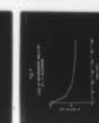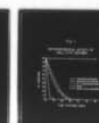AD-A061 401   STRITCH SCHOOL OF MEDICINE MAYWOOD ILL   DEPT OF MICRO--ETC   F/G 6/5
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Antidermatophytic and antidermatomycotic agents
Dermatophytic arthrospores in pathogens

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Lytic enzymes from various biological courses have been shown to effectively kill arthrospores of T. mentagrophytes which are resistant to the lethal action of chemotherapeutic agents. The lytic principles of the enzymes seem to be 1:3-glucanases and chitinase. Unlike their hyphal form or microconidium, the substrates of these enzymes, glucans and chitin, comprised the bulk portion of the thick wall and the enzymes seem to be able to act directly from the surface of the cells causing injuries to the cell wall and the cytoplasmic membrane of the spores. At appropriate

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concentrations, dormant arthrospores could be killed by these enzymes within a few minutes. If used topically in combination with other drugs, these enzymes may prove to be useful antidermatomycotic agents. The relatively stable nature of these enzymes coupled with the commercial availability further enhances this possibility. A series of animal experiments are now underway to test this practical applicability. The effectiveness of glutaraldehyde as an antidermatophytic and antidermatomycotic agent are now being tested in guinea pigs and we expect that the results of the tests will become available in a few months.

During the course of this investigation, we found that the dermatophyte T. mentagrophytes accumulated enormous quantities of carotenoids only during arthrosporulation. Although the significance of this observation is not clear at this time, we are exploring a possibility that the accumulation of such pigments within arthrospores may be related to their resistance to ultraviolet light.

In another series of experiments, we isolated the cell wall of T. mentagrophytes arthrospores and chemically characterized its component. This is an essential step to test feasibility of using arthrospore walls as a source of vaccine and this possibility is being further explored in this laboratory.

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6 CONTROL OF DERMATOMYCOSES BY

PHYSICAL, CHEMICAL AND BIOLOGICAL AGENTS.

by

10 H. J. Blumenthal and T. Hashimoto

Department of Microbiology
Loyola University Stritch School of Medicine
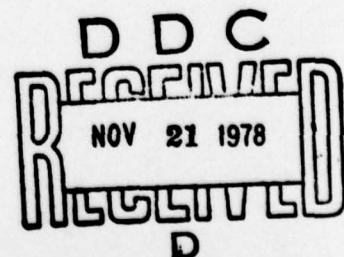Maywood, Illinois   60153

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Several lines of evidence listed below have led us to believe that the key to the radical cure of dermatomycoses and the control of ringworm infections in communal life is to develop effective methods that kill dermatophytic arthrospores in infected lesions or in our environment.

1. Arthrospores are the only dormant or resistant form of dermato-phytes produced in lesions. The presence of abundant arthro-spores in active and chronic lesions has been well documented in the literature.

2. Once arthrosporulated, the dermatophyte Trichophyton mentagro-phytes cells become extremely resistant to several antifungal agents commonly used in the chemotherapy of dermatomycoses (T. Hashimoto and H. J. Blumenthal, Appl. Environ. Microbiol. 35:273-277, 1978). In other words, most lesions containing arthrospores are highly recalcitrant to chemotherapy.

3. Arthrosporulation of the dermatophyte T. mentagrophytes is in fact significantly stimulated by the presence of sublethal doses of antifungal agents such as clotrimazole, nystatin and griseofulin. (Emyanitoff and Hashimoto, 1978, submitted for publication in Can. J. Microbiol.) The use of insufficient doses of antifungal agents rapidly converts susceptible hyphae to resistant arthrospores.

4. Arthrospores are fully capable of germinating into infectious hyphae by the normal skin constituents. In fact, some of the aged arthrospores are quite capable of transforming into infectious hyphae in the presence of water only. (T. Hashimoto and H. J. Blumenthal, Infect. Immun. 18:479-486).

These observations may account for our common experience that the radical cure of dermatomycoses is difficult to achieve despite the extensive use of various chemotherapeutic agents. We now strongly believe that neither radical cure nor the control of ringworm infections in communal life would be possible unless effective methods for killing arthrospores are made available for general use.

Weighing these factors carefully we have decided to concentrate our research effort on the following two specific areas: (1) test-ing of various physical, chemical and biological agents on arthro-spores of T. mentagrophytes and (2) elucidating the morphological and physiological basis of arthrospore resistance to various agents. The latter approach is considered important because such studies may provide useful clues to the development of effective arthro-sporocidal agents.

I. Physical, chemical and biological agents with arthrosporocidal activity-potential candidates for antidermatomycotic agents.

We took several different approaches to develop methods that effectively inactivate dermatophytic arthrospores in vitro and in vivo.

1. Topical application of moist and warm temperature prior to and during active chemotherapy.

The rationale behind this approach is based on our observation that dormant and activated arthrospores are able to germinate and transform into hyphae which are highly susceptible to antifungal chemotherapeutic agents. It appears to us that the continuous application of either topical or systemic chemotherapeutic agents to dried (most physicians advise to keep the lesions as dry as possible!) skin lesions is a futile attempt. This approach will be tested in an animal model and an in vivo experiment is now in progress.

2. Topical application of chemicals with arthrosporocidal activity.

The arthrosporocidal activity of dialdehyde(glutaraldehyde) has been thoroughly tested and established in our laboratory (Technical report #2). The therapeutic as well as antiseptic effect of this compound against arthrospore bearing lesions is now being evaluated in our laboratory.

3. Topical application of enzymes that actively digest the cell wall of arthrospores.

As described later, one of the cellular factors contributing to the poor penetrability of reagents into arthrospores appears to be the thick wall. It seems reasonable to search for enzymes that digest off the thick wall thus making arthrospores more susceptible to antifungal chemotherapeutics or unable to survive in osmotically unstable environments. Although we indicated this possibility in our earlier report, a more likely applicability of such enzymes in the treatment of dermatomycotic lesions emerged when we elucidated the structure and chemical composition of the wall of T. mentagrophytes arthrospores. The chemical analysis of purified cell wall of T. mentagrophytes revealed that glucans (mostly β:3 linked) and chitin are the major wall constituents. Interestingly, these glucan-chitin components are directly exposed to the surface of the wall and are readily accessible to the lytic activity of exogenous glucanase and chitinase. Based on this reasoning, we have tested the arthrosporocidal effects of various lytic enzymes and the results of such tests will be described in the following section.

Sporocidal Activity of Cell Wall Lytic Enzymes

METHODS OF EXPERIMENT

Gluculase (snail intestinal juice) was purchased from Endo Laboratories, and β-1:3 glucanase was prepared from an imperfect fungus and provided by Dr. Nagasaki. These enzymes were relatively stable when stored at 4°C. (No significant loss of activity was seen for 6 months). Arthrospores were produced and purified according to the method described earlier (Technical Report #2).

Approximately 10^7 ml of arthrospores were mixed with enzyme solutions and incubated at 30°C for predetermined periods. In some experiments, the enzymes were diluted with 0.05 M citrate phosphate buffer (pH 5.5) to obtain desired concentrations. Aliquots of the mixture were filtered through millipore filter (pore size 0.45 μm) and cells retained on the filter were washed with sterile Sabouraud dextrose broth. Washed cells were incubated in Sabouraud dextrose broth for 15 hours and the viability of enzyme treated arthrospores was determined microscopically as described earlier (Hashimoto and Blumenthal, Appl. Environment. Microbiol. 35:273-277, 1978). For each sample, a total of 200 spores was examined and the percentage of survived cells was calculated.

RESULTS

Typical survival curves of T. mentagrophytes arthrospores exposed to glusulase and purified β-1:3 glucanase are shown below. Phase contrast photomicrographs of normal and glusulase treated arthrospores are shown in Fig. 2. The iso-cell walls of T. mentagrophytes arthrospores were highly susceptible to the lytic action of β-1:3 glucanase (Fig. 3) and chitinase (data not shown) which were the two major lytic principles of glusulase.

SIGNIFICANCE

The susceptibility of fungal spores to lytic enzymes is dependent on their chemical composition as well as on their location within the wall. The whole spores may not be lysed by lytic enzymes when the substrate is surrounded by an impervious layer resistant to the enzymes. On the other hand, if the entire spore wall is made of materials sensitive to the lytic action of given enzymes, spores may be lysed rapidly regardless of its thickness. The ultrastructural and chemical studies showed that the majority, if not all, of the arthro-
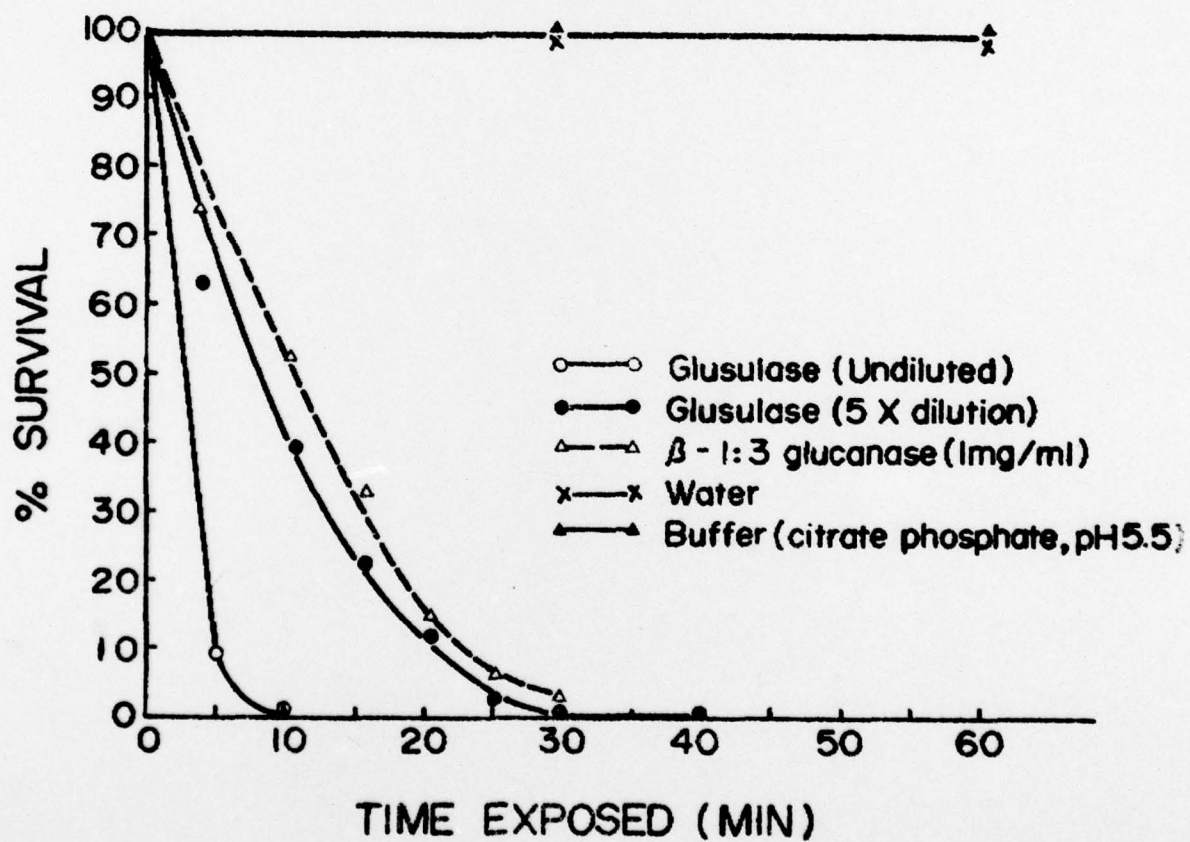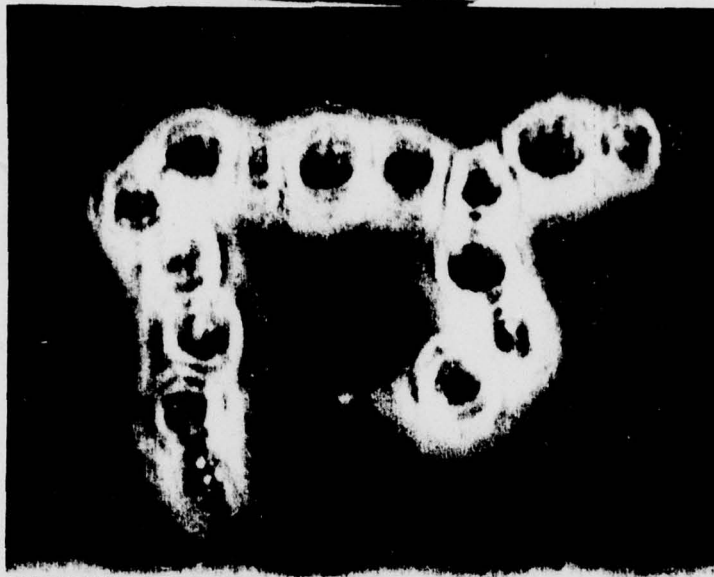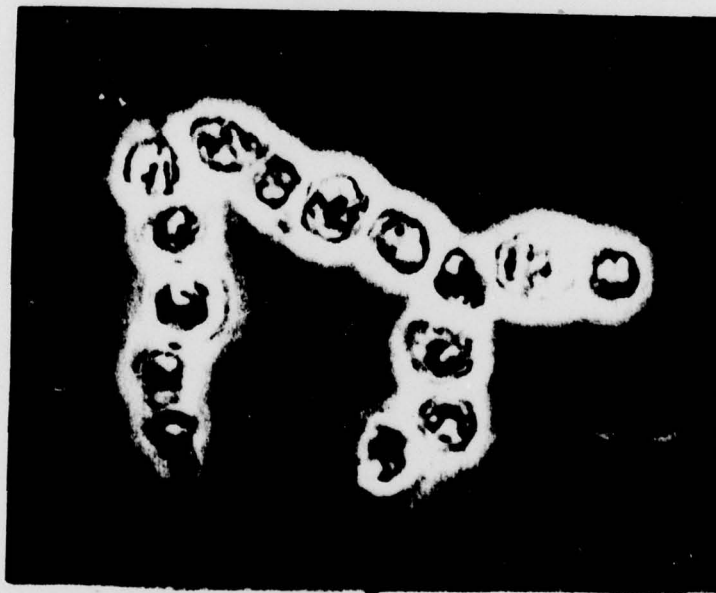
Fig 1

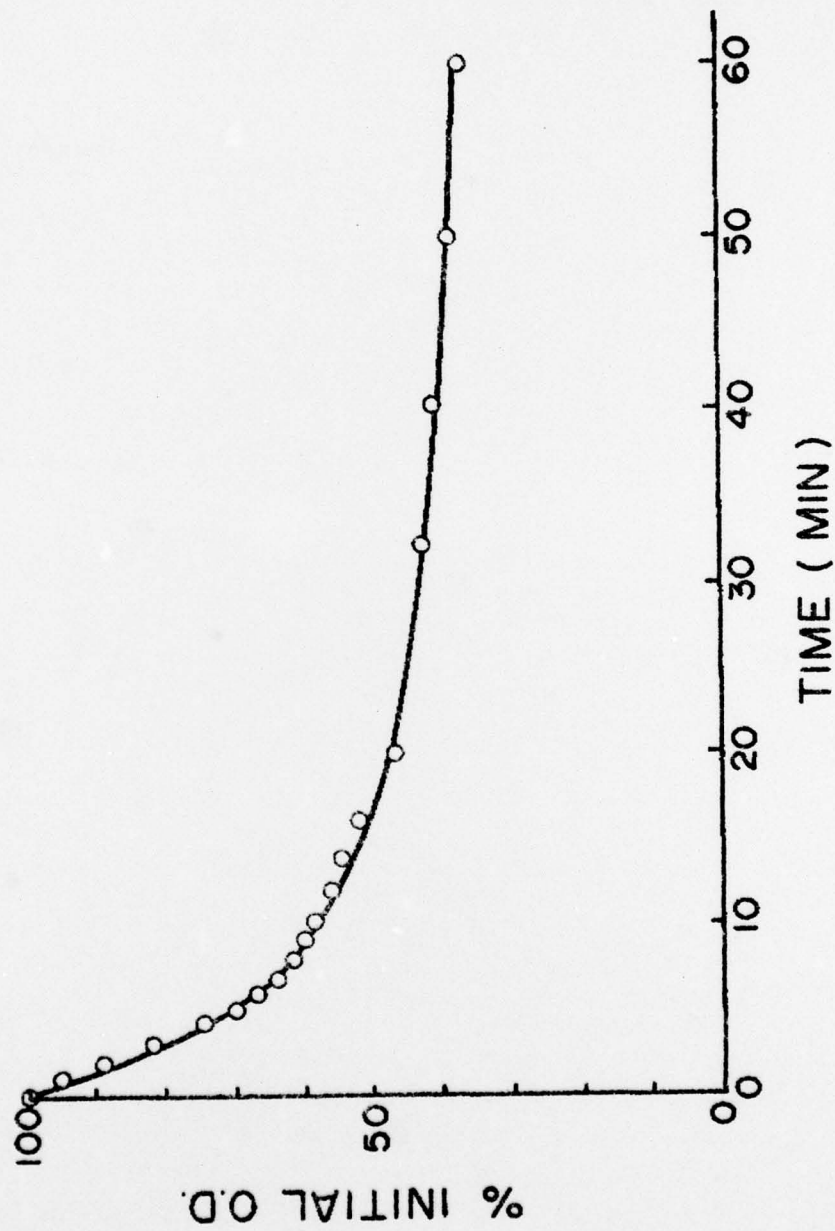ARTHROSPOROCIDAL ACTIVITY OF
WALL LYTIC ENZYMES

Fig. 2

6

Before treatment

10 min treatment
by glusulase

Fig 3

LYSIS OF ARTHROSPORE WALLS BY
β 1:3 GLUCANASE

spore wall of T. mentagrophytes is made of β-1:3 glucans and chitin, and these components are directly exposed to the surface of the wall suggesting an easy accessibility of glucanase and chitinase to the major cell wall components. Since electron microscopy of glusulase or β-1:3 glucanase killed arthrospores demonstrated that they still retained a considerable amount of the wall materials, it appears that the injuries in the cytoplasmic membrane resulting from the partial disintegration of the wall are the primary cause of death of enzyme treated arthrospores. These observations coupled with the relatively stable nature of the enzymes, make these enzymes prospective therapeutic agents in the topical treatment of dermatomycoses. Very recently, a possibility of systemic and topical use of wall lytic enzymes for the treatment of deep seated and superficial mycotic infections has been proposed by Davies and Pope (Nature 173:235-236, 1978).

II. Structural and physiological properties uniquely associated with arthrosporulation of T. mentagrophytes.

In order to find some unique structural and physiological characters that may be responsible for the resistance of dermatophytic arthrospores, we examined T. mentagrophytes arthrospores by means of electron microscopy and analyzed the chemical composition of certain subcellular components. The results so far obtained indicate that there are at least two structural components that characteristically associated with arthrospores. It may be plausible that the inhibition of the development of these structures or the specific destruction of these cellular components might lead to the rapid killing of arthrospores.

1. Arthrospore wall.

Electron microscopy of T. mentagrophytes clearly revealed that the wall of arthrospores is several times thicker than that of vegetative hyphae. During arthrosporulation, cells synthesize a thick new wall layer immediately beneath the existing hyphal wall. By the time spores became mature most of the outer wall was lost and the cytoplasm was surrounded by a new thick (sometimes it exceeds 1 μm!) wall synthesized de novo during sporulation. During this ultrastructural study, we experienced considerable difficulties in fixing and embedding mature arthrospores. We attributed these difficulties to the thick wall of arthrospores. The penetration of the fixatives and other reagents seemed to be hampered by the impervious wall. This speculation was confirmed by our subsequent observation that the spheroplasts of arthrospores could be readily fixed for thin sectioning. Although further

experimentation is needed to draw a more definitive conclusion, the resistance of arthrospores to various antifungal agents may well be related to the presence of the unique spore wall.

2. Lipid and carotenoid granules.

One of the most striking observations made during this study was the demonstration of carotenoid pigments within the lipid granules of T. mentagrophytes arthrospores. In T. mentagrophytes these pigments were shown to occur only in arthrospores. No trace of such pigments was found in either the hyphal or microconidial form of this dermatophyte. Although the significance of these pigments in the resistance of arthrospores is not immediately clear, we believe that these observations are highly significant because carotenoid pigments in plants are considered to be vital for the protection of the photosynthetic apparatus from ultraviolet injury. In view of this possible significance the full technical detail related to this work will be included in this report.

MATERIALS AND METHODS

Fungus strain. T. mentagrophytes ATCC 26323 was used throughout this investigation.

Preparation of arthrospores. Arthrospores of T. mentagrophytes were produced by a slight modification of the method described earlier.[6] Approximately 0.15 to 0.2 ml of a microconidial suspension (2×10^6 spores per ml of distilled water) was inoculated on a cellulose dialysis membrane (Union Carbide Corp., New York, N.Y.), which had been placed on Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) containing 0.1% sodium acetate. The dialysis membrane was previously boiled in 1% sodium bicarbonate solution for 10 min rinsed with distilled water, and subsequently sterilized by autoclaving. After the inoculated plates were incubated at 37°C for 36 h, 2 ml of Sabouraud dextrose broth (Difco) was added aseptically over the dialysis membrane. By this time, hyphae emerging from microconidia adhered to the membrane, and the addition of the broth caused no separation of hyphae from the membrane. The plates were placed in a large covered glass jar (21 cm diameter, 25 cm high) and further incubated under saturated humidity at 37°C for an additional week. By the end of the incubation period, essentially all hyphae were transformed into arthrospores.

The arthrospores were readily removed from the cellulose membrane by gently scraping with a spatula. The harvested arthrospores were washed in distilled water three times by means of centrifugation (1,500 x g, 15 min). In some experiments, young hyphae or arthrosporulating

hyphae were collected similarly from the cellulose surface after specified periods of incubation, washed, and stored as described above.

Quantitation of arthrospore formation. The degree of arthrospore formation was determined according to the method of Timberlake and Turian. Cells removed from the membrane culture were placed on a glass microscope slide and examined microscopically using an oil immersion objective (x100; numerical aperture 1.25; Nikon). The percentage of hyphal tips containing arthrospores was determined after examining 200 tips.

Extraction and quantitation of pigments. The pigments were repeatedly extracted from cells with methyl alcohol at 25°C. The extracts containing pigments were pooled and saponified in methanol at 25°C for 15 h. The pigmented fraction was collected in hexane by means of phase separation technique. The epiphase fraction containing pigments was concentrated under vacuum. When the pigments were not used immediately, they were stored under vacuum in the dark at -20°C.

The relative amounts of carotenoid pigments contained in cells undergoing arthrosporulation were determined by measuring absorbance at 460 nm of the pigments collected in hexane, and was expressed as percentage relative to the amount of pigments obtained from mature arthrospores (8 days old).

Thin-layer chromatography. The pigment solution in hexane was applied on alumina thin-layer sheets (no. 6063, Eastman Organic Chemicals, Rochester, N.Y.), or on silica gel thin-layer sheets (no. 6061, Eastman Organic Chemicals). Development of thin-layer plates was carried out at 25°C in the following solvent systems: hexane-benzene-n-butanol (10:1:1) or hexane-benzene (10:1.5). Colorless spots were visualized either by illumination of chromatograms with UV light (Mineral light, model R52, equipped with filter no. 20119 for short wave length and no. 20118 for long wave length: Ultraviolet Products, Inc., San Gabriel, Calif.) or spraying with the reagents specified below. To ascertain the nature of the pigments, chromatograms were sprayed with various reagents as specified in Table 2. Authentic carotenoids, such as α and β carotene, were purchased from Sigma Chemical Co. (St. Louis, Mo.). Lycopene was isolated from tomato according to the method described by Weedon (22). Phytoene and phytofluene were similarly prepared from Rhodotorula rubra.

Carr-Price test for carotenoids. The Carr-Price test for carotenoids was performed by spraying chromatograms with 20% antimony trichloride (J. T. Baker Chemical Co., Phillipsburg, N.J.) in chloroform.

Spectroscopy. For spectroscopic study of individual carotenoids, the extract was streaked on thin-layer plates and distinctly separated lines of pigments after development were eluted in hexane. Absorption spectra of individual carotenoids in hexane were examined in a Cary 15 spectrophotometer.

Reagents. All chemicals used in the present investigation were of reagent quality. All solvents were either chromatographic or spectroscopic quality, depending on the purpose of the experiments and were used without further purification.

Dry-weight determination. Dry weight of cells on extracted materials was determined by desiccating samples in preweighed aluminum planchets over P_2O_5 placed in vacuum at 25°C for 72 h.

Cytological techniques. The light microscope appearance of the wet-mounted cells was examined with either an ordinary or phase-contrast microscope using oil immersion objectives. Photomicroscopy was made on panchromatic film (Kodak Plus X) with a Nikon camera equipped with an automatic exposure system. Thin sections and freeze-etched replicas of arthrospores were prepared by the methods described earlier. Specimens were examined with a Hitachi HU-11A electron microscope operating at 50 kV.

RESULTS

Identification and characterization of pigments. The thin-layer chromatographic and spectroscopic properties of the pigments extracted from mature (8 days old) arthrospores of T. mentagrophytes are shown in Table 1. The results of various color reactions performed directly on thin-layer chromatograms are summarized in Table 2. The characteristic color of these pigments on thin-layer chromatogram sheets tended to fade when the pigments were dessiccated in air or exposed to intensive light. Although it was not possible to firmly establish the identification of a few minor pigments because of the lack of certain authentic carotenoids, the data presented in Tables 1 and 2 are compatible with our conclusion that the majority of the pigments occurring in arthrosporulating T. mentagrophytes are indeed carotenoid in nature. It is clear from Table 3 that the pigments isolated from arthrospores of T. mentagrophytes were not xanthomegnin. To our knowledge, this is the first demonstration of carotenoid pigments in the group of dermatophytic fungi.

Kinetics of growth, arthrosporulation and pigment formation. Under our experimental conditions, arthrosporulation began as soon as the maximum hyphal growth had been attained and intracellular pigment granules became evident upon the completion of multiple segmentation of hyphae (Fig. 4). During the maturation of arthrospores, cells transformed from rectangular ot oval shape and formed short

TABLE 1. *Thin-layer chromatographic and spectroscopic characteristics of epiphase carotenoids found in T. mentagrophytes arthrospores*

| Identification | Rate of flow (R_f) | | | | Absorption maxima in hexane[a] (nm) |
| | Alumina | | Silica gel | | |
	Solvent A[b]	Solvent B[c]	Solvent A	Solvent B	
Ergosterol (non-carotenoid)	0.28	0	0.48	0	271, 282, 294
Unidentifiable spot	0.32	0	0.51	0	403, 425, 450
Lycopene	0.40	0.02	0.77	0.33	443, 469, 500 (443, 469, 500)
Unidentifiable spot	0.47				440, 465, 496
Neurosporene	0.57	0.09	0.80	0.43	414, 438, 467 (414, 437, 466)
γ-Carotene	0.57	0.06	0.80	0.49	431, 458, 487 (434, 459, 490)
ζ-Carotene	0.61	0.14	0.84	0.53	379, 399, 424 (379, 400, 424)
Unidentifiable spot	0.67	0.14	0.84	0.59	426, 449, 475
β-Carotene	0.67	0.20	0.84	0.64	428, 450, 478 (430, 450, 477)
Phytofluene	0.73	0.33	0.87	0.60	339, 348, 367 (331, 348, 367)
Phytoene	0.78	0.59	ND[d]	0.83	277, 286, 298 (275, 286, 298)

[a] Figures in parentheses represent values reported by others (3, 5). Major peaks are underlined.
[b] Solvent A: hexane–benzene–butanol (10:1:1).
[c] Solvent B: hexane–benzene (10:1.5).
[d] ND, Not detectable.

TABLE 2. *Color reaction of epiphase carotenoids of T. mentagrophytes arthrospores with various reagents*

| Identification	Color	Carr-Price test for carotenoids[a]	2 N NaOH[a,b]	70% $HClO_4$	0.5% Mg acetate[c] (methanolic)
Ergosterol (non-carotenoid)	Colorless	−	−	Red	−
Unidentifiable spot	Yellow	+	−	Blue	−
Lycopene	Pink	+	−	Blue	−
Unidentifiable spot	Light pink	±	−	Blue	−
Neurosporene	Yellow	+	−	Blue	−
γ-Carotene	Orange	±	−	Blue	−
ζ-Carotene	Yellow	+	−	Blue	−
Unidentifiable spot	Orange	+	−	Blue	−
β-Carotene	Light yellow	+	−	Blue	−
Phytofluene[d]	Colorless	±	−	Yellow	−
Phytoene[d]	Colorless	±	−		−

[a] −, No change in color. ±, weakly positive; +, positive.
[b] Xanthomegnin became purple when sprayed with 2 N NaOH.
[c] Xanthomegnin and quinone derivatives turned to purple to violet.
[d] Phytoene strongly absorbed UV light. Phytofluene fluoresced under long-wavelength UV light.

TABLE 3. *Some major differences between carotenoid and quinone pigments*[a]

Pigment	Absorption spectra (visible light)	Carr-Price test	Alkali solubility (2 N NaOH)	Color change in:		R_f (TLC)[b] in polar solvent benzene-acetone (3:1)
				2 N NaOH	0.5% Mg acetate	
Carotenoids (α- or β-carotene)	Usually three characteristic peaks	+	–	–	–	1.0
Xanthomegnin[c]	One broad peak	–	+	+	+	<1.0

[a] Results expressed as –, no change in color; +, positive.
[b] Rate of flow on thin-layer chromatographic (TLC) sheet (silica).
[c] A representative quinone pigment from *Microsporum cookei* (provided by Y. Nozawa).

Fig. 4
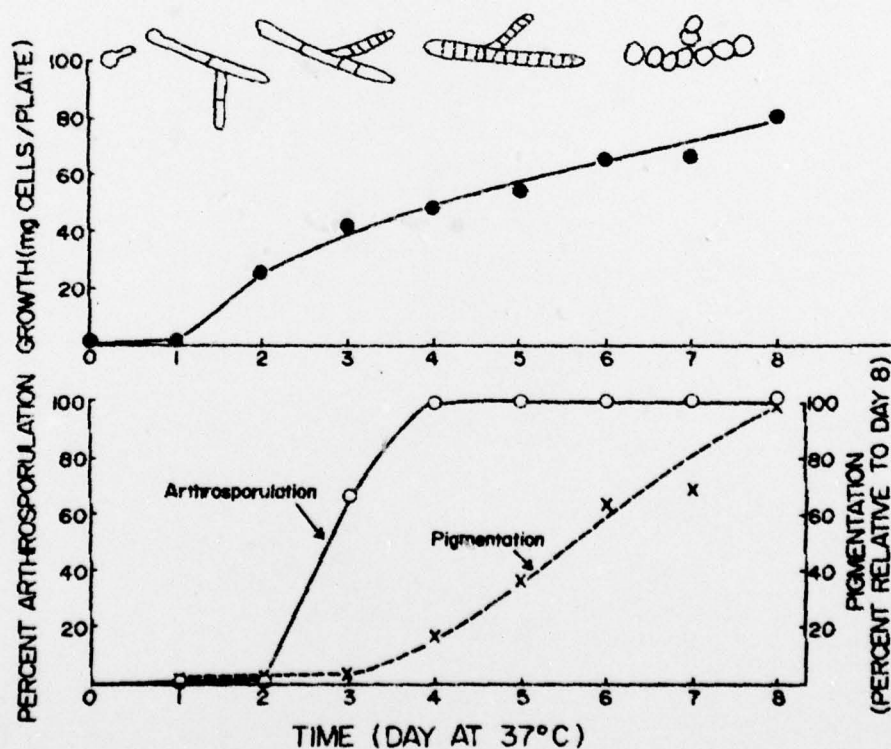
4. Carotenoid pigment formation during arthrosporulation of T. mentagrophytes. The appearance of cells is depicted in the upper graph, and the relationship of carotenoid formation to arthrosporulation is shown in the lower graph.

chains or were released as single cells as a result of disarticulation of connecting septa (Fig. 4). During this maturation process, arthrospores continuously accumulated intracellular pigments, reaching a maximum in about 8 days.

Although carotenoid pigments could not be demonstrated in any states of growth (hyphae or microconidia) other than during arthrosporulation, carotenogenesis does not seem to be an essential event for either the induction or the completion of arthrosporulation. Complete arthrosporulation took place when this fungus was grown on Sabouraud-sodium acetate medium at 39°C (data not shown). Downy strains, naturally occurring asporogenic variants of T. mentagrophytes, were able neither to arthrosporulate nor to form carotenoid pigments under all conditions we tested. Light was not required for the induction of carotenoid biosynthesis in arthrosporulating T. mentagrophytes.

SIGNIFICANCE AND DISCUSSION

The data presented in Table 1 and 2 clearly indicate that the pigments associated with arthrosporogenesis of T. mentagrophytes are carotenoids and not quinone derivatives. In addtion to the positive test for carotenoids (Carr-Price test) and the typical carotenoid three-peak light absorption spectra (Table 1), the insolubility of the pigments in 2 N NaOH and their failure to change color in alkali or in magnesium acetate solution render it highly unlikely that these pigments from T. mentagrophytes arthrospores are quinone derivatives as generally reported for dermatophytes. The authentic fungal quinone pigment xanthomegnin reacted quite differently from the T. mentagrophytes pigments in both the physical and chemical tests (Table 3).

Considering the rather ubiquitous occurrence of carotenoid pigments in fungi, it is not surprising to find carotenoids in a dermatophyte. However, the unusual finding that carotenogenesis in this fungus occurs only during arthrosporulation suggests a reason why carotenoid pigments have not been previously detected in T. mentagrophytes. Recent studies (R. Emyanitoff, Ph.D. dissertation, Loyola University, 1978) have shown that the induction of arthrosporulation in this dermatophyte is possible only under specific nutritional and physiological conditions and that arthrospores would not be formed under most routine growth conditions. Mares et al. recently observed either yellow or orange substance in T. mentagrophytes growing under a condition which, in our judgement, was conducive to arthrosporulation. Without attempting chemical analysis, they assumed the pigment to be a "compound of quinone structure since similar substances are common in the pigmented strains of dermatophytes." In fact, many strains of derma-

tophytes are known to produce pigments which are of quinone deriva-
tives. Among these, the most common pigment is xanthomegnin, the
major pigment produced by T. megnini. Xanthomegnin was subsequently
found in many other strains of dermatophytes as well as in other
fungi. It appears that the unique association of carotenogenesis
with arthrosporulation and an a prior assumption by some workers
that dermatophytes produce only quinone type pigments are jointly
responsible for undue delay in demonstrating carotenoids in dermato-
phytes. It is anticipated that with the elucidation of specific
conditions for arthrosporulation, similar carotenoids may be found
in other dermatophytic fungi.

Despite the intimate association of carotenogenesis and arthro-
sporulation in this fungus, the former does not seem to be an
essential event for the completion of arthrospore formation, although
the properties of arthrospores with or without carotenoids need
further examination. Morphologically normal arthrospores could be
produced when the fungus was arthrosporulated at 39°C instead of 37°
C. Such arthrospores were almost completely devoid of carotenoid
pigments (Hashimoto and Mock, unpublished data).

III. Preparation of the cell walls of T. mentagrophytes hyphae and
     arthrospores as possible sources of vaccine.

The existence, in man, of specific acquired immunity to super-
ficial cutaneous infections (dermaotmycoses) has been debated since
Bloch and Massini reported that guinea pigs became resistant to
reinfections following the first experimental Trichophyton infection.
Although there are numerous reports that immunization against derma-
tomycoses is an ineffective and unpractical approach to the prophy-
laxis of dermatomycoses (reviewed by Grappel et al., Immunology of
Dermatophytes and Dermatophytosis, Bacteriol. Rev. 38, 222-250,
1974), the data obtained from a recent human experimentation do
indicate that individuals experimentally infected with T. mentagro-
phytes spores become either immune to reinfection or at least
become more resistant to reinfection (Jones et al. Arch. Dermatol.
109:840-848, 1974).

Since arthrospores of dermatophytes are now considered the
primary source of infection in man, we strongly feel that the problems
of acquired immunity and prophylaxis against dermatomycoses should be
reexamined at this time. No workers in the past have explored the
pathogenic or immunological roles of arthrospores in ringworm infec-
tions. We believe that if arthrospores introduced to skin wounds are
arrested before transforming into ineffective hyphae by either speci-
fic antibodies or cell mediated immunity, such individuals are less
likely to develop active infections. Because dermatophytic arthro-
spores are characterized by the presence of thick cell walls and
these walls are the first cellular component coming in contact with

the host defense system, we reasoned that the immunological and chemical characterization of arthrospore cells merits a thorough investigation.

Isolation and purification of T. mentagrophytes arthrospore walls.

Probably because of the unusual thickness and spherical shape of the wall, our attempts to disrupt arthrospores of T. mentagrophytes by means of several conventional methods (sonication, grinding, Mickle technique and freeze and thawing) were proven to be ineffective. We found that repeated passages through a French pressure cell (Aminco, J4-3339) using 40,000 psi caused the complete disruption of mature arthrospores. In most cases, 7-10 passages were sufficient to break more than 99% of arthrospore populations. Arthrospore walls were separated from broken cells by repeated centrifugation at 4°C and the walls were further purified by gradient centrifugation in renografin. Purified cell walls were free from ergosterol (membrane constituent) and RNA (ribosomes). Electron microscopy of wall preparations confirmed that the preparation is essentially free from cytoplasmic materials.

Chemical composition.

For the analytical methods, see our earlier publication (Wu-Yuan and Hashimoto, J. Bacteriol. 129:1584-1592, 1977). The chemical analysis of purified cell walls of T. mentagrophytes arthrospores revealed the following composition.

Protein	3%
Neutral sugars* (as glucose)	53%
Chitin	30%
Lipid	< 1.0%
Others	15%

* Glucose is the major monosaccharide in this wall. Mannose and galactose are also present as minor components.

We are now accumulating a large quantity of purified cell walls of T. mentagrophytes arthrospores for more thorough chemical analyses and immunological characterization.

Publications (Nov. 1, 1977 - Oct. 31, 1978)

1. Hashimoto, T. and H. J. Blumenthal. 1977. Factors affecting germination of Trichophyton mentagrophytes. Infect. Immun. 18: 479-486.

2. Hashimoto, T. and H. J. Blumenthal. 1978. Survival and resistance of Trichophyton mentagrophytes arthrospores. Appl. Environ. Microbiol. 35:273-277.

3. Hashimoto, T., J. H. Pollack and H. J. Blumenthal. 1978. Carotenogenesis associated with arthrosporulation of Trichophyton mentagrophytes. Accepted for publication in J. Bacteriol. To be published in December 1978.

4. Emyanitoff, R. G. and T. Hashimoto. The effect of temperature, incubation atmosphere and medium composition on arthrospore formation in the fungus Trichophyton mentagrophytes. Submitted for publication in Canadian J. Microbiol.