shocked *Physarum* plasmodia, independent of developmental state. This set of heat shock proteins has molecular weights of 85,000, 78,000, 75,000, 73,000, 69,000, 18,000, and 14,000 daltons. An additional heat shock protein with a molecular weight of 82,000 daltons is occasionally evident. The major heat shock protein is that of 69,000 daltons.

Although the synthesis of heat shock proteins appears to be independent of developmental state, the response to heat shock in terms of the normal protein synthesis appears to differ as a function of developmental state. Thus sporulating plasmodia at different stages of development all reduce the synthesis of normal proteins during a 37°C heat shock, while growing plasmodia synthesize both heat shock proteins and normal proteins at 37°C. The response of growing plasmodia to a 37°C heat shock resembles that of sporulating plasmodia to a 32°C heat shock when both heat shock proteins and normal proteins are also synthesized simultaneously.

Fig.	Treatment*	Heat shock proteins ($\times 10^3$ Kd)	Major	
			hsp	Actin
86	0/22, 0/37, 7/22, 7/37.	77, 74, 69, 18, 14.	69	46
87	0/22, 0/32, 0/37, 10/22, 10/37.	85, 78, 75, 73, 70, 17, 14.	70	44
89	0/22, 0/32, 0/37.	86, 82, 78, 75, 73, 69, 18, 14.	69	45
90	2/22, 2/37.	76, 71, 68, 20.	68	45
91	gp/22, gp/37.	85, 82, 78, 75, 73, 69, 19, 15.	69	45

* developmental state (hours postillumination)/treatment temperature; (gp) growing plasmodia.

Table VI. Summary of *Physarum* heat shock responses.

SECTION 4. DISCUSSION

4.1 Normal development of *Physarum* sporangia.

The differentiation of surface plasmodia of *Physarum polycephalum* into mature sporangia can be readily induced experimentally. Plasmodia first become competent for sporulation following a prolonged period of starvation in darkness and in the presence of nicotinamide. Sporangial development itself can then be induced by a short period of illumination. In the experiments reported here, using *Physarum polycephalum* strain CL-2, plasmodia are starved for 72 hours and then illuminated for the following 4 hours. The subsequent sporangial development leading to fully mature sporangia is completed approximately 19 hours after the end of the illumination period. The timing of events in this developmental sequence is highly reproducible when the same experimental conditions are used. The results presented here relating to the timing of morphological changes that occur during the development of sporangia are also in agreement with those obtained for other strains of *Physarum* under slightly different experimental conditions (Guttes *et al.*, 1961; Daniel and Rusch, 1962; Daniel and Baldwin, 1964; Sauer *et al.*, 1969).

Although the first morphological change associated with sporangial development is shown here to occur at approximately 5 hours postillumination with the formation of nodules, it has been established by refeeding experiments that plasmodia become committed to sporulation soon after the illumination period (Daniel and Rusch, 1962; Sauer *et al.*, 1969). At the ultrastructural level, it has been shown that the light necessary for induction of sporangial development causes the formation of calcium-rich deposits in mitochondria and cytoplasmic vacuoles (Daniel and Järlfors, 1972b). However the first major change in ultrastructure becomes evident with the onset of cleavage. The process of cleavage appears to proceed by the formation of a vacuolar network that cuts off irregularly-shaped masses of cytoplasm (protospores) and these subsequently develop into mature spores. The results presented here for the development of *Physarum* strain CL-2 sporangia indicate that cleavage begins between 9 and 11 hours postillumination and is completed by

15 hours postillumination, when the cytoplasm of the sporangial head has cleaved entirely into protospores. This process is, to some extent, non-synchronous with some areas of the sporangial head containing almost fully delimited protospores at 11 hours postillumination, while other areas are only beginning to cleave at 13 hours postillumination. This aspect of cleavage has been reported for at least one other strain of *Physarum* (Laane and Haugli, 1976).

While most nuclei within the head of a sporangium become associated with areas of cytoplasm to form protospores, other nuclei appear to degenerate. Although the extent of nuclear degeneration during development was not determined here, it has been reported on the basis of light microscopical observations that up to one-fourth of the nuclei are degenerating at any one time during the period of several hours preceding spore formation (Guttes *et al.*, 1961). At all stages of development up to cleavage, nuclei remain unchanged in their appearance in the electron micrographs presented here. Their appearance is typical of interphase nuclei in growing plasmodia of a number of different strains of *Physarum polycephalum* (Guttes *et al.*, 1968; Goodman and Rusch, 1970; Lord *et al.*, 1977; Wille and Steffens, 1979; Cadrin *et al.*, 1981) as well as sporulating plasmodia of *Arcyria cinerea*, a member of a separate order of myxomycetes (Mims, 1972a). However at 15 hours postillumination, when cleavage is complete, the nuclei have undergone a change in appearance suggesting that a mitosis has begun between 13 and 15 hours postillumination. Mitosis has been reported to occur just prior to cleavage (Randall and Lynch, 1974) and at the beginning of cleavage in *Physarum* (Sauer *et al.*, 1969), and just prior to cleavage in *Arcyria cinerea* (Mims, 1972a). The completion of mitosis, as indicated by the reappearance of the nucleolus and chromocentres within each nucleus, has in this case certainly occurred by 19 hours postillumination and perhaps by 17 hours postillumination. In growing *Physarum* plasmodia, estimates of the duration of mitosis vary from 76 minutes (Wolf *et al.*, 1979) to 115 minutes (Turnock, 1979).

Following the completion of cleavage, the protospores round up and spore wall material is produced so that at 19

hours postillumination, structurally mature spores are evident. The results presented here for *Physarum* strain CL-2 development indicate that the spines are probably the first component of the spore wall to be laid down, beginning during the period from 15 to 17 hours postillumination. Thus spine formation appears to coincide with the rounding up of the protospores. The timing of spine formation in *Physarum* development therefore resembles that of *Physarella oblonga* development (Bechtel, 1977) rather than that of *Didymium iridis* and *Physarum flavicomum* development, where spine formation occurs while cleavage is still in progress, within the narrow cleavage furrows and where membranes of adjacent protospores touch (Aldrich, 1974; Aldrich and Blackwell, 1974). However it was often observed that spines were formed on adjacent spores at points of contact between them (e.g. Fig. 18) and therefore it is possible that the spines of *Physarum polycephalum* spores do form at the final points of attachment between protospores as has been described for *Arcyria cinerea* (Mims, 1972b). The deposition of spore wall material itself appears to lag behind the formation of the spines such that at 17 hours postillumination, when spine formation is almost complete, spore wall material has just begun to be deposited. This is in agreement with an earlier study of *Physarum* spore development (Randall and Lynch, 1974). By 19 hours postillumination, the deposition of spore wall material appears to be complete.

The mechanism whereby spore wall material is deposited during spore development in *Physarum* is unclear. It has been shown that melanin is responsible for the dark colour of the spore wall, constituting approximately 15% of the spore wall material (McCormick *et al.*, 1970). The reactions which lead to the production of melanin from its precursors are catalysed by a number of enzymes collectively referred to as polyphenol oxidase (Czaninski and Catesson, 1974). A kinetic study of polyphenol oxidase activity revealed a very active enzyme, capable of oxidising L-dihydroxyphenylalanine (L-DOPA), in sporulating *Physarum* plasmodia (Chet and Hütterman, 1977). The activity of this enzyme was at a maximum at 8 hours postillumination and decreased significantly after melanin synthesis (Chet and Hütterman, 1977). In the

experiments aimed at localizing polyphenol oxidase reported here, electron opaque vacuoles first become apparent within sporangia at 15 hours postillumination, before the formation of spines on the spore wall. Although these vacuoles are significantly less electron opaque in controls incubated in the absence of substrate, no change is apparent in those controls incubated with diethyldithiocarbamate (DDC) which has been reported as an inhibitor of polyphenol oxidase (Czaninski and Catesson, 1974; Eppig, 1974). A similar pattern is apparent at both 17 hours, when melanization appears to have begun, and 19 hours postillumination, when melanization appears to be complete. At both these latter times, a similar pattern of electron density is seen in the spines and in the spore walls as is seen in the vacuoles. Only rarely were the vacuoles seen to fuse with the spore membrane. Clearly, if the electron opaque areas do represent sites of polyphenol oxidase activity within the developing spore, the enzyme here has a somewhat different time course of activity than that described by Chet and Hütterman (1977). The reasons for this discrepancy are unknown. However L-DOPA also acts as a substrate for the enzyme peroxidase which is not inhibited by DDC (Czaninski and Catesson, 1974; Eppig, 1974). Peroxidase has also been shown to be present in spores of *Agaricus bisporus* (Rast *et al.*, 1981). Because peroxidase inhibited controls were not incorporated into the experiments reported here, the electron opaque areas cannot conclusively be identified as sites of polyphenol oxidase activity although this does remain a possibility. Therefore it cannot be assumed that the electron opaque vacuoles are necessarily associated with the deposition of spore wall material. A lack of peroxidase inhibited controls in the experiments reported by Chet and Hütterman (1977) may also be contributing to the discrepancy in the results for polyphenol oxidase activity during development.

It has been reported that the spines of *Phyisarum flavicomum* and *Didymium iridis* are deposited by the fusion of polysaccharide-containing vesicles, formed by the Golgi apparatus, with the outer protospore membrane (Aldrich, 1974; Aldrich and Blackwell, 1974). Although this mechanism was considered unlikely for the spine formation of

Physarella oblonga spores, vesicles associated with the Golgi apparatus were clearly implicated in the secretion of the inner spore wall in this organism (Bechtel, 1977). However no structures resembling Golgi bodies were evident during the deposition of spine or spore wall material in the present study of *Physarum polycephalum* development.

Although no specific structures can be directly associated with the spore wall formation during the development of *Physarum*, rough endoplasmic reticulum does first become apparent at 15 hours postillumination and remains evident until at least 19 hours postillumination. Thus the formation of rough endoplasmic reticulum do appear to parallel spine and spore wall formation. However the nature of the relationship, if indeed such a relationship exists, in this case remains unknown. It has been reported that endoplasmic reticulum changed from the smooth to the rough state during elaboration of the cell wall during spherule formation in *Physarum polycephalum* (Goodman and Rusch, 1970). In addition, endoplasmic reticulum has been associated with the developing spines of *Arcyria cinerea* spores (Mims, 1972b).

The formation of the spore wall during the development of *Physarum* reported here is complete by 19 hours postillumination and thus the spore is morphologically mature at this time. However further ultrastructural changes can be observed within the spore nucleus at 48 hours postillumination. These changes almost certainly indicate that meiosis is in progress at this stage of development. It has been concluded that meiosis occurs in spores of most species of myxomycetes between 12 and 18 hours after cleavage is complete (Aldrich and Blackwell, 1974). In spores of a heterothallic strain of *Physarum*, meiosis was shown to occur approximately 20 hours after spore cleavage (Laane and Haugli, 1976), while in an apogamic strain of *Physarum*, meiosis was shown to occur approximately 24 hours after cleavage (Laane *et al.*, 1976).

A number of other changes in ultrastructure also become evident throughout the developmental sequence leading to fully mature *Physarum* spores. At all times up to the completion of cleavage, but before spine formation has begun,

mitochondria maintain a consistent appearance, with each mitochondrion containing numerous tubular cristae and a centrally located nucleoid. Thus the appearance of mitochondria during this period resembles that typical of mitochondria in growing *Physarum* plasmodia (Daniel and Järlfors, 1972a; Kuroiwa *et al.*, 1977). While the spore wall is being formed, the typical internal structures can no longer be seen within the mitochondria. However once the spores are morphologically mature, these structures reappear. Within nuclei, bundles of microtubules are evident at 5 hours postillumination (data not shown) and again in fully mature spores at both 96 hours and 7 days postillumination (Figs. 22, 23, and 24). However the significance of these microtubules is unknown. Such structures have previously been reported to occur in sporulating *Physarum* plasmodia and in this case they were associated with meiosis (Laane *et al.*, 1976). In general, the presence of microtubules within the nucleus is usually associated with either meiosis or mitosis (Dustin, 1980). However these processes could not be associated with the presence of microtubules in the present study.

The morphology of mature *Physarum* spores is clearly revealed in the scanning electron micrographs presented here, as is the sporangial peridium containing these spores. Spores often have a somewhat collapsed appearance in preparations for the scanning electron microscope compared with the spherical shape of spores mounted in water and viewed in a light microscope. However it is probable that the appearance of spores in the scanning electron microscope merely reflects the natural desiccated state of the spores rather than being the result of improper dehydration, as has been suggested (Kislev and Chet, 1973).

4.2 The effects of heat shock on ultrastructure of developing *Physarum* sporangia.

Transmission electron microscopy of thin sections of heat shocked, developing *Physarum* sporangia reveals a number of ultrastructural abnormalities which seem to be invariant features of heat shock, regardless of the stage of development at which the heat shock is given. Soon after the

heat shock, mitochondria become more rounded in appearance and small vacuoles develop within them. Subsequently the ordered internal structure of the mitochondria disappears and they undergo a process of degeneration. When plasmodia heat shocked at 8 hours postillumination eventually recover from the heat shock, normal mitochondria are again apparent. Similarly, when recognizable spores are produced following a 12 hours postillumination heat shock, these contain normal mitochondria. However when no recovery of development occurs, as after a 10 hours postillumination heat shock and in areas of sporangia after a 12 hours postillumination heat shock, complete degeneration of mitochondria is apparent.

Mitochondrial abnormalities have been previously reported to occur in growing *Physarum* plasmodia following a 10 minute heat shock at 42°C (Lomagin, 1978). However in this case, mitochondria exhibited a more irregular outline than normal following the heat shock, and the changes induced by the heat shock were not as pronounced as those reported in the present study. A return of mitochondria to their normal appearance occurred within approximately 9 hours following the heat shock (Lomagin, 1978). Abnormalities have also been reported to occur in mitochondria of the chrysophycean flagellate, *Poterioochromonas malhamensis*, following heat shock (Schnepf and Schmitt, 1981). The repair of *P. malhamensis* mitochondria was largely completed within one hour following the heat shock, and this repair was shown to be dependant upon new protein synthesis following the heat shock (Schnepf and Schmitt, 1981). Abnormalities similar to those reported in the present study of heat shocked *Physarum* are also evident within mitochondria of heat shocked *Drosophila* cells in published electron micrographs, but receive no mention from the authors (Velazquez *et al.*, 1980).

Nuclear ultrastructure also appears to be affected by heat shock and the pattern of recovery or degeneration of the nuclei is the same as that of mitochondria. Soon after the heat shock, the nucleolus and chromocentres become more diffuse than in control nuclei, although the typical interphase appearance is maintained. In addition, macro-tubular structures often become apparent for the first time soon after the heat shock. These structures persist within

the nuclei of heat shocked plasmodia but are never apparent within the nuclei on non-heat shocked plasmodia. Very similar structures have been reported to occur in more than 39% of the nuclei of growing *Physarum* plasmodia shifted to 31°C, and in this report the microtubular origin of these structures was hypothesized (Del Castillo *et al.*, 1978). Structures described as macrotubules have also been reported to occur within nuclei of *Physarum* plasmodia where mitosis was blocked by CO₂-induced anaerobiosis (Wille and Steffens, 1981). However in the latter case, bundles of macrotubules as reported here to occur in heat shocked *Physarum* plasmodia undergoing development, were not reported. The initial heat shock-induced abnormalities within the nucleus are followed by later changes which appear to represent nuclear degeneration. The nuclear membrane develops a number of breaks and these are accompanied by areas of clearing within the nucleus. At later stages, almost complete degeneration of nuclei is evident. However where some recovery in development does occur following an 8 hours postillumination heat shock and in areas of sporangia produced following a 12 hours postillumination heat shock, normal nuclei are again evident. An additional abnormality seen within nuclei of developing plasmodia heat shocked at 12 hours post-illumination is the vacuolated appearance of these nuclei soon after the heat shock. Heat shock of growing *Physarum* plasmodia has previously been shown to induce abnormalities within nuclei (Lomagin, 1978), as has heat shock of *Poteroochromonas malhamensis* (Schnepf and Schmitt, 1981). However the specific abnormalities reported by these authors were not observed within the nuclei of *Physarum* plasmodia heat shocked during development.

Clearly recovery of developmental capacity following the induction by heat shock of ultrastructural abnormalities in developing *Physarum* plasmodia is possible. However, such recovery is evidently dependant upon the stage of development attained by the plasmodia at the time of the heat shock. In the present study, the ultrastructural details of developing plasmodia heat shocked at 8, 10, and 12 hours post-illumination only were examined. Of these, plasmodia heat shocked 8 hours postillumination eventually recovered their

developmental capacity while plasmodia heat shocked at 12 hours postillumination exhibited a partial recovery in synchrony with normal development. Although the ultra-structural details of plasmodia heat shocked at earlier stages of development were not examined, it can be assumed that similar abnormalities are induced by the heat shock. However it is evident that plasmodia heat shocked early in development are able to repair the heat shock-induced abnormalities much more quickly so that subsequent development suffers only a slight delay.

4.3 The effects of heat shock on protein synthesis in *Physarum*.

Sporulating *Physarum* plasmodia at a number of different stages of development as well as plasmodia undergoing active growth, all respond to a heat shock at 37°C with the synthesis of a set of apparently novel proteins that are either not normally synthesized at 22°C or are synthesized only at relatively low levels. The synthesis of these heat shock proteins appears to be a consistent response of *Physarum* plasmodia to the elevated temperature and, from a combination of the results of a number of separate experiments, it is evident that the *Physarum* heat shock proteins have approximate molecular weights of 85,000, 78,000, 75,000, 73,000, 69,000, 18,000, and 14,000 daltons. In all experiments, the 69,000 dalton protein is the major heat shock protein synthesized while in a minority of experiments, additional heat shock proteins are also synthesized. In parallel with the synthesis of heat shock proteins by sporulating plasmodia there is a considerable reduction in the synthesis of normal (22°C) proteins during a heat shock at 37°C. This is particularly pronounced for the synthesis of the protein actin which is, in most cases, the predominant protein synthesized by sporulating plasmodia at the normal incubation temperature. Thus the overall protein synthetic response of sporulating plasmodia to heat shock at 37°C is very similar to the heat shock response of *Drosophila* as well as those of many other organisms (see Introduction). Although the majority of *Physarum* heat shock proteins differ in molecular weight from those of *Drosophila*,

the major heat shock protein of *Physarum* closely resembles in molecular weight the 70,000 dalton, major heat shock protein of *Drosophila*.

Although plasmodia undergoing active growth do synthesize heat shock proteins when heat shocked at 37°C, the synthesis of those proteins normally synthesized at 22°C is apparently not reduced. Similarly, when sporulating plasmodia are heat shocked at 32°C, both normal proteins and heat shock proteins are synthesized concurrently. Clearly the minimum temperature required to induce the synthesis of heat shock proteins is lower than the minimum temperature required to repress the synthesis of normal proteins and the latter temperature differs for growing and sporulating plasmodia. Thus the synthesis of normal proteins by growing plasmodia but not sporulating plasmodia, when heat shocked at 37°C, probably reflects differing sensitivities to elevated temperatures at different stages of the life cycle of *Physarum*. In this respect the heat shock response of *Physarum* appears to differ from that of *Drosophila*. The concurrent synthesis of normal proteins and heat shock proteins at certain temperatures by *Physarum* supports the view that the heat shock proteins themselves are not responsible for the repression of those genes active before the heat shock (Arrigo, 1980).

When sporulating plasmodia at an early stage of development (2 hours postillumination) are heat shocked at 37°C and then returned to 22°C, the synthesis of heat shock proteins, and particularly that of the 69,000 dalton heat shock protein, continues for 2 to 3 hours following the return. Although the synthesis of heat shock proteins does decrease markedly during this period, there is no significant resumption of normal protein synthesis and the total protein synthesis at the end of this period remains much reduced. Thus these results indicate that the delay in resumption of normal protein synthesis by sporulating *Physarum* plasmodia, following a heat shock at 37°C, exceeds 3 hours. However, the atypical developmental arrest associated with this experiment (see Section 3.2.3) suggests that this conclusion may not be valid where only the typical delay in subsequent development occurs following heat shock

at this stage of development. Following the heat shock of *Dictyostelium* cells, the synthesis of the major heat shock protein continues at a high rate for 4 hours and then gradually returns to the initial low level during the subsequent 4 hours (Loomis and Wheeler, 1980). Similarly, on return to the normal incubation temperature, heat shocked *Drosophila* cells continue to synthesize heat shock proteins for approximately 8 hours (Arrigo *et al.*, 1980) while normal patterns of protein synthesis occur 4 hours after heat shock of HeLa cells (Slater *et al.*, 1981) and chinese hamster ovary cells (Bouche *et al.*, 1979). The typical developmental response of sporulating *Physarum* plasmodia heat shocked early in development is a delay in subsequent development not exceeding 2 hours (see below). If the resumption of normal development following the heat shock of *Physarum* is dependant upon the resumption of normal protein synthesis, then it is to be expected that normal patterns of protein synthesis would be evident before 3 hours following the heat shock of plasmodia at this stage of development.

4.4 The effects of heat shock on development of *Physarum*.

When sporulating *Physarum* plasmodia are heat shocked during the early stages of sporangial development, i.e. at 0, 2, 4, and 6 hours postillumination, development subsequent to the heat shock in all cases suffers a slight delay. However, approximately 100% of the sporangia heat shocked at these times complete the sporulation process to produce mature sporangia and spores that are indistinguishable from those produced by non-heat shocked plasmodia. In addition, the germination frequencies of mature spores produced by plasmodia heat shocked during early development, where determined, are not significantly different from those of non-heat shocked plasmodia. Since sporangial development was recorded photographically at 2 hour intervals in the experiments reported here, it is not possible to accurately quantify the actual extent of the delays in subsequent development induced by these heat shocks. However for heat shocks at all times during early sporangial development, such delays did not exceed 2 hours.

Of the heat shock treatments administered during the early stages of development, the patterns of normal protein

synthesis and of heat shock protein synthesis were characterized by fluorography at 0 hours postillumination only (Figs. 86, 87, and 89; Table 3). From these results, it is evident that plasmodia normally synthesize a large number of proteins, the predominant protein being actin. When plasmodia at this stage of development are heat shocked, the synthesis of normal proteins is considerably reduced and the synthesis of heat shock proteins occurs instead. This change is most pronounced with the reduction in intensity of the actin protein band and the appearance of the 69,000 dalton heat shock protein band during heat shock. A very similar change occurs in the synthesis of actin and the 70,000 dalton, major heat shock protein following heat shock of *Dictyostelium* cells (Loomis and Wheeler, 1980). Considering the evidence from the work on heat shock responses of many other organisms, especially *Drosophila* (reviewed in Introduction), it can be assumed that while developing *Physarum* plasmodia are at the elevated temperature, those genes active in the developmental process before the heat shock are no longer transcribed and specific heat shock genes are transcribed instead. However, upon return to the normal incubation temperature, the transcription of heat shock genes ceases and the transcription of genes in the developmental program is able to resume. Thus normal development can subsequently recommence after some additional delay, with the lack of transcription of normal genes during the heat shock not affecting subsequent development. Contributing to the delay in subsequent development may be the requirement for new protein synthesis, outside normal protein synthesis for development, to repair structural defects caused by the elevated temperature. Consequently, the net effect of heat shocks during the early stages of *Physarum* development is only a delay in subsequent development upon return to the normal incubation temperature. It has been reported that the development of *Dictyostelium* cells resumes with less than a 4 hour lag following heat shock (Loomis and Wheeler, 1980). Similarly, the development of *Drosophila* pupae is delayed following heat shock (Lindsley and Poodry, 1977; Mitchell and Lipps, 1978), as is the embryogenesis of *Drosophila* (Dura, 1981). However in the latter case, the recovery of development did not seem to be

dependant upon the recovery of normal protein synthesis (Dura, 1981).

In contrast, sporulating *Physarum* plasmodia heat shocked at 8, 10, and 12 hours postillumination respond quite differently to the heat shock in terms of their subsequent development. Heat shocks at 8 hours postillumination appear to produce a complete developmental arrest accompanied by some degeneration at the morphological level of both plasmodial strands and immature sporangia. This degeneration is also evident in the ultrastructure of these plasmodia. However, development does recommence after approximately 48 hours postillumination, albeit non-synchronously, and the resulting sporangia and spores appear quite normal. Thus the developmental arrest caused by heat shocks at this stage of development cannot be associated with complete death of the plasmodium. Heat shocks at 10 hours postillumination also produce a complete developmental arrest at approximately that stage of development attained by plasmodia at the time of the heat shock. Again some degeneration of the immature sporangia is evident in both their morphology and ultrastructure. No further development is evident subsequent to a heat shock at 10 hours postillumination. Heat shocks at 12 hours postillumination do not affect the morphology of sporangia, these having attained their mature form by the time of the heat shock. However, it is apparent that those areas of each sporangium that have not cleaved into protospores at the time of the heat shock develop no further and subsequently degenerate after the heat shock, while those areas that have cleaved into protospores do undergo some further development. Such development leads to the production of mature spores which appear somewhat abnormal but which are, apparently, at least as viable as control spores. The responses to heat shocks at 8, 10, and 12 hours postillumination are total, with all plasmodia at each time point/heat shock responding in the typical manner. As a result of the developmental arrest caused by heat shocks at each time point, sporangia appear grossly abnormal relative to non-heat shocked plasmodia. The actual extent of the abnormalities is dependant upon the stage in the developmental process attained by plasmodia at the time of the heat shock.

Of the heat shock treatments of developing *Physarum* plasmodia that induce a partial or total arrest in subsequent development, the patterns of normal and heat shock protein synthesis were characterized at 7 and 10 hours postillumination (Figs. 86 and 87; Table 3). From these results it is evident that plasmodia at 7 hours postillumination normally synthesize a variety of proteins similar to those synthesized by plasmodia during early development. When these plasmodia are heat shocked, the typical set of heat shock proteins is synthesized. In spite of the lack of a pretreatment step at the elevated temperature to allow for completion of on-going protein synthesis before the addition of labeled amino acids for this experiment, a significant reduction in the synthesis of actin is evident. Thus it is apparent that plasmodia at this stage of development also respond to heat shock with a reduction in the synthesis of those proteins being synthesized before the heat shock. At 10 hours postillumination, the overall rate of protein synthesis by plasmodia undergoing normal development is considerably reduced relative to that of plasmodia at 0 hours postillumination. In addition, the synthesis of actin no longer appears to predominate. When these plasmodia are heat shocked, the typical set of heat shock proteins is again synthesized. However, with normal protein synthesis at a low level at this stage of development, it is difficult to determine what changes occur in the synthesis of these proteins in response to the heat shock.

Thus, while the developmental consequences of heat shock on sporulating *Physarum* plasmodia can be divided into two broad categories, i.e. 0 to 6 hours postillumination and approximately 8 to 12 hours postillumination, the effects of heat shock on protein synthesis at all stages of development appear to be relatively constant. The major change that occurs in protein synthesis as sporulation proceeds appears to be a reduction in total protein synthesis by 10 hours postillumination although it is possible that this reflects an accelerated rate of degradation of newly synthesized or total proteins. However it is not known if the apparent low level of normal protein synthesis is a general feature of the later stages of sporangial development. Clearly, there

must be fundamental differences between plasmodia in these two phases of the sporulation sequence for there to be differences in the developmental consequences of heat shock. It was originally reported that 60% of those sporangia that formed subsequent to 30 minute, 37°C heat shocks of developing *Physarum* plasmodia in the period from 5 to 9 hours postillumination were abnormal whereas 3% were abnormal following heat shocks in the period from 0 to 4 hours postillumination and none were abnormal following heat shocks in the period from 10 to 15 hours postillumination (Sauer *et al.*, 1969). Although the precise timetable of morphogenesis in *Physarum* is a function of the prior starvation conditions (Wilkins, personal communication), the results presented by Sauer *et al.*, (1969) suggest that, under their conditions of starvation, the period from 5 to 9 hours postillumination is a particularly sensitive one during the development of sporangia. This is confirmed by the results presented here, with the sensitive period beginning between 6 and 8 hours postillumination. The absence of abnormalities following heat shocks in the period from 10 to 15 hours postillumination (Sauer *et al.*, 1969) is, in the absence of a definition of the abnormalities observed, more difficult to reconcile with the results presented in this report. However it is probable that heat shocks applied after approximately 13 hours postillumination would, for the developmental timetable presented here, have little effect on the subsequent external appearance of sporangia.

Specific abnormalities have been reported to occur in a number of organisms following heat shocks applied during developmentally sensitive periods, most notable of which are the heat shock-induced phenocopies of *Drosophila* (reviewed in Introduction). As is evident from the results presented here, heat shocks applied to sporulating *Physarum* plasmodia at specific stages of development can induce the production of abnormal sporangia and such abnormalities are specific for a heat shock at a particular stage of sporangial development. Although phenocopies of *Drosophila* are specific abnormalities within an otherwise normal adult and the abnormalities reported here for *Physarum* appear to involve the complete developmental arrest of the organism, the

underlying molecular basis for the two phenomena is probably the same. Thus the developmental arrest of sporulating *Physarum* plasmodia may be due to inhibition of normal gene expression, the arrest occurring through the lack of expression of specific genes necessary for continuation of the developmental program.

As mentioned above, sporulating *Physarum* plasmodia at some point in their development become committed to sporulate. Prior to this point in development, plasmodia can resume growth rather than continue with the sporulation process if refeed with glucose, but beyond this point sporulation is irreversible (Sauer *et al.*, 1969). Clearly this point in development represents a transition from a reversible to an irreversible developmental program. From the results presented here, it is evident that a transition point in development occurs between 6 and 8 hours post-illumination such that heat shocks before this point merely delay subsequent development whereas heat shocks after this point appear to arrest development. However the relationship between the point of commitment determined by refeeding experiments to that determined by heat shock is unclear.

In addition to its effects on transcription of the genome, heat shock also directly affects translation of mRNA species such that during the heat shock, heat shock mRNA is translated while normal mRNA is not (reviewed in Introduction). Therefore it is possible that the effects of heat shock on developing *Physarum* plasmodia reported here reflects solely the disruption of protein synthesis in response to heat shock. If this were so, then inhibition of protein synthesis with cycloheximide at a time in the developmental process when plasmodia are particularly sensitive to heat shock (approximately 7 - 8 hours post-illumination) should reproduce, to some extent, the effects of heat shock on subsequent development. When such an experiment was carried out, some inhibition of the developmental process was achieved but the effects were much milder than those induced by heat shock and involved mainly a delay in subsequent development; subsequent sporangia and spores were essentially normal (Wilkins, personal

communication). Thus it is evident that a disruption in translation alone cannot account for the developmental effects of heat shock on *Physarum*. However elsewhere it has been established that there is a complete dependance of the *Physarum* sporulation process on continued protein synthesis (Sauer *et al.*, 1969), which led these authors to conclude that translation must be involved continuously in the process of differentiation. In this case, disruption of protein synthesis at various stages of development either prevented further development or led to the formation of abnormal sporangia (Sauer *et al.*, 1969). The repair of the structural damage of *Poterioochromonas* *lhamensis* cells induced by heat shock has also been shown to be dependant upon protein synthesis after the heat shock (Schnepf and Schmitt, 1981). Therefore it is possible that heat shock of developing *Physarum* plasmodia has a more permanent effect on protein synthesis past the point of commitment than before it and consequently the synthesis of repair proteins necessary before further development can occur is possible prior to the point of commitment but not after it. Alternatively, the synthesis of developmentally important proteins may be impossible following heat shocks at certain stages of development.

There is strong evidence to suggest that a mitosis occurs in the developmental sequence leading to mature *Physarum* spores at approximately 15 hours postillumination. The molecular events that establish the synchrony of mitosis must begin well before the actual mitosis occurs. It is possible that heat shock arrests plasmodial development when applied after the point of commitment by disrupting the synthesis of a protein or proteins necessary for synchronous mitosis to occur. It has been established that two polypeptides, identified as microtubular proteins, are synthesized by growing *Physarum* plasmodia over a period preceding mitosis (Laffler *et al.*, 1981; Turnock *et al.*, 1981). The synthesis of these microtubular proteins is thought to be a possible component within the chain of molecular events that establishes the high mitotic synchrony in growing plasmodia (Laffler *et al.*, 1981). Nuclear actin has also been shown to be involved in the formation of micro-

tubules within the nuclei of *Physarum* and may be involved in mitosis itself (Jockusch *et al.*, 1971). Since nuclear actin constitutes approximately 4% of the nuclear proteins of *Physarum* (Jockusch *et al.*, 1974), its role within the nucleus is probably an important one. Furthermore, the normal process of spindle microtubule assembly appears to be essential for signalling the onset of mitosis in *Physarum* (Hebert *et al.*, 1980). Therefore disruption of the synthesis of proteins essential for the correct functioning of microtubules may be responsible for the formation of abnormal microtubules (macrotubules) reported here to occur within the nuclei of developing *Physarum* plasmodia subjected to heat shock. Such a disruption may also explain the arrest in subsequent development that occurs when plasmodia are heat shocked after what appears to be a point of developmental commitment. Support for this comes from the finding that a number of microtubule-disrupting drugs have effects on protein and RNA synthesis that parallel the effect of heat shock in *Physarum* (Bernstam *et al.*, 1980).

When developing *Physarum* plasmodia are heat shocked at 12 hours postillumination, some areas of each sporangium appear to be arrested in development and subsequently degenerate while other areas continue to develop and subsequently produce spores. The spores so formed are somewhat abnormal, being larger, more irregular in size and shape, and having incompletely developed spore walls, but they appear to be at least as viable as normal spores. It is probable that those areas of each sporangium least affected by heat shock at this stage of development have already cleaved into protospores at the time of the heat shock. Consequently they may be more resistant to the effects of the elevated temperature. Alternatively, since the process of cleavage is not entirely synchronous, the areas least affected by heat shock may have reached a stage in the developmental program that is no longer sensitive to permanent interruption by heat shock. The abnormalities that are induced in spores following a 12 hours postillumination heat shock may, as with complete developmental arrest, have a molecular basis resembling that of *Drosophila* phenocopy induction. Thus the expression of genes involved in spore

wall thickening and in the establishment of normal spore shape and size may occur in the normal developmental sequence around 12 hours postillumination; heat shock induces abnormalities by preventing the normal transcription of these genes. During the course of normal development, spore wall thickening and establishment of the typical spore shape and appearance occur within the period from 15 to 19 hours postillumination, or some 3 to 7 hours after the proposed time when the relevant genes are transcribed. Storage of gene transcripts for subsequent translation has been shown to occur during sea urchin oogenesis and embryogenesis (Hough-Evans *et al.*, 1977) and it is conceivable that a similar storage occurs during the development of *Physarum* plasmodia, thus accounting for the lag between gene expression and the actual events in the developmental process.

While the effects of 37°C heat shocks on both protein synthesis and development of sporulating *Physarum* plasmodia are characterized in the present study, the developmental consequences of 32°C heat shocks are not so clear. Heat shocks of sporulating plasmodia at 32°C elicit the synthesis of heat shock proteins but do not appear to affect the synthesis of normal proteins. It is reported here that developmental abnormalities are induced following normal initiation of sporulation when starved plasmodia are maintained at 32°C from 48 to 60 hours after plating out. However these abnormalities were not well characterized. In other organisms, pretreatment at a temperature that induces the synthesis of heat shock proteins without affecting normal protein synthesis enables the organism to survive an otherwise lethal heat shock (see Introduction). For *Physarum* this phenomenon needs further investigation. In the protein labeling experiments for heat shocks at 32°C reported here, plasmodia were maintained at 32°C for a total of 90 minutes. As a consequence successful pretreatment of *Physarum* to avoid developmental abnormalities induced by subsequent 37°C heat shocks may also require longer periods of incubation at 32°C to maximize heat shock protein synthesis.

4.5 Conclusion.

In response to heat shock, or perhaps environmental stress in general, many organisms synthesize a novel set of proteins, the heat shock proteins, while the synthesis of normal proteins is repressed. In *Drosophila* this response involves the repression of preexisting transcription at the elevated temperature, the simultaneous transcription of a specific set of heat shock genes, and the selective translation of the resulting heat shock mRNAs.

The protein synthesis of sporulating *Physarum polycephalum* plasmodia responds to heat shock at 37°C in a similar manner to that of other organisms. However the precise pattern of heat shock protein synthesis in *Physarum*, relative to the synthesis of normal proteins, appears to be modulated by the developmental state of the organism. It is likely that the predominant heat shock protein synthesized by *Physarum*, with a molecular weight of approximately 69,000 daltons, is closely related to the major heat shock proteins of both *Drosophila* and *Dictyostelium*. Growing *Physarum* plasmodia also respond to heat shock at 37°C with the synthesis a novel set of heat shock proteins and these appear to be identical to those of sporulating plasmodia. However the synthesis of normal proteins by growing plasmodia during heat shock appears to be unaffected by the elevated temperature. The pattern of proteins synthesized by growing plasmodia heat shocked at 37°C does resemble that of sporulating plasmodia heat shocked at 32° and therefore it is probable that this reflects differing sensitivities to elevated temperature at different stages of the life cycle of *Physarum*. Thus, while the induction of heat shock protein synthesis in *Physarum* appears to be independent of the particular developmental program, the response of normal protein synthesis to heat shock appears to be modulated by the developmental program.

Heat shock of sporulating *Physarum* plasmodia at specific stages of development can also induce specific abnormalities in the sporangia that are formed subsequent to the heat shock. The induction of developmental abnormalities is also dependant upon the developmental program and is probably associated with a disruption of transcription and translation

brought about by the heat shock in a manner resembling phenocopy induction in *Drosophila*. However for *Physarum*, the precise molecular basis for the induction of developmental abnormalities remains to be elucidated.

There is mounting evidence to indicate that the heat shock response is a universal response of eukaryotic cells to stress and that the response itself is important in enabling the cell to survive that stress. The heat shock response also provides the means whereby specific genes can be expressed experimentally and thus heat shock can assist in elucidating the mechanisms involved in gene expression.

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