Nutrition of Systemic and Subcutaneous Pathogenic Fungi

GERALD L. GILARDI

Microbiology Department, Hospital for Joint Diseases, New York, New York

INTRODUCTION

Many studies on the nutritional behavior of bacteria have appeared in the past few decades. The reasons for these studies are apparent: to examine the accessory growth factors essential for bacteria and to increase knowledge of the basal nutritional requirements necessary for their development.

While nutritional investigations with bacteria have been progressing rapidly, comparatively few recent studies have been carried out with fungi. The earliest nutritional studies with fungi were performed primarily on saprophytic yeasts and molds, with little attention paid to the pathogenic fungi. At first, nutritional studies concerned with pathogenic fungi consisted of studies with complex nonsynthetic media to de-

termine the types and abundance of growth obtained and efficiency of the media in isolation. Later, nutritional investigations with pathogenic fungi were concerned with defining basal nutritional requirements and accessory growth factors necessary to replace media containing complex mixtures of organic substances of unknown chemical composition. This has resulted in cultivation of most pathogenic fungi on much simpler, chemically defined culture media.

This review is concerned with those studies which define the essential growth factors and nutritional characteristics of fungi responsible for the systemic and subcutaneous mycoses of man. Hence, the areas of primary concern covered by the review include vitamin and amino acid requirements, basal media required for growth, effect of chemical and physical agents on growth, studies of carbon and nitrogen assimilation, and differences in nutritional requirements between the mold form and the yeast form of dimorphic fungi.

NUTRITION OF PATHOGENIC YEASTS

Candida

Vitamin requirements. Most strains of the genus *Candida* do not grow in a glucose-ammonium-inorganic salts medium (GASM) unless they are supplemented by yeast extract. Vitamin-requirement studies show that *C. albicans* is completely deficient for biotin (9, 13, 14, 23, 24, 26, 34, 42, 43, 56, 57, 60, 61, 95) and partially deficient for thiamine; that is, thiamine has an additive effect, enhancing growth (13, 14, 34, 42, 57, 60, 61, 95). Koser et al. (34) found pantothenic acid to be required or stimulatory for three strains of *C. albicans.* Various biotin analogues replace biotin, including biocytin, *N*-biotinyl-β-alanine, *N*-biotinyl-L-aspartic ethyl ester, desthiobiotin, biotin-D-sulfoxide, homobiotin, and oxybiotin; aspartic acid partially replaces biotin, and biotin sulfone, biotin diamine sulfate, biotinol, and norbiotin show little activity (26, 42).

Amino acid requirements. Several of the vitamin-requirement studies have been performed in a glucose-asparagine-inorganic salts medium (13, 14, 57), and one in a more complex medium containing casein hydrolysate, several amino acids, purines, and pyrimidines (34). But *C. albicans* does grow in a simple GASM containing biotin with no exogenous supply of any amino acid required, indicating that all amino acids can be synthesized in the presence of biotin (1, 60).

Miyashita, Miwatani, and Fujino (61) found that, in the presence of suboptimal amounts of biotin, six amino acids (aspartic acid, glutamic acid, arginine, proline, serine, and alanine) have a biotin-sparing effect.

Factors influencing dimorphism. Extensive investigations have been concerned with those factors which influence yeast (Y) form, pseudohyphae, and chlamydospore development in yeastlike fungi, including *C. albicans.* No attempt has been made here to cover the literature in this area since there are many fine reviews on dimorphism (25, 63, 65, 69, 70, 93, 100, 101, 107), including the more recent reviews by Skinner and Fletcher in 1960 (101) and Nickerson in 1963 (65).

In general, the conditions favoring Y-form development include a fermentable carbohydrate source such as glucose (30, 32, 67, 68, 100, 103), the presence of cysteine (67, 68, 70, 93), the presence of glutathione (67), increased oxygen tension (93, 100), and the absence of such growth factors as potassium, phosphorus, and biotin (56).

The conditions favoring pseudohyphae development include an assimilable but not so readily fermentable carbohydrate such as sucrose (56), the presence of polysaccharides (67, 68, 93), the presence of certain growth factors such as potassium, phosphorus, and biotin (56), reduced oxygen tension (30, 93, 100), starvation media (30, 100), and liquid media in general (56, 100).

From the review of the literature there seems to be little agreement among the various investigators concerning the influence of pH (30, 33, 56, 93, 100) and temperature (30, 56, 93, 100). Scherr and Weaver (93) reported that the Y form is present at pH 7.0, and pseudohyphae are present at lower pH levels. Skinner (100), however, found pseudohyphae formation at high pH levels. This was confirmed by Johnson, Guzmon, and Agurlera (30) at pH 7.4 to 9.6. They found the Y form at pH 2.5 to 6.4. McClary (56) said that extreme pH ranges almost invariably produce the Y form, and that a pH of 5 is optimal for pseudohyphae development. In the review by Skinner (100), high temperature (37 C) was stated to favor pseudohyphae production. Johnson et al. (30) found both Y forms and pseudohyphae developing at 37 C. McClary (56) found temperatures of 25 to 30 C to favor pseudohyphae production, and temperatures of 37 to 40 C to favor Y-form development.

Chlamydospore production has been attributed to the presence of polysaccharides as the carbon source (67, 68, 93), the absence of reducing sugars (67, 68, 79), the presence of aminopterin (67), a deficiency in available phosphorus (56), the absence of sulfhydryl groups (68, 70), and extreme pH ranges (56).

Media have been developed for demonstrating

the production of spores by chlamydospore-producing species of *Candida* (*C. albicans, C. stellatoidea*). Nickerson and Mankowski (68) obtained abundant chlamydospore production in a medium containing purified polysaccharides (free from reducing sugars) such as glycogen, soluble starch, and dextrin. A chlamydospore-producing medium containing zein, the basic protein of corn meal, was developed by Reid, Jones, and Carter (79). They concluded that a partial explanation for enhanced formation in the zein-agar lies in the absence of reducing sugars in this medium. Enhanced chlamydospore production has been observed in a semisolid medium containing polyoxyethylene sorbitan monooleate (Tween 80) (96) and on Loewenstein's medium moistened with Old Tuberculin (50).

Investigations have been directed towards an understanding of metabolic differences between the Y form and the pseudohyphae form of growth. On the basis of the evidence obtained from these investigations, summarized by Morris (63), Ward (107), Skinner and Fletcher (101), and Nickerson (65), pseudohyphae production results from an impaired cell-division mechanism resulting from an apparent breakdown of intracellular sulfhydryl maintenance. Conversely, a specific disulfide-reducing process must be maintained if the budding phase is to occur.

Agents demonstrating beneficial or deleterious effects on growth. The addition of chemical agents to basal media has been noted to exert either a stimulatory or inhibitory effect on growth of fungi. Marwin (53–55) found four nonionic surface-tension reductants, Nonisol 100, Polyethylene glycol 400 monolaurate, Pluronic L 64, and Mulsor 224, to have a stimulatory effect on the growth of several pathogenic fungi, including *C. albicans*. *p*-Hydroxymethyl benzoic acid (108) and thiourea (22) are fungistatic to *C. albicans*, whereas hydrogen sulfide (7), sodium acetate (74), and ascorbic acid (73) have fungicidal effects.

Assimilation patterns. Most authorities agree that *C. albicans* assimilates glucose, galactose, sucrose, and maltose, but not lactose or potassium nitrate (23, 44, 60). Other studies reveal that raffinose (23) and starch (56) are not assimilated and that fructose, mannose (56, 60), ethyl alcohol, glycerol, and succinic acid (56) are assimilated as sole sources of carbon and that ammonium sulfate, asparagine, and urea (23) are assimilated as sole sources of nitrogen. Kapica and Blank (32) reported that keratin is assimilated as the sole source of nitrogen. They pointed out that this may not be due to keratinolytic activity, since *C. albicans* might grow on a nitrogen-free medium by fixing atmospheric nitrogen. They

further questioned whether the breakdown of keratin is due to acid hydrolysis, rather than enzymatic activity which might supply the yeast with soluble forms of nitrogen.

Miyashita et al. (61) showed that amino acids utilized as sole sources of nitrogen include aspartic acid, glutamic acid, arginine, proline, serine, alanine, histidine, methionine, and tryptophan. They found that the amino acids utilized as sole sources of carbon include aspartic acid, glutamic acid, and arginine, the latter three being utilized as the sole source of both carbon and nitrogen. In addition to the amino acids so far mentioned, Johnson et al. (30) found that the following amino acids are assimilated as the sole source of nitrogen: phenylalanine, aminoacetic acid, tyrosine, leucine, lysine, threonine, norleucine, isoleucine, and valine. They found that cystine, homocystine, hydroxyproline, and ornithine are not assimilated.

Cryptococcus

Vitamin and amino acid requirements. The growth requirements of *Cryptococcus neoformans* have been determined in a chemically defined medium by several investigators. In a review on the vitamin requirements of fungi, Robbins and Kavanagh (80) stated that the yeast grows well in the presence of thiamine but can also grow, although poorly, in a glucose-asparagine-ammonium lactate-inorganic salts medium without the addition of thiamine. Reid (78) and Arêa Leão and Cury (2) have grown the yeast in a glucose-asparagine-inorganic salts medium containing thiamine. But several investigators (27, 41, 94, 105) have grown the yeast in a simple GASM to which they added thiamine. Arêa Leão and Cury (2) found an absolute requirement for thiamine, and Littman (41) an absolute requirement for thiamine and its moieties, thiazole and pyrimidine.

According to Schmidt et al. (94), amino acids, purines, and pyrimidines are not essential for growth, with amino acids exerting merely a slight stimulatory effect on growth.

Agents demonstrating beneficial or deleterious effects on growth. The nonionic surface-active agents described as having a stimulatory effect on growth of *C. albicans* have a similar effect on *C. neoformans* (52–54). In studies on the inhibition of growth of *C. neoformans*, *p*-hydroxymethyl benzoic acid (108) and thiourea (22, 104) were found to be effective. Tager, Hales, and Danowski (104) found that the fungistatic activity of thiourea is reversed upon the addition of —SH-containing compounds such as cysteine. They concluded that thiourea inactivates the —SH radical through some process such as oxidation,

thus suppressing growth of the organism, and that this is reversed upon the addition of compounds which replace or reconstitute —SH groupings.

Assimilation patterns. Most authorities agree that *C. neoformans* assimilates glucose, maltose, sucrose, and galactose, but not lactose and potassium nitrate (5, 6, 44). But Kao and Schwarz (31) showed that potassium nitrate and lactose provide sufficient nutriment for slight growth. This is at variance with the opinion of Benham (5), who reported no growth on media containing these substances as the sole source of carbon and nitrogen. The assimilation of amino acids and carbohydrates as the sole source of carbon has been studied by Littman (41). Of the amino acids and derivatives investigated, he found that the yeast assimilates members of the glutamic acid family: glutamic acid, glutamine, proline, serine, and asparagine. His studies show that 25 other sources are not assimilated. In his investigation of carbohydrates, all hexoses, a number of pentoses (ribose, xylose), disaccharides (maltose, sucrose, trehalose, cellobiose), and polysaccharides (dextrin, starch) are assimilated. Other compounds assimilated include ethyl alcohol, glyceraldehyde, glucuronolactone, inositol, glucosamine, and the Krebs cycle intermediate, α-ketoglutaric acid. Compounds which he found are not assimilated include β-glycerophosphoric acid, glucose-1-phosphate, glutaric acid, glycogen, and lactose.

NUTRITION OF DIMORPHIC FUNGI

Blastomyces

Vitamin requirements. Vitamin requirement studies of the genus *Blastomyces* have produced variable results. Halliday and McCoy (29) found both the Y and mold (M) forms of *B. dermatitidis* completely deficient for biotin. They observed that optimal growth of recently isolated strains is obtained with less biotin than is required for optimal growth of old laboratory strains. Substances which have been implicated in biotin metabolism, including oleic acid, aspartic acid, methionine, and pimelic acid, were found to have no effect on growth; desthiobiotin can be replaced for biotin. Other investigators (2, 28, 40, 66, 88, 102) found no growth-factor requirements necessary for growth of either form of *B. dermatitidis*.

Arèa Leão and Cury (2) found two strains of the M form of *B. brasiliensis* (*Paracoccidioides brasiliensis*) to be completely deficient and one strain partially deficient for thiamine, but Nickerson and Edwards (66), Salvin (88), and Gilardi and Laffer (28) found no vitamin requirements for either form. In those cases where vita-

mins are not required, it is also noted that the vitamins are not stimulatory for growth (28, 88).

Halliday and McCoy (29) pointed out that other investigators have performed vitamin studies with media solidified with agar in cotton-plugged tubes, which could allow contamination with biotin from the cotton plug or agar. They used a liquid medium in aluminum-capped test tubes for their studies. Salvin (88) obtained good growth in sealed tubes, with a semisolid medium containing washed agar. Gilardi and Laffer (28) used a liquid synthetic medium in metal-capped tubes, confirming the results of Salvin (88).

Amino acid requirements. Many nutritional studies have been performed with media containing organic nitrogen sources. The M form of *B. dermatitidis* has been grown on media containing neopeptone plus Tryptone (58), peptone, casein hydrolysate (40), ammonium acetate, acetamide (102), asparagine (2), and glycine (66). But the M form does not require an organic nitrogen source, and can be grown on a GASM (40, 66, 102). The M form of *B. brasiliensis* has been cultured on a medium containing asparagine (2) and glycine (66), but has also been grown on a simple GASM (66, 88).

The Y form of these fungi has been grown primarily on solid media, because difficulty has been encountered in obtaining Y-form growth in liquid media (40). Y-form growth has been obtained in semisolid media (88) or liquid media (29, 66, 89) to which vitamins (29, 66, 89) and organic nitrogen sources (66, 89) have been added, but recent studies show that the Y form grows in a liquid GASM without the addition of organic nitrogen or vitamins (28). The various studies indicate that amino acids are not required, but are stimulatory for growth of both the Y and M forms (1, 28, 40).

Factors influencing dimorphism. Dimorphism in *Blastomyces* occurs on a minimal medium, and is apparently independent of nutrition and a function only of the temperature of incubation (40, 64, 66, 88). However, Bullen (11) found that an increased concentration of CO_2 appears to be essential for the development of the Y form. The change in morphology upon conversion to the M form was considered by Nickerson and Edwards (66) to result from the selective inhibition of cell division, without simultaneous inhibition of growth, with selective inhibition being dependent only on temperature. They further considered that there is competition for a common substrate between the enzymes responsible for M-form development and the enzymes responsible for Y-form development. They assumed that a normally higher affinity of the mold-enzyme

system for the common substrate is offset at higher temperatures by an increasing rate in its reversible thermal inactivation, thus explaining the observed dependence of the cell-division mechanism on the maintenance of an elevated temperature.

Physical requirements. The optimal temperature for growth of the M form is stated to be 25 C (91) and 31 to 33 C (40); for the Y form, 35 to 37 C (40) and 37 C (66, 91). Optimal pH for growth of the M form is 4.5 to 7.5 (40); for the Y form, 5.5 to 8.5 (28, 40). The fungi grow only under aerobic conditions (28).

Agents demonstrating beneficial or deleterious effects on growth. Gilardi and Laffer (28) noticed a tendency of the Y form to produce a granular type of growth and to grow along the side of the culture tube above the liquid medium, suggesting that the yeast requires a surface or some particulate matter on which to grow. They found that adding agar to the medium does not enhance growth nor does it minimize the granulation or the growth along the side of the tube. The incorporation of agar into a liquid medium was found by Salvin (88) to enhance growth.

The addition of Tween 80 as a dispersing agent does not minimize the granular effect, but it has an inhibitory effect retarding growth. Halliday and McCoy (29) found Tween 80 to stimulate growth in the presence, but not the absence, of biotin, which is required for growth of their strain.

These investigators further observed that in shake cultures the growth still appears along the side of the culture tube above the liquid medium, and, because of the shaking, is more pronounced than in quiescent cultures; but the granular type of growth evident in quiescent cultures is virtually eliminated. They noticed that more luxuriant growth occurs in a shorter period of time in shake cultures compared to quiescent cultures, and in some cases compared to agar slant cultures. Salvin (89) also noticed luxuriant growth when cultures were kept in constant rotation.

The nonionic surface-active agents which are stimulatory for *C. neoformans* are also stimulatory for *B. dermatitidis* (53–55). Oxaloacetic acid (29), thiourea (22, 28), p-aminobenzoic acid (8), p-hydroxymethyl benzoic acid (108), as well as sulfur-containing compounds such as cysteine, glutathione, methionine, and thioglycolate (28), have an inhibitory effect on growth of *Blastomyces*. It was found that the Y form, in the presence of tyrosine, is converted to the M form at 35 C (28).

Assimilation patterns. Stewart and Meyer (102) found that the M form of *B. dermatitidis* is able to utilize ammonium acetate or acetamide as the sole source of both carbon and nitrogen in

liquid medium. Archibald and Reiss (1) found the M form to be capable of assimilating the following amino acids as sole sources of nitrogen on solid medium: cysteine, glutathione, glycine, alanine, leucine, valine, glutamic acid, aspartic acid, asparagine, serine, threonine, arginine, histidine, and phenylalanine. They observed poor growth with isoleucine, glutamine, lysine, tyrosine, tryptophan, proline, and hydroxyproline. They obtained no growth with methionine or p-aminobenzoic acid. In these studies, they pointed out that the agar was not purified and the mycelial inoculum was not washed.

Salvin (88) found that the Y form of *B. dermatitidis* gives slight growth with ammonium sulfate, potassium nitrate, tyrosine, and arginine, and good growth with glycine, alanine, serine, valine, aspartic acid, glutamic acid, proline, and hydroxyproline as the sole source of both carbon and nitrogen in semisolid medium. He found that the Y form of *B. brasiliensis* gives good growth with glycine, serine, glutamic acid, aspartic acid, proline, and hydroxyproline as the sole source of both carbon and nitrogen. He found no carbohydrate to be required for growth of the Y form of *B. dermatitidis*.

Gilardi and Laffer (28) found that the Y form of both *B. dermatitidis* and *B. brasiliensis* assimilates the same 21 carbon and 25 nitrogen substrates in liquid shake cultures. They found that the only carbohydrates assimilated as sole sources of carbon are the hexoses. They further found that glutamic acid, the imino acids proline and hydroxyproline, some aliphatic amino acids (alanine, glycine, serine, and valine), as well as the substituted amino acid asparagine, the amino acid relative β-alanine, and the amino acid metabolites pyruvic acid and lactic acid are assimilated as sole carbon sources. They also found three intermediates of the Krebs cycle (α-ketoglutaric acid, oxaloacetic acid, and succinic acid) to be assimilated.

They found that the only inorganic nitrogen compounds assimilated as sole sources of nitrogen are ammonium salts. They further found that some aliphatic amino acids (glycine, alanine, valine, serine, and isoleucine), the imino acids proline and hydroxyproline, the acidic amino acids aspartic acid and glutamic acid, and the basic amino acids histidine, arginine, and lysine are assimilated as sole nitrogen sources. The amino acid relatives citrulline, ornithine, and β-alanine, the substituted amino acids asparagine and glycylglycine, and the carbohydrate derivative glucosamine, as well as urea, were also found to be assimilated.

Similarity of the species. A comparison of the results obtained from studying these fungi shows

that there is no difference between the two species; any variation which the investigators could see occurred only at the culture level. It is concluded that there is no justification for placing the two species into separate genera, as proposed by some investigators; the fungi are close phylogenetically and belong in the same genus (28, 88).

Coccidioides

Vitamin and amino acid requirements. Coccidioides immitis does not require any vitamin or amino acid supplement (2, 4, 81, 102), and will grow in a simple GASM (4, 102).

In vitro cultivation of parasitic form. Nutritional studies have been primarily concerned with the in vitro cultivation of the spherules (parasitic form) of *C. immitis*. Media that have been employed contain complex organic substances such as coagulated egg albumin (36) and coconut milk (12). Conant and Vogle (16) obtained spherules in cultures transferred to Sabouraud medium after exposure to Tween 80. Lubarsky and Plunkett (48) obtained spherules in Tyrode's solution, containing chick embryo extract and rooster serum and aerated periodically with measured amounts of O_2 and CO_2. Larsh, Hinton, and Silberg (37) produced spherules in HeLa tissue cultures containing human serum. Northey and Brooks (72) developed a medium consisting of pyridoxine and a 2% solution of Edamine, a lactalbumin hydrolysate containing various amino acids and peptides, which enhances conversion to the parasitic form.

Other studies have been concerned with obtaining a chemically defined medium for spherule production. Baker and Mrak (3) obtained spherules on a solid acetate-ammonium-inorganic salts medium containing cupric sulfate. The first production of spherules in a liquid chemically defined medium, containing glucose, ammonium acetate, and inorganic salts, was obtained by Converse (17). Converse (18, 19) and Converse and Besemer (20) noticed that most satisfactory production is obtained from young (7- to 14-day) cultures in a medium containing 0.34% solids (total nutrient content) with optimal pH between 6.0 and 8.0, incubated at 37 C.

Converse (18) pointed out that shaking has a marked effect on spherule production. He noticed a decrease in yield in shake cultures, whereas stationary incubation produces a high yield of spherules. Many other investigators obtained good yields of spherules in shake cultures (38, 39, 45, 46, 72). Lones and Peacock (45) and Northey and Brooks (72) demonstrated that shaking is essential for spherule production.

Increased temperature (between 34 and 40 C) induces spherulation (10, 17, 18, 19, 20, 46, 48,

72), but Converse and Besemer (20) stated it is not the only factor influencing development. The studies by Breslau and Kubota (10) also indicated that temperature is a critical factor, but not the only factor for spherulation. They found that, below 40 C, the hyphae remain viable and grow, but at 40 C the hyphae degenerate (yet, the spherules survive). Whereas arthrospores germinate at lower temperatures, they found that arthrospores transform directly into spherules at 40 C, and the few viable hyphae quickly degenerate.

The addition of chemical agents to basal media enhances the conversion and maturation of the spherules. Some of these agents include pyridoxine (72), sodium and calcium ions (20), sodium bicarbonate (18, 20), sulfhydryl compounds (20), various metabolic inhibitors including sodium fluoride, sulfaguanidine, lithium chloride (20), and copper sulfate (3, 20), and hemoglobin and its derivatives such as hemin, biliverden, and bilirubin (72).

A marked stimulation of spherules has been noticed in cultures containing oleic and linoleic acids (20), Tamol N (19, 20, 45, 72), and Tween 80 (16, 20, 72). Converse and Besemer (20) stated that the stimulatory action may result from a change in the permeability of the spherule wall or may be due to the fact that these substances serve as growth factors or as detoxifying agents. Northey and Brooks (72) demonstrated that Tamol and Tween 80 enhance maturation of spherules, as reported by others (19, 20, 45), but maturation is found to be possible without the addition of a surface-active agent and to be nearly equal to that obtained with Tamol. Converse (19) was able to obtain an essentially pure (hyphae-free) culture of the spherules and to perpetuate them through several serial transfers, by inoculating arthrospores into his basal medium supplemented with Tamol N and incubating on a shaker for 72 hr at 34 C. Spherules have been continuously maintained for more than 4 years by Breslau and Kubota (10) in a medium lacking a surface-active agent. They used a modified Converse medium (less NaCl) diluted 1:25, in a modified Lubarsky and Plunkett (1955) culture tube incubated at 40 C. For continuous cultures, the investigators removed old medium and replaced it with fresh medium previously bubbled with a mixture of 20% CO_2 and 80% air.

Investigations have demonstrated the favorable effects of metabolic or bubbled CO_2 on the growth of the parasitic form (10, 45, 48). Lubarsky and Plunkett (48) were able to obtain spherules in tissue culture media supplemented with various animal sera aerated periodically with 23% O_2 and 77% CO_2. Lones and Peacock

(45) showed that Converse's medium, supplemented with Tamol N and 10% CO_2 and incubated on a rotary shaker at 35 C, enhances spherule production. They noticed that enhanced spherulation is demonstrated with large inocula (2.0 mg per ml), without bubbled CO_2, but not with small inocula (0.2 mg per ml) unless CO_2 is supplied. They conclude that the favorable effect of the larger inoculum is due to a greater concentration of metabolic CO_2. Breslau and Kubota (10) showed that a modified Converse medium bubbled with 20% CO_2 and incubated at 40 C enhances spherule production. They observed that, when arthrospores are incubated without CO_2, the inoculum germinates and the hyphae grow; with CO_2, the inoculum transforms into spherules. Furthermore, they observed that, when spherules are subcultured into fresh medium without bubbled CO_2, they germinate, and that, when a part of the old medium is removed and replenished with CO_2-bubbled fresh medium, germination is eliminated. In contrast to the previous studies, Northey and Brooks (72) found that a medium containing increased CO_2 has neither a beneficial nor a deleterious effect. Converse (18), in earlier studies, stated that the increased yield in stationary cultures may be due to an accumulation of CO_2 in the medium, and that the addition of sodium bicarbonate to the medium can be a substitute for shaking, with the same results. But, in later studies by Converse and Besemer (20), a requirement for added CO_2 was not demonstrated. They observed optimal spherulation to occur in atmospheres containing 20% O_2, in the absence of added CO_2. Furthermore, they found that a concentration of 10% CO_2 has no effect, but a CO_2 concentration greater than 10% inhibits spherulation. According to Lones and Peacock (45), the explanation that added CO_2 is not required for spherulation may be in differences in the size and metabolic activity of the inoculum and volume of the culture.

Converse and Besemer (20) found that sulfhydryl inhibitors inhibit spherulation, but that the inhibition is reversed by the addition of glutathione. Because of this observation, they suggested that the disulfide-sulfhydryl metabolic process similar to that associated with other dimorphic fungi may be operative in *C. immitis*.

Converse and Besemer (20) summarized the findings of their studies by saying that spherule production may be due to one or several factors and that the tonicity of the growth environment and the presence of sulfhydryl groups may be the most critical factors. They emphasized that the basic mechanism underlying conversion has not yet been elucidated.

Agents demonstrating beneficial or deleterious

effects on growth. Various agents have been found to have a deleterious effect on the growth of *C. immitis.* Thiourea (22) and *p*-hydroxymethyl benzoic acid (108) are fungistatic to the arthrospores of *C. immitis.* The addition of supplemental lactose or galactose to a basal medium enhances conversion of arthrospores to spherules, but not maturation, whereas glucose inhibits maturation (72). The addition of cysteine results in the formation of very large abortive spherules that fail to mature; methionine and thioglycolate inhibit spherulation (20). Northey and Brooks (72) found that, although the addition of thioglycolate to the original inoculum retards conversion, addition to a 12-hr-old culture enhances maturation of spherules. The sulfhydryl inhibitors, *p*-chloromercuribenzoate and iodoacetate, inhibit spherulation, but the inhibition is reversed by the addition of glutathione (20).

Vaccine preparation. With the formulation of chemically defined media for the growth of the parasitic form, it has been possible to develop a coccidioidal endospore-spherule vaccine. Levine, Cobb, and Smith (38, 39) described the preparation of such a vaccine by use of a modified Converse medium, containing 25% more acid phosphate, inoculated with arthrospores, and incubated at 37 C for 2 to 3 days. They harvested the crop of endospores by filtration, and used the filtered endospores as inoculum for fresh cultures. By repeating this procedure several times, they obtained a relatively hyphae-free endospore phase.

Metabolic properties of spherules. Lones and Peacock (46), in an examination of the metabolic properties of washed spherules, showed that respiration is increased by the addition of glucose and that most of the glucose utilized is assimilated. In contrast, stimulation of respiration of the hyphal form by added glucose is obtained only after starvation of the cells. The organism is capable of anaerobic metabolism, with the formation of CO_2 and ethanol, but this activity, which is stimulated by starvation, is present in a much lower degree in hyphae than in spherules.

Assimilation patterns. Ammonium salts and urea, but not nitrates, serve as the sole source of nitrogen; glucose and acetate, but not urea or formic acid, serve as the sole source of carbon (102). Acetamide, as well as all amino acids studied, serve as the sole source of both carbon and nitrogen. Baker and Smith (4) found the following utilized as the sole source of carbon: all hexoses, xylose, maltose, cellobiose, trehalose, raffinose, α-methyl glucoside, salicin, amygdalin, starch, dextrin, inulin, ethyl alcohol, glycerol, erythritol, mannitol, sorbitol, acetate, propionate, caproate, lactate, pyruvate, succinate,

fumarate, malate, glycine, alanine, cystine, acetamide, and asparagine. They observed no growth with arabinose, sucrose, lactose, melizitose, cellulose, inositol, formate, butyrate, oxalate, tartrate, gluconate, citrate, or urea. The organic acids which do not support growth are not toxic, since these investigators observed that the spores germinate and develop until the reserve food material in the spore is exhausted. Baker and Smith (4) found the following utilized as the sole source of nitrogen: nitrate, in contrast to the results of Stewart and Meyer (102), ammonium chloride, acetamide, asparagine, glycine, alanine, glutamic acid, tyrosine, and cystine. They observed that nitrite is not utilized. Lones and Peacock (47) found that the spherule form assimilates glucose (as the sole source of carbon) but not mannitol, as does the filamentous form. They found that neither the spherules nor the filamentous form utilizes citrate or sorbitol. However, Baker and Smith (4) found that sorbitol is assimilated by the filamentous form.

Histoplasma

Vitamin requirements. Histoplasma capsulatum has been grown on comparatively complex solid and liquid media containing meat infusion (84), blood (15, 84), potato flour plus egg (35), neopeptone, and Tryptone (11, 58, 59, 85). Many of these and other complex media have been used for the conversion and maintenance of the Y form (11, 15, 35, 59). Larsh et al. (37) obtained Y-form conversion in HeLa tissue cultures containing human serum. Zarafonetis (109) obtained the Y form in Dubos medium supplemented with albumin.

Nutritional studies have been concerned with obtaining a better understanding of the factors influencing growth and dimorphism by studying the basal nutritional requirements. Vitamin-requirement studies of *H. capsulatum* show variable results. The M form has been found to require no vitamins (2, 77, 87), to require thiamine (2, 90), biotin (87), and pantothenate (83), and to be partially deficient for biotin and niacin (90). The Y form has been found to require thiamine (77), biotin (77, 87), and thioctic acid (77).

Amino acid requirements. The amino acid requirements of the M form have been reported to be complex and variable for different strains (83). However, Salvin (87) has grown the M form with ammonium as the sole nitrogen source, but the Y form requires a sulfide or sulfhydryl group, such as in cysteine. Other investigations also find that the Y form has a requirement for sulfhydryl groups (75, 76, 91, 92). In addition to a requirement for cysteine, Pine (75) found that aspartic acid and glutamic acid are stimulatory for growth.

Glutathione is able to replace the —SH requirement but not cysteine, so Pine suggests that cysteine is not a requirement for —SH groups alone.

The simplest basal medium for the growth of the M form is the GASM used by Salvin (87), with biotin added for biotin-requiring strains. The Y form is grown on a glucose-cysteine-inorganic salts medium containing biotin (87, 91, 92).

Physical requirements. The Y form grows both aerobically and anaerobically (85), but Pine (75) found both the Y and M forms to be obligate aerobes. The optimal pH for growth of the Y form is between 6.3 and 8.1 (85).

Factors influencing dimorphism. Dimorphism in *Histoplasma* is a function of temperature (11, 59, 77, 85, 91, 92), vitamins (77, 87, 89), and sulfhydryl groups (75, 76, 87, 91, 92). The optimal temperature for Y-form development is 37 C (11, 59, 77, 85, 91, 92), but some strains will maintain the Y form at 25 C (77, 91, 92). Y-form development also depends on the presence of vitamins such as biotin (77, 87), thiamine, and thioctic acid (77). In addition to vitamins, Salvin (87) found —SH groups to be necessary for Y-form development. Salvin demonstrated that this requirement is best fulfilled with an organic molecule of small size, such as an amino acid. He found that if the molecular size is too great, as in the tripeptides, some hyphae accompany the Y growth. Pine (75) stated that cysteine is required to initiate growth of the Y form; the —SH groups maintain the viability of the fungus until growth commences. Pine (77) found that the Y form will grow at 25 C when cysteine is present in the medium. He stated that, given the correct medium, both forms might grow under apparently optimal and identical conditions at 25 C. Hence, he believed that the Y form is not merely a morphological expression of an increased temperature. He explained this by suggesting that two different metabolic reactions occur for each form, reactions which are competitive but not necessarily mutually exclusive. Scherr (92) also found that the Y form is maintained at 25 C when cysteine is added to the medium. He stated that the concentration of cysteine is more critical for the maintenance of the Y form than the temperature of 37 C; the role of the temperature appears to be the maintenance of optimal conditions for the activity of enzymes which direct M to Y transition.

Agents demonstrating beneficial or deleterious effects on growth. Chemical and physical factors are known to enhance the growth of the Y form. Pine (75) found that albumin stimulates growth. He observed that a high concentration of oleic

acid inhibits growth, but this effect is reversed by the addition of albumin. This is attributed to the fact that albumin is able to detoxify fatty acids (59, 75, 109). Rowley and Pine (83) found that the addition of KH_2PO_4 inhibits germination of the Y form, but this is reversed upon the addition of albumin or starch.

Rowley and Huber (82) did not find any deleterious effect upon the addition of agar to their basal medium, but Pine (76) observed that the addition of agar to basal medium retards growth of the Y form. He found that the addition of albumin or starch reverses the inhibition, and he suggested that agar also contains a toxic substance such as a fatty acid. Salvin (85) found that no growth will occur in a liquid medium unless the viscosity is increased by 0.175% agar or silica gel. He attributed this to the fact that a growth substance is provided or a specific O_2 or CO_2 tension is produced. McVickar (58) stated that agar detoxifies the environment and enhances Y-form growth. Scherr (92) believed that agar or silica gel reduces the oxidation-reduction potential to optimal conditions for growth of the Y form.

Carbon dioxide is reported to enhance growth (11, 75), but Salvin (85) reported that CO_2 tension is of no importance. Without making any addition to the basal medium, Salvin (89) obtained extensive growth of the Y form when his liquid medium was kept in constant rotation.

Studies have also been concerned with the effect of various agents on the M form. Various nonionic surface-active agents, when incorporated into basal medium, have been observed to enhance growth of the M form (53–55). Diethylthiourea (87), thiourea (22), and p-hydroxymethyl benzoic acid (108) have been reported to be fungistatic to the M form of *H. capsulatum*.

Assimilation patterns. Scheff (90) found that the M form assimilates glucose, maltose, sucrose, lactose, fumaric acid, and succinic acid, but not starch, as the sole carbon source. He observed that a carbohydrate is not essential, since growth takes place in its absence, with asparagine, fumaric acid, or succinic acid being used as the carbon source. He found that the M form assimilates ammonium sulfate, aspartic acid, asparagine, succinic acid, and fumaric acid as the sole source of nitrogen. Archibald and Reiss (1) observed that the M form can not grow with methionine as the sole nitrogen source and gives only poor growth with cysteine, lysine, histidine, tryptophan, phenylalanine, and p-aminobenzoic acid. Sixteen other amino acids in their investigation were assimilated as the sole nitrogen source.

Salvin (87), in an extensive survey on the ability of the Y form to assimilate various inorganic and organic nitrogen compounds as the sole source of nitrogen, found that none of the inorganic compounds and only three organic compounds, cysteine, cystine, and glutathione, are assimilated. He also noted that the M form assimilates inorganic ammonium, but not nitrate or nitrite compounds.

Sporotrichum

Vitamin requirements. Only a few investigations of *Sporotrichum schenckii* have been reported. Robbins and Kavanagh (80) stated that the M form grows in the presence of thiamine, and can also grow, but to less degree, in a liquid glucose-ammonium lactate-asparagine-inorganic salts medium without thiamine. Other investigations indicate that both the Y and M forms require thiamine (2, 14, 51, 52), and that the M form requires no vitamins (49).

Amino acid requirements. Most nutritional studies have been performed with media containing complex organic nitrogen sources such as a mixture of proteose-peptone, neopeptone, and Tryptone (86), neopeptone plus Tryptone (85), asparagine (2, 14, 52), Casamino Acids plus cystine, caseinate (49), casein hydrolysate (51, 52), and individual amino acids (1). The M form has been grown on a medium containing ammonium as the sole nitrogen source (51), but the Y form requires organic nitrogen. In addition to cultivation on agar-containing media, both the Y and M forms have been grown in liquid media by several investigators (49, 51, 52, 86).

Factors influencing dimorphism. Nutritional studies have been concerned with the factors responsible for the conversion and maintenance of the Y form. Salvin (86) grew the Y form in a semisolid peptone medium and found the following conditions favoring Y-form development: optimal temperature of 37 C, 0.1 to 0.3% concentration of agar, pH of 8.2, and 60 to 80% CO_2 tension. He noted that the absence of agar, an acid environment, and a high concentration of glucose results in a conversion to the M form. Bullen (11) was able to obtain the Y form on a peptone medium incubated at 37 C in an atmosphere of 15% CO_2. He concluded that a certain concentration of CO_2 is essential for Y-form development. Norden (71) stated that the Y form is obtained on complex organic media by increasing the CO_2 tension. Mariat and Drouhet (51, 52) grew the Y form in a liquid glucose-casein hydrolysate-inorganic salts medium containing thiamine and biotin. A temperature of 37 C, a pH of 7.1, and the addition of biotin to the basal medium were found to favor Y-form development; mechanical agitation or increased CO_2 tension was found to be essential for Y-form growth.

Agents demonstrating beneficial or deleterious

effects on growth. The nonionic surface-active agents which are stimulatory for *C. neoformans* are also stimulatory for the M form of *S. schenckii* (53–55). Thiourea (22) and *p*-hydroxymethyl benzoic acid (108) were found to be fungistatic for the M form of this fungus.

Assimilation patterns. Archibald and Reiss (1) found that the M form grows not at all or poorly with aspartic acid, *p*-aminobenzoic acid, lysine, or histidine as the sole source of nitrogen, but grows well with 19 other amino acids studied.

Mariat and Drouhet (51, 52) performed an extensive comparison of the effect of various nitrogen sources as the sole source of nitrogen (inorganic salts, casein hydrolysate, 22 individual amino acids) on growth in shake, quiescent liquid, and solid cultures. They found that, regardless of the nitrogen source, the M form develops in quiescent liquid and solid cultures, and develops in shake cultures in the presence of ammonium sulfate, ammonium nitrate, aspartic acid, asparagine, serine, and the sulfur-containing amino acids. They found that the Y form develops in shake cultures in the presence of casein hydrolysate, casein hydrolysate plus amino acids, and the following individual amino acids: alanine, arginine, glutamic acid, glycine, isoleucine, leucine, norleucine, norvaline, ornithine, phenylalanine, threonine, and valine. When the investigators increased the CO_2 tension of the culture environment, the Y form developed in still or shake liquid cultures and solid cultures. They noted that, without increased CO_2 tension, only liquid shake cultures supplied with specific amino acids allow Y-form development. Mariat and Drouhet concluded that the amino acids, through decarboxylation, serve as a source of CO_2 required for Y-form development, in place of atmospheric CO_2, and that the accumulation of CO_2 is probably favored by shake cultures.

NUTRITION OF THE DEMATIACEOUS FUNGI

The dematiaceous fungi which have undergone nutritional studies include the fungi of chromoblastomycosis and related dematiaceous saprophytes and pathogens. Five species of dematiaceous fungi are now recognized as causing chromoblastomycosis. These are *Phialophora verrucosa, Cladosporium carrionii, Fonsecaea pedrosi, F. compacta,* and *F. dermatitidis.* Representatives of dematiaceous fungi which serve as agents of mycetoma include *Phialophora jeanselmei* and *Cladosporium gougerotii.* Saprophytic dematiaceous fungi include *Cladosporium sphaerospermum, C. elatum, Phialophora obscura, P. richardsiae,* and *Pullularia pullulans.*

Vitamin-requirement studies indicate that thiamine is required by *F. pedrosi, F. verrucosa*

(2, 14), *F. compacta,* and *P. jeanselmei* (2). A species identified by Arêa Leão and Cury (2) as *Cladosporium wernecki* requires no vitamin for growth. In recent studies, Silva (99) found that a B-vitamin supplement is essential for growth of strains of *P. richardsiae* and *P. obscura,* stimulates growth of strains of *F. pedrosi, F. compacta, F. dermatitidis, C. carrionii, C. gougerotii, C. sphaerospermum, C. elatum, P. verrucosa,* and *P. jeanselmei,* and has no effect on the growth of strains of *P. pullulans* nor on other strains of *P. jeanselmei* and *C. gougerotii.*

Silva (99) found organic nitrogen to be essential for the growth of a strain of *P. jeanselmei,* to stimulate the growth of strains of *P. verrucosa* and *P. obscura,* and to have no effect on growth of any other strains studied. Those strains not requiring vitamin supplement or organic nitrogen can be grown on a GASM.

Silva (99) observed that alterations in the basal medium are able to influence the type of sporulation. By increasing the concentration of inorganic nitrogen, substituting ammonium chloride for sodium nitrate, or adding yeast extract, she was able to increase the pseudo-Acrotheca type of sporulation. No substance was found that consistently stimulated either the *Cladosporium* or *Phialophora* type of sporulation.

Montemayor (62) reported an optimal temperature of 20 to 25 C for saprophytic cladosporia, of 30 C for agents of mycetoma, and of 37 C for agents of chromoblastomycosis. Silva (98, 99) reported the optimal temperature in the last-named group to range between 25 and 35 C. She concluded that the faster growth rate at 25 C of saprophytic cladosporia than that of the fungi of chromoblastomycosis does not provide a reliable criterion for differentiation of these two groups. Furthermore, she observed that the absence of growth at 30 C is a good sign of a saprophyte, but growth at this temperature is not found to be an exclusive characteristic of pathogenic cladosporia.

Cycloheximide is known to inhibit saprophytic fungi, but Silva (99) found that, with the exception of one isolate of *P. obscura,* all saprophytic isolates grow well on this antibiotic. She concluded that cycloheximide is not suitable for separating a saprophyte from a pathogen.

The factors responsible for the conversion to the parasitic form of the fungi of chromoblastomycosis are unknown. Serial streaking of fungi on Francis glucose-cystine-blood-agar (97) and Cystine-Heart-Hemoglobin agar (99) incubated at 37 C induces partial conversion to the parasitic form. Complete suppression of the filaments has been obtained only with *P. jeanselmei* and *C. gougerotii* (99). Partial conversion also has been

obtained in the chick embryo chorioallantoic membrane and in the pupae of silkworms (97).

Montemayor (62) reported the following carbon and nitrogen assimilation patterns: the agents of chromoblastomycosis assimilate glucose, maltose, sucrose, galactose, and asparagine, but not lactose, urea, ammonium sulfate, or potassium nitrate; the agents of mycetoma assimilate glucose, maltose, sucrose, galactose, urea, asparagine, ammonium sulfate, and potassium nitrate, but not lactose; the saprophytic cladosporia assimilate all the above-mentioned substrates. Silva (98) reported that the agents of chromoblastomycosis assimilate glucose, fructose, xylose, arabinose, sucrose, maltose, and starch, but not lactose or cellulose.

NUTRITION OF ALLESCHERIA (MONOSPORIUM)

A few studies have been performed on one of the causative agents of maduromycosis, Allescheria boydii (Monosporium apiospermum). It has been cultured on a glucose-asparagine-inorganic salts medium containing biotin (2, 21, 106). Villela and Cury (106) reported a pH of 4 to 5 and room temperature as optimal conditions, with no growth occurring at 37 C. They noticed that nicotinic acid and casein hydrolysate enhance growth, that cysteine and glutathione have no effect on growth, and that pyridoxine depresses growth. In their study of compounds known to replace biotin requirements, they found that pimelic acid, aspartic acid, oleic acid, and sorbitan monooleate are not effective. However, they found that the analogues desthiobiotin and o-heterobiotin do partially replace biotin, but do not show an additive response when added to biotin. Wolf (108) observed that p-hydroxymethyl benzoic acid has a fungistatic effect on this fungus.

CONCLUSIONS

Data in Table 1 show that there is wide variation in the results obtained from vitamin-requirement studies among different strains of the same species. This is true in Blastomyces, Histoplasma, Sporotrichum, and some agents of chromoblastomycosis. The requirements of Cryptococcus, Candida, Coccidioides, and Allescheria appear to be well substantiated. Cryptococcus requires thiamine, Candida and Allescheria require biotin, and Coccidioides has no requirement.

Few definite statements can be made concerning differences between the Y and M forms with respect to their vitamin requirements. The Y form of H. capsulatum definitely requires a B vitamin, but the M form probably requires no vitamin. The Y form of S. schenckii is stimulated by biotin, but biotin has no effect on the M form.

Definitive requirements for these organisms will be obtained only after repeated experimentation by various investigators using a large number of strains. An all-purpose basal medium containing biotin and thiamine would probably support the growth of the majority, if not all, of the strains of pathogenic fungi which have been cultivated on artificial media to date.

The majority of the fungi will grow on a basal medium supplied with inorganic ammonium salts. There are some important exceptions. The Y form of H. capsulatum requires a sulfhydryl compound. The Y form of S. schenckii requires organic nitrogen, as does the filamentous form of A. boydii and P. jeanselmei. A difference is noted between the Y and M forms of dimorphic fungi in regard to nitrogen requirements, with the Y form demonstrating a more exacting requirement, requiring organic nitrogen. In most cases where organic nitrogen is not required, it is found to be stimulatory.

At present, little is known about the nutritional requirements of the parasitic form of the agents of chromoblastomycosis. With this exception, synthetic basal media have been developed which will support the growth of all the fungi included in this review. An all-purpose basal medium incorporating the same nitrogen source would be of only partial value, since some organisms require sulfhydryl compounds and others have demonstrated either inhibited or retarded growth when —SH-containing compounds are incorporated in the medium.

On the basis of evidence obtained to date, dimorphism in C. albicans is controlled through disulfide - sulfhydryl maintenance. Sulfhydryl maintenance is required for Y-form development, whereas the disulfide form favors production of the filamentous form. Dimorphism in Blastomyces is probably a function only of the temperature of incubation. Temperature is a critical factor controlling conversion in C. immitis, H. capsulatum, and S. schenckii, but other factors, including disulfide-sulfhydryl maintenance, CO_2 tension, presence of B vitamins, and tonicity of the growth medium, influence dimorphism in these organisms as well. Little is known concerning the factors that control dimorphism in the agents of chromoblastomycosis.

Physical and chemical modifications of the environment and basal medium have demonstrated a beneficial effect on growth (Table 2). Besides alterations in the pH of the medium and temperature of incubation, changes in the atmosphere of incubation produce beneficial effects. Aeration with CO_2 or mechanical agitation (shake cultures) is beneficial for Blastomyces, C. immitis, H. capsulatum, and S. schenckii. Shaking

TABLE 1. *Summary of nutritional requirements of pathogenic fungi as reported in the literature*

Organism	Vitamin requirements	Nitrogen requirements	Basal medium
Candida albicans............	Biotin (numerous)* Thiamine (stimulatory; numerous) Pantothenate (3)*	Inorganic ammonium salts	Glucose Ammonium-inorganic salts Biotin
Cryptococcus neoformans......	Thiamine (numerous)	Inorganic ammonium salts Amino acids (stimulatory)	Glucose Ammonium-inorganic salts Thiamine
Blastomyces dermatitidis......	*Yeast form* No requirement (22) Biotin (6) *Mold form* No requirement (7) Biotin (6)	*Yeast form* Inorganic ammonium salts Organic nitrogen (stimulatory) *Mold form* Inorganic ammonium salts Organic nitrogen (stimulatory)	Glucose Ammonium-inorganic salts
B. brasiliensis...............	*Yeast form* No requirement (11) *Mold form* No requirement (2) Thiamine (2) Thiamine (stimulatory; 1)	*Yeast form* Inorganic ammonium salts Organic nitrogen (stimulatory) *Mold form* Inorganic ammonium salts Organic nitrogen (stimulatory)	Glucose Ammonium-inorganic salts
Coccidioides immitis..........	*Parasitic form* No requirement (numerous) *Filamentous form* No requirement (numerous)	*Parasitic form* Ammonium acetate *Filamentous form* Inorganic ammonium salts	*Parasitic form* Glucose Ammonium acetate Inorganic salts *Filamentous form* Glucose Ammonium-inorganic salts
Histoplasma capsulatum......	*Yeast form* Biotin (9) Thiamine (8) Thiotic acid (1) *Mold form* No requirements (24) Thiamine (2) Biotin (1) Pantothenate (1) Niacin (stimulatory; 1) Biotin (stimulatory; 1)	*Yeast form* Sulfhydryl compounds Aspartic acid and glutamic acid (stimulatory) *Mold form* Inorganic ammonium salts	*Yeast form* Glucose Cysteine Inorganic salts Biotin or thiamine *Mold form* Glucose Ammonium-inorganic salts

(*continued*)

TABLE 1—*Continued*

Organism	Vitamin requirements	Nitrogen requirements	Basal medium
Sporotrichum schenckii	*Yeast form* Thiamine (2) Biotin (stimulatory; 2) *Mold form* Thiamine (4) No requirement (1)	*Yeast form* Organic nitrogen *Mold form* Inorganic ammonium salts Organic nitrogen (stimulatory)	*Yeast form* Glucose Casein hydrolysate Inorganic salts Thiamine *Mold form* Glucose Ammonium-inorganic salts
Allescheria boydii	Biotin (5)	Asparagine	Glucose Asparagine Inorganic salts Biotin
Fonsecaea pedrosoi	*Mold form (all species)* Thiamine (2) B vitamins (stimulatory; 2)	*Mold form (all species)* Inorganic ammonium salts	*Mold form (all species)* Glucose Ammonium-inorganic salts (B-vitamin supplement and organic nitrogen for deficient strains)
F. compacta	Thiamine (1) B vitamins (stimulatory; 2)	Inorganic ammonium salts	
F. dermatitidis	B vitamins (stimulatory; 1)	Inorganic ammonium salts	
Cladosporium carrionii	B vitamins (stimulatory; 2)	Inorganic ammonium salts	
C. gougerotii	No requirement (1) B vitamins (stimulatory; 1)	Inorganic ammonium salts	
C. sphaerospermum	B vitamins (stimulatory; 1)	Inorganic ammonium salts	
C. elatum	B vitamins (stimulatory; 1)	Inorganic ammonium salts	
Phialophora jeanselmei	Thiamine (3) No requirement (3) B vitamins (stimulatory; 1)	Organic nitrogen required	
P. richardsiae	B vitamins required (1)	Inorganic ammonium salts	
P. verrucosa	Thiamine (2) B vitamins (stimulatory; 2)	Organic nitrogen (stimulatory)	
P. obscura	B vitamins required (1)	Organic nitrogen (stimulatory)	
Pullularia pullulans	No requirement (1)	Inorganic ammonium salts	

* Number of strains demonstrating stated requirement.

of the medium is reported as essential for the production of spherules of *C. immitis* and for the development of the Y form in *S. schenckii*. The beneficial effect in the latter two fungi is due to the increased CO_2 tension. Either CO_2 or O_2, or both, may stimulate growth of the other fungi. It should be remembered that other experiments have shown that increased CO_2 tension has no effect on the growth of *H. capsulatum* and may inhibit spherulation in *C. immitis*. The role of O_2 and CO_2 tension in the growth of fungi requires clarification.

TABLE 2. *Summary of factors influencing dimorphism of pathogenic fungi, and physical and chemical agents affecting growth of pathogenic fungi, as reported in the literature*

Organism	Factors influencing dimorphism	Agents demonstrating a beneficial effect on growth	Agents demonstrating a deleterious effect on growth
Candida albicans............	Disulfide-sulfhydryl maintenance	*Yeast form* Fermentable carbohydrate Presence of —SH compounds Increased O_2 tension *Filamentous form* Absence of —SH compounds Decreased O_2 tension Assimilable carbohydrates Polysaccharides Phosphorus Potassium Biotin Starvation media Liquid media	*p*-Hydroxymethyl benzoic acid Thiourea Hydrogen sulfide Sodium acetate Ascorbic acid
Cryptococcus neoformans......		Nonionic surface-active agents	*p*-Hydroxymethyl benzoic acid Thiourea
Blastomyces dermatitidis......	Temperature CO_2 tension	*Yeast form* Semisolid media (agar) Tween 80 Aeration (shake cultures) *Mold form* Nonionic surface-active agents	*Yeast form* Tween 80 Oxaloacetic acid Sulfur-containing compounds Tyrosine *Mold form* *p*-Hydroxymethyl benzoic acid
B. brasiliensis...............	Temperature	*Yeast form* Aeration (shake cultures)	*Yeast form* Tween 80 Sulfur-containing compounds *Mold form* *p*-Aminobenzoic acid
Coccidioides immitis..........	Temperature Tonicity of media Sulfhydryl compounds	*Parasitic form* Pyridoxine Sodium, calcium ions Bicarbonate Sulfhydryl compounds Metabolic inhibitors Hemoglobin Oleic, linoleic acids Tween 80, Tamol N Metabolic or bubbled CO_2	*Parasitic form* Supplemental glucose Sulfur-containing compounds Shake cultures (aeration) Sulfhydryl inhibitors *Filamentous form* *p*-Hydroxymethyl benzoic acid Thiourea

(continued)

TABLE 2.—*Continued*

Organism	Factors influencing dimorphism	Agents demonstrating a beneficial effect on growth	Agents demonstrating a deleterious effect on growth
Histoplasma capsulatum	Temperature B vitamins Sulfhydryl compounds	*Yeast form* Albumin Starch Agar (semisolid media) Silica gel CO_2 tension Aeration (shake cultures) *Mold form* Nonionic surface-active agents	*Yeast form* Potassium dihydrogen phosphate Oleic acid Agar *Mold form* p-Hydroxymethyl benzoic acid Thiourea Diethylthiourea
Sporotrichum schenckii	Temperature CO_2 tension	*Yeast form* Biotin Shake cultures (aeration) Amino acids CO_2 tension Agar (semisolid media) pH *Mold form* Nonionic surface-active agents	*Mold form* p-Hydroxymethyl benzoic acid Thiourea
Allescheria boydii		Nicotinic acid Casein hydrolysate	p-Hydroxymethyl benzoic acid Pyridoxine
Cladosporium species	Unknown	*Parasitic form* Cystine heart-hemoglobin agar Francis cystine-blood-agar	

Difficulty has been encountered in obtaining Y-form growth in liquid media, but good growth is obtained as long as the basal requirements are fulfilled. Physical factors will enhance the growth. The addition of agar or silica gel to a semisolid medium is favorable to Y-form growth of *H. capsulatum* and *S. schenckii*. Luxuriant growth of *Blastomyces* is obtained in a basal liquid medium incubated with mechanical agitation. Mechanical agitation or increased CO_2 tension enhance Y-form development in *S. schenckii* and *H. capsulatum* in liquid media.

Various surface-active agents such as Tween 80 and Tamol N, and fatty acids such as oleic acid and linoleic acid, enhance growth. This has been attributed to the ability of these agents to allow the nutrient material of the culture medium to come in more intimate contact with the cell, to increase the permeability of the cell, and to facilitate faster utilization of the nutrients, with consequent stimulation of growth. They have also been described as serving as detoxifying agents. The role of albumin, starch, and agar in enhancing growth is attributed to the fact that these agents also detoxify the environment. But it should be emphasized that there are instances where Tween 80, agar, and oleic acid demonstrate deleterious effects on growth.

Table 2 shows that the majority of agents demonstrating a deleterious effect on growth are reducing agents or sulfur-containing compounds. The inhibitory effect of the reducing agents may be brought about by the lowering of the oxidation-reduction potential to the point where growth is retarded or cannot be initiated. This would explain the action of cysteine, thioglycolate, glutathione, and ascorbic acid. The sulfur radical as found in methionine, cystine, and thiourea may block the metabolism of the organism by inactivating enzyme systems through oxida-

tion of sulfhydryl-active sites or by functioning as a chelating agent.

Assimilation patterns at present are too sketchy to make many conclusions. With respect to carbon assimilation, these fungi are capable of assimilating all of the hexoses. Lactose is usually not available, whereas the assimilation of other disaccharides, namely, maltose, sucrose, trehalose, and cellobiose, is variable. Variable results are also noticed with regard to other oligosaccharides, the pentoses, and the polyvalent alcohols. Carbohydrate derivatives assimilated by one or more species include gluconic acid lactone, glyceraldehyde, pyruvic acid, and lactic acid. Krebs cycle intermediates assimilated include α-ketoglutaric acid, succinic acid, fumaric acid, and oxaloacetic acid. Various amino acids, amino acid derivatives, and fatty acids are also capable of being used as the sole carbon source.

Inorganic nitrogen compounds assimilated as the sole source of nitrogen include only ammonium salts. Nitrates, nitrites, and hydroxylamine are not available. Many of the amino acids and amino acid derivatives are assimilated. Some of the more complex organic compounds assimilated as sole sources of nitrogen include citrulline, ornithine, asparagine, glutamine, glucosamine, glutathione, glycylglycine, β-alanine, urea, and acetamide.

The nutritional behavior of ascosporogenous yeasts and the dermatophytes is used in their classification. Eventually, it may be possible to classify systemic and subcutaneous fungi according to their nutritional characteristics. Before this is possible, definitive vitamin requirements and more complete assimilation patterns must be obtained.

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