ERADICATION OF CANDIDA ALBICANS FROM THE GASTROINTESTINAL TRACT OF MICE AND WOMEN

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ABSTRACT

ERADICATION OF *CANDIDA ALBICANS* FROM
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By

Linda Sue Olsen

Candidosis, most commonly caused by *Candida albicans*, is an infection of endogenous nature, originating in most cases from the intestine. A mouse model was developed to study removal of the intestinal carrier state of *C. albicans*. Nystatin and crystal violet were compared for efficacy in eradication of intestinal *C. albicans* in the mouse. Crystal violet was found to be effective, while nystatin was not. Human studies were also conducted to determine if gentian violet would remove intestinal carriage of *C. albicans*. Removal of the intestinal reservoir of *C. albicans* could result in a long-lasting period without candidosis. All persons taking 6-10 days of the prescribed therapy became culturally negative for intestinal *C. albicans* and remained negative at least one month after completion of therapy.

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INTRODUCTION

Candida albicans is a commensal yeast in the intestine of 25-50% of healthy persons [19,112]. It is passed from mother to neonate at birth during passage through the birth canal [51,102]. The incidence of *Candida* infections and the change from commensal to parasite are increased in debilitated persons as well as in those with altered physiological states [92,112].

It is thought that the intestine is a reservoir for infection of other parts of the body by *Candida albicans* [17,67,102]. In debilitated persons systemic dissemination may occur. More commonly the gut may seed the perianal area causing cutaneous candidosis of both infant and adult. The intestine is also a reservoir for the infection of the vagina and reinfection after topical treatment [110].

To eliminate the reservoir, *C. albicans* must be eradicated from the alimentary tract. Sternberg *et al.* (1954), as well as other investigators, have suppressed the intestinal *Candida* population by administration of nystatin tablets or capsules. However, they failed to completely eradicate the *Candida* population of the

alimentary tract as evidenced by reappearance of the yeast soon after treatment ended [78,98].

Current therapy for candidosis is aimed at the site of infection rather than the intestinal source of the infection. Even when oral intestinal therapy with nystatin, the current drug of choice, is combined with topical therapy, the goal and result is merely to return the population of *C. albicans* to commensal proportions.

The mouse has been a useful model for the study of pathogenesis and treatment of various forms of candidosis. In the present study, a mouse model for intestinal colonization by *C. albicans* is described. This model was used to compare two antimycotic agents, crystal violet and nystatin, with regard to efficacy in removal of an intestinal population of *C. albicans*. The model is potentially useful in the laboratory study of human intestinal candidosis and the treatment and prevention of candidoses arising from intestinal sources.

The study was extended to humans, in which the purpose was to determine if the alimentary population of *C. albicans* could be eradicated through use of gentian violet.

LITERATURE REVIEW

Candida albicans

There are 8 species of *Candida* which have been repeatedly associated with human disease. Of these, *Candida albicans* is the most pathogenic and most commonly encountered in disease [92]. Its synonyms are *Oidium albicans*, Robin 1853; *Monilia albicans* (Robin) Zopf 1890; *Syringospora robinii*, Quinquad 1869; *Endomyces pinoui*, Castellani 1922; *Candida genitalis*, Batista and Silveria 1962; and *Candida intestinalis*, Batista and Silveria 1959. Lodder lists a total of 100 synonyms [61].

Candida albicans is a yeast-like fungus, which reproduces by budding and forms a white, creamy, smooth, opaque colony on Sabouraud's dextrose agar after 3 days at 25 C. At one month it is "creamy, glistening, waxy, soft, smooth to somewhat reticulated; old stocks wrinkled and folded with spicules." Microscopic cellular morphology after 3 days at 25 C in Sabouraud's dextrose broth consists of globose, short, ovoid (5-7 μm), sometimes elongate yeasts (4-6 x 6-10 μm) [92]. When incubated in serum for 2 h at 37 C, the blastospores form germ tubes (Reynold's-Braude phenomenon), a characteristic of *C. albicans* not seen in most other yeasts or other

species of *Candida* associated with human disease [91,101]. This phenomenon is probably due to the low glucose, high undigested protein content of serum. Sabouraud's dextrose broth is 1% peptone (enzyme-digested denatured proteins) and 4% glucose. The nitrogen in serum occurs as protein and the glucose content is less than 0.1% [16]. Germ tube formation is a rapid, reliable diagnostic technique for identification of *C. albicans* [101].

Candida albicans is the only yeast occurring in man which usually forms chlamydospores on chlamydospore agar. Chlamydospores are "thick-walled, non-deciduous, intercalary or terminal asexual spores made by the rounding up of a cell or cells" [2]. The word chlamydospore is derived from the Greek word *chlamys*, meaning mantle or sheath. Chlamydospores of *C. albicans* are large (7-17 μm), thick-walled cells which appear at the tips and sides of hyphae. The chlamydospore is borne on an elongated suspensor cell or a long filament. Both of these cells are produced by budding. Material is deposited inside giving rise to a thick wall. Proto-chlamydospore cells which look as though they will become chlamydospores must bud once more and the budded cell becomes the chlamydospore. They concentrate glycogen and other cellular material which migrates into the chlamydospore [46]. Chlamydospores are generally formed in the absence of an easily utilizable carbon source. An increase in glucose produces a decrease in chlamydospore

formation [81]. Glucose at a concentration of 5 mg/ml has been shown to inhibit chlamydospore production [46]. Chlamydospore formation may be enhanced by inoculation of media free of reducing sugars. Nickerson-Mankowski chlamydospore agar or cornmeal agar plus Tween-80 is used for this purpose. These media are inoculated by furrowing an inoculum into the medium, thus effecting a reduced oxygen tension in the organism's immediate environment.

Electron micrographs of chlamydospores have shown them to be double-layered, consisting of a thin, electron-transparent outer layer surrounding a thick electron-dense inner layer. The thickness of the inner layer increases with age. It is 0.4 μm in mature cells. The outer layer is continuous with the cell wall of the suspensor cell. A membrane seems to separate the inner layer from the cytoplasm. It has no structurally distinguishable cytoplasmic components [46].

Tsuchiya has done serologic studies on the different species of *Candida* [108]. There are many antigens associated with the species of this genus. Charts of the antigenic structures of each species as determined by absorbed antisera have been compiled by several researchers [35,108,113]. As a result of these studies, as well as others, *C. albicans* can be divided into 2 serologic groups, A and B, of equal pathogenicity [92].

Carbohydrate fermentation, assimilation, and mycelial morphology may also be useful in identification of *C.*

albicans. *Candida albicans* ferments glucose and maltose with acid and gas production, while only acid is produced as sucrose is fermented. It assimilates glucose, galactose, maltose, and sucrose [6,92].

There is no known perfect stage of *C. albicans* [92].

Epidemiology, Ecology, Distribution

Candida albicans is part of the normal flora of the alimentary tract, including the oral cavity, of 25-50% of healthy adult humans [19,112]. The prevalence of *C. albicans* in the normal vagina of healthy, nonpregnant women has been reported as 5-20% [68,102,112,113], but is increased to 15-50% in healthy pregnant women [19,42, 112,113]. In the oral cavity of the newborn the occurrence has been reported to be about 4% [102]. In the feces of 2- to 6-week-old infants the prevalence of *C. albicans* is 25% [23], which is similar to that in the vagina of pregnant women and the feces of healthy adults [112].

Before an oral flora of the neonate has been established, even a few organisms indicate imminent thrush [102]. Kozinn *et al.* [51] established that the organism may be swallowed at birth as the fetus passes through the birth canal. This route of infection not only contributes to the incidence of thrush but also allows the organism to colonize the intestine. Thrush has also been found to be passed by cross-contamination to other infants

and personnel during a nursery epidemic [113]. Intestinal colonization of the infant is closely associated with clinical symptoms, including diarrhea, general irritability, and perianal colonization resulting in diaper rash [102].

Although other species of *Candida* may be found on normal skin, *C. albicans* is not part of the normal skin flora. In one study it was only recovered 3 times in 2444 scrapings from the skin of 118 normal people [32]. Some reseachers, however, report that a tropical environment or contact with infected patients resulted in increased recovery of *C. albicans* from the skin [69].

Candida albicans is a common inhabitant of the alimentary tract of many mammals and birds. Van Uden *et al.* [110,111] have done extensive studies in this area and have recovered the organism from horses, sheep, goats, swine, African bush pigs, African pied crows, baboons, and African wart hogs.

Candida albicans has rarely been isolated from soil. When found, it probably represents fecal contamination [92].

The population of *C. albicans* in the adult intestine is affected by other intestinal flora and by diet [92,110]. The lactobacilli and other lactic acid producing bacteria act to decrease the yeast population. Competition for nutrients, oxidation-reduction potentials, and secreted inhibitory factors may also act to control the yeast population. Diet influences the population of *C. albicans* in

that the type of available nutrients affect growth. Poor oral hygiene or use of small amounts of antibiotics may increase the number of organisms in the oral cavity, but does not usually result in clinical symptoms [92].

Pathogenicity

Candida albicans is part of the normal flora of many humans. It is not known whether the presence of the organism is useful to humans [112]. It is an opportunist, taking advantage of altered host states. Candidosis may be a primary or secondary infection. Although other members of the genus may be involved, *C. albicans* is most commonly the causative organism. Physiologic changes or certain states predispose to candidosis. Among these are oral contraception [47,87], infancy, pregnancy, antibiotic therapy, diabetes and other endocrine disorders, immunosuppressive therapy, immune deficiencies, malignant diseases, and other debilitated states [92,112]. Candidosis may occur in infants during the process of establishing normal flora [92,102]. During pregnancy, oral contraception, or diabetes, the glycogen content of the vaginal epithelium is raised, resulting in optimal conditions for growth of *Candida*. The estrogen component of oral contraceptives seems to alter carbohydrate metabolism, causing an increase in blood glucose levels due to a slower disappearance rate than in non-oral contraceptive users [97]. Antibiotic therapy, especially

if prolonged, alters the microbial flora, thus removing the system of bacterial checks and balances, allowing overgrowth of *C. albicans* [95]. Experiments in mice suggest that antibiotics may also increase the pathogenicity of *C. albicans* by allowing direct invasion through the renal capsule [114]. Corticosteroids and antibiotics may suppress the host's immune response, allowing proliferation of *Candida* [95]. Immunosuppressive agents, as cytotoxins, and other drugs which alter the host's defense mechanisms may lead to candidosis [112].

Candidosis encompasses a wide spectrum of symptoms, diseases, and pathology ranging from superficial to systemic involvement. Allergies to *C. albicans* also cause clinical manifestations [17,92).

Normal human serum contains a factor which is not an antibody but is lethal to *Candida*[62]. Some patients with cirrhosis, hepatitis, diabetic and non-diabetic renal disease, and with mucocutaneous or systemic candidosis show a reduction in the amount of this factor [62]. Iron-unsaturated transferrin [24] and lactoferrin [50] have been shown to inhibit growth of *C. albicans in vitro*. This inhibition is decreased when transferrin and lactoferrin are saturated with iron. Another serum factor called clumping factor, present in normal serum, promotes germ tube formation and causes clumping if many *Candida* organisms are present. This factor is not lethal to *C. albicans*. It is inhibited by IgG antibodies for *C.*

albicans; however, this IgG does not affect the organism's growth [13]. Although some of these factors may exhibit anti-candidal effects *in vitro*, their *in vivo* role has not been elucidated.

Circulating antibody does not appear to play a major role in candidal immunity [112]. The major host defense lies in the cellular immune system supported by serum opsonization factor and the candidacidal capacity of polymorphonuclear leukocytes [8,49,59]. This is supported by the low incidence of candidoses in Bruton-type agammaglobulinemia, in which cellular immunity is intact but humoral immunity is defective, and the high incidence of mucocutaneous candidosis in persons with Swiss-type agammaglobulinemia or DiGeorge syndrome, a thymus abnormality [38,92].

Candida albicans may exist in 2 phases: a yeast (Y) form or a mycelial, pseudomycelial (M) form, or both. It was once thought that only the mycelial phase was the invasive form; however, it has been shown that the yeast phase may also be invasive [75]. The transition from Y to M form is due to available nutrients and environmental factors [16,71,80,94]. The yeast phase is the form which initiates the lesion. There is a change from Y to M form as tissue invasion begins [112]. When cell division, but not growth, is inhibited, elongation occurs, resulting in a pseudomycelium [44,103]. The ratio of M form to Y form increases as the lesion gets older [92]. It has been

suggested that the mycelium may have a capsular aggressin which is cytotoxic to vascular endothelium [33]. Cellular components may cause inflammatory and toxic responses in the host [65].

As with other commensal organisms, if *C. albicans* should occur out of its usual habitat or in greater numbers than normal, it may produce disease. Its growth is better in some organs than in others. The kidney is especially favorable to growth of *C. albicans*. Other strains of the organism will grow well in the brain and myocardium. The skin is not particularly favorable for growth, nor are the lungs, except when damaged [112].

Candidal Vaginitis

Candidal vaginitis is characterized by the presence of a yellow to white milky or curdy discharge, patches of gray-white pseudomembrane on the vaginal epithelium, marked pruritus, inflammation, and a 3.8-5.0 pH range [10,92]. "The lesions vary from a slight eczematoid reaction with minimal erythema to a severe disease process with pustules, excoriations, and ulcers." Extension to perineum, vulva, and the entire inguinal area may occur [92].

Oral contraception [47,87], pregnancy, diabetes, corticosteroid therapy, antibiotic therapy, and immune deficiencies predispose to vaginal candidosis [112]. It

is more common premenstrually due to the increased estrogen level [19].

Treatment is usually topical. Nystatin, 0.25-2.0% gentian violet solution, and candicidin, among other antifungals, have been used for treatment of candidal vaginitis, but recurrent infections are common. A high percentage of patients with recurrent vaginal candidosis have *C. albicans* in oral smears [4]. Sexual transmission is usually not a problem unless the male is uncircumcized [12]. Balanitis, probably of an allergic nature, occurs rarely in men whose spouses have candidal vaginitis. It disappears when the spouse's vaginitis is treated [92].

Experimental Murine Infections

The mouse has proven to be a useful model for the study of pathogenesis and treatment of candidoses. Many investigators have induced experimental candidosis in mice. The great majority of these studies were conducted using the intraperitoneal or intravenous route to establish infection. Immunity to *C. albicans* [48,76,77] as well as enhancement of virulence by various agents [54] has been studied in the mouse. Even vaginal candidosis has been established in mice [104].

In most mice, *C. albicans* shows a predilection for the kidney. It has been suggested that this is due to the slower clearance of the organism from the kidney than from other organs. Penetration of the renal tubules is

followed by proliferation in intratubule sites. While other organs have been found to mount a cellular response which clears the organism, the renal intratubule position apparently is not accessible by normal cellular defenses. The result is intratubule proliferation of the organism leading to death [63].

The effects of cortisone [64,96], various antibiotics [95,96], and artificial diabetes [3] have been studied in mice and were found to enhance candidosis. Experimentally induced mouse thigh lesions have been used as a model for the study of the efficacy of various antifungal drugs and for the study of the effects of various bacterial antibiotics and cortisone on the lesions [82,83,105].

Establishment of intestinal colonization by oral administration of *C. albicans* has been used by few investigators as a model. Those using this technique reported no particular difficulties [64,96].

Nystatin

In 1949, Hazen and Brown isolated the first antifungal antibiotic, which they called fungicidin, from a soil actinomycete, *Streptomyces noursei* [36]. The drug was later named nystatin and is produced by E. R. Squibb & Sons under the trade name Mycostatin.^{R} It was recovered from the surface growth of the organism after fermentation. The intracellular product was shown to be both fungistatic and fungicidal. Nystatin is highly insoluble

in water and only slightly soluble in the lower aliphatic alcohols, but this solubility is enhanced if water is added. It is highly soluble in propylene glycol, N,N-dimethylformamide, or dimethylsulfoxide. The antifungal activity is broad, while there is no antibacterial activity [36].

The empirical formula of nystatin is $C_{46}H_{77}NO_{19}$. There are four C-methyl groups and one amino sugar moiety, mycosamine, in the molecule. Nystatin is a tetraene, having four conjugated double bonds [11,39].

In its purest form, nystatin exists as fine needles, but is commonly a white, amorphous or partly crystalline powder that is unstable in the presence of light, oxygen, and extremes of pH. This instability is due to unsaturated and unstable parts of the nystatin molecule. Nystatin is also unstable in suspension but will remain active in water for 2 weeks if refrigerated. In the dry crystalline form it is stable at room temperature [39].

Nystatin formulated for clinical use has a potency of 3500 units/mg. This drug has broad spectrum antifungal properties against both pathogenic and saprophytic fungi *in vitro* [30]. The minimal inhibitory concentration (MIC) for *C. albicans* and *Candida* spp. is 1.56-6.25 μg/ml [20]. Nystatin also has an inhibitory effect on *Trichomonas vaginalis* and will kill *Leishmania* [39]. The presence of blood or plasma decreases its activity [30].

In vivo resistance has not been demonstrated with *C. albicans*; however, gradual repeated exposure *in vitro* may result in resistant strains [60,86]. This is especially true of species of *Candida* other than *C. albicans*. Organisms treated in this way also demonstrate resistance to amphotericin B, another polyene antifungal agent.

Nystatin binds to sterols present in the cell membrane of a susceptible organism [56,58]. This binding causes a change in permeability of the cell membrane, resulting in leakage of cellular components, including essential ions such as potassium [55,86]. Inhibition of endogenous respiration and aerobic and anaerobic glucose utilization also occur [20,57,58]. Impairment of intracellular protein synthesis results from interference with phosphate utilization and accelerated degradation of adenosine triphosphates. Stimulation of oxygen uptake coupled with inhibition of respiration result in death of cells. Nystatin is not bound by bacteria or resistant organisms [56,58].

Nystatin can be administered orally to laboratory animals in dosages of 800,000 units/kg body weight without toxicity. The LD_{50} for the crude substance given to mice is 20-26 mg/kg injected intraperitoneally. It is less toxic when administered subcutaneously [36]. Parenteral administration of 1.2 mg/kg was tolerated for 30 days in dogs [39]. In man, doses of up to 12 g/day orally for 6 months were administered with no side effects other than

transitory nausea and diarrhea [79]. Nystatin is not absorbed from the gastrointestinal tract; thus, nearly all of the drug is excreted in the feces [30]. Intramuscular and intravenous administration have consistently been associated with severe systemic symptoms, and local inflammation at the site of injection. When given parenterally, shaking, chills, fever, and malaise accompanied the first injection. Sclerosing of veins was also noted. Severe pain and tenderness at the site of injection resulted when given intramuscularly [37]. Nystatin has been used as an aerosol containing 25,000 units and as an eye drop suspension of 20,000 units/drop without toxicity. Topical application in a concentration of 100,000 units/g of vehicle has been used without untoward effects. No sensitivities have been reported [30,39].

Since nystatin produces toxic effects when given parenterally and is not absorbed through the gastrointestinal tract or the epidermis, the primary use is in the therapy of superficial candidosis and conjunctival infections caused by sensitive fungi [39]. Nystatin is available in oral and vaginal tablets, oral suspension, ointments, creams, and powders. Oral tablets contain 100,000 units each; the oral suspension contains 100,000 units/ml; ointments, creams and powders contain 100,000 units/g of vehicle. For adults, the oral dose is 500,000 units to 1,000,000 units 3 times daily; for children, 100,000 units 3 or 4 times daily. No toxicities or

sensitivities have been reported from oral or topical administration [30,39]. No irritation of skin or mucous membranes and no toxic effects on the blood or blood-forming organs have been reported [30]. Since nystatin has no effect on bacteria, secondary bacterial infections are not encouraged by its use [30,39]. Nystatin has been used to treat all types of cutaneous candidoses [9,18, 43,115], intestinal candidosis in infants and adults [18,21], oral candidosis [21,3152], esophageal candidosis [29,34], vaginal candidosis [4,7,45,85,116], paronychia [99], urinary tract candidosis [18,21,41], pulmonary candidosis [5,18,72] and aspergillosis [27], ocular candidosis [74,93], and keratitis from aspergillosis [27]. It is used as prophylaxis against yeast overgrowth in persons on corticosteroid therapy [30]. A combination of tetracycline and nystatin is available to prevent overgrowth of *Candida* during tetracycline therapy [30, 39,40]. Nystatin is also available as a sterile powder for use in the laboratory as an antifungal agent in bacteriological samples and media, especially in tissue culture techniques [39,70,73,89].

Gentian Violet

Gentian violet (crystal violet, methyl violet, methylrosaniline chloride) is one of the methylrosaniline dyes. Introduction of methyl groups into the amino radicals of pararosaniline hydrochloride (fuscin) results in the

formation of methylrosaniline dyes. The methylrosaniline dyes used in therapy are mixtures of the hexamethyl, pentamethyl, and tetramethyl derivatives. Crystal violet is more refined, consisting of the hexamethyl derivative. Methyl violet is the pentamethyl derivative. Gentian violet is a dark green powder with a metallic sheen. It is moderately soluble in water, alcohol, and glycerine [30,107]. These solutions are stable [84,100]. The old proprietary name for gentian violet is pyoktanin [109].

Gentian violet is both bacteriostatic and bactericidal to gram positive bacteria, but gram negative and acid-fast bacteria are resistant to it, presumably due to differences in cell wall structure. Its mechanism of action is unknown [26,30,100].

Gentian violet has been administered intravenously to laboratory rabbits in doses of 10 mg/kg body weight with no toxicity. All mucous membranes were instantly stained purple, but the stain disappeared [15]. Rabbits that received 20 mg/kg body weight intravenously died in 5 hours [117]. In humans, gentian violet is nontoxic and nonirritating when used topically or orally. Transitory nausea, vomiting, and diarrhea may occur with oral use, but no sensitivities have been noted [30,39,88]. Gentian violet has been administered to children and adults intravenously in doses of 5 mg/kg body weight or less, which were well tolerated and without toxicity [117]. An infant received 3 mg/kg intravenously, which was well tolerated; however, 5 mg/kg

as the initial dose caused a depression in the white blood cell count [15]. Tattooing of the skin was reported when gentian violet was applied to granulation tissue [100]. Not much is known about the metabolism of gentian violet except that little is absorbed when given by mouth.

Gentian violet is available in powder and solution, which is 1% of the dye in 10% alcohol. A concentration of 1:500 to 1:100 is used for direct application to tissues. The concentration is reduced to 1:1000 for instillations in closed cavities [30,39,88]. Enteric coated tablets containing 25 mg are available as an over-the-counter drug for treatment of enterobiasis. The adult dosage is 50 mg 3 times daily for 10 days. Vaginal tampons containing 5 mg gentian violet to be inserted for 3 to 4 hours 1 or 2 times daily for 12 days are available for treatment of vaginal candidosis. Vaginal tablets containing 2 mg gentian violet or inserts containing a 0.4% gentian violet solution to be inserted 1 or 2 times daily for 12 and 14 days, respectively, are also available for treatment of vaginal candidosis [88].

Gentian violet was the first reasonably effective treatment for strongyloidiasis. It may be used in treatment of enterobiasis. There are newer, more effective drugs presently available for treatment of these helminthiases, so gentian violet is not the current drug of choice in either infection [30,39]. Some therapeutic uses of gentian violet include topical treatment of

pyogenic infections, geotrichosis [26], Vincent's infection [14,100], leg ulcers [53,90,106], chronic and irritative lesions [30], paronychia due to *Candida* [28], oral thrush [17], diaper rash due to *C. albicans* [25], topical therapy for candidal vaginitis [4], pruritus ani [100], and other nondisseminated candidoses [1]. Intravenous use has been recommended in cases of systemic candidosis [25,100]. Crystal violet is used in many bacteriologic media for the inhibition of gram positive organisms.

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ARTICLE I

Eradication of *Candida albicans* from the Gastrointestinal Tract of Mice: A Comparison of Nystatin and Crystal Violet

By

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(Article is to be submitted to Journal of Bacteriology)

ARTICLE I

Abstract

A model for experimental intestinal colonization with *Candida albicans* was developed in the mouse. This model was then used to compare nystatin and crystal violet with regard to efficacy in their ability to eradicate *C. albicans* from the alimentary tract. Crystal violet successfully eliminated *C. albicans* from the mouse gastrointestinal tract as evidenced by no growth of this yeast from feces when placed into culture media. Nystatin failed to eliminate, but did reduce, the intestinal population of *C. albicans*. Intestinal colonization lasted at least nine weeks in control animals. Systemic dissemination probably did not occur as no evidence of growth of yeast resulted when the macerated kidneys were placed into culture media. The results obtained may have an important influence on human therapy.

Introduction

Candida albicans is a commensal yeast in the intestine of 25-50% of healthy persons [2,27]. It is passed from mother to neonate at birth during passage through the birth canal [9,24]. The incidence of *Candida* infections

and the change from commensal to pathogen are increased in debilitated persons as well as in those with altered physiological states. Examples of conditions which predispose to candidosis are: oral contraception [8,17], pregnancy, infancy, diabetes, immunosuppressive therapy, immune deficiencies, and antibiotic therapy [19,27].

It is thought that the intestine is a reservoir for infection of other parts of the body by *C. albicans* [1, 12,24]. In debilitated persons, systemic dissemination may occur. More commonly, the gut may seed the perianal area causing cutaneous candidosis of both infant and adult. The intestine is also a reservoir for the infection of the vagina and reinfection after topical vaginal treatment [26].

To eliminate the reservoir, *C. albicans* must be eradicated from the alimentary tract. Since *C. albicans* is most frequently acquired at birth [9,24] and is not normally found in non-animal environments [19], eradication should be long lasting. Sternberg *et al.* [22], as well as other investigators, have suppressed the intestinal *C. albicans* population in mice and humans by administration of nystatin. However, they failed to completely eradicate the *C. albicans* population of the alimentary tract as evidenced by reappearance of the yeast soon after treatment ended [13,22].

Crystal violet is an old drug which is currently on the market as gentian violet and is used for therapy of candidosis. It is effective against organisms with gram positive cell walls. Not much is known about its metabolism, but it is known that little of the drug is absorbed when given by mouth. Its mechanism of action is unknown [4,5,18,23].

Nystatin is an antifungal compound derived from the soil actinomycete *Streptomyces noursei*. It is a polyene with broad spectrum antifungal activity but shows no effect on bacteria [6]. Since this drug is not absorbed from the gastrointestinal tract or the epidermis [5,7], the primary therapeutic use is in the treatment of superficial candidosis and conjunctival infections due to sensitive fungi [7]. Nystatin is bound to sterols of the cell membranes present in susceptible organisms. This binding causes a change in permeability of the cell membrane, resulting in leakage of cellular components, especially essential ions such as potassium [10,16]. Stimulation of oxygen uptake, coupled with inhibition of respiration, results in cell death [3].

The mouse has been a useful model for the study of pathogenesis and treatment of various forms of candidosis. Most investigators have induced systemic candidosis through intraperitoneal or intravenous inoculation. The effects of cortisone [11,21] and various antibacterial compounds [20,21] have been studied in mice. An experimentally

induced mouse thigh lesion has been used as a model for the study of the effects and interactions of various antimycotic and antibacterial agents and cortisone [14,15,25]. Establishment of intestinal colonization by oral administration of *C. albicans* has been used by only a few investigators, who reported no particular difficulties [21,22].

In the present study, a mouse model for intestinal colonization by *C. albicans* is described. This model was used to compare two antimycotic agents, nystatin and crystal violet, with regard to efficacy in removal of an intestinal population of *C. albicans*. The model is potentially useful for a comparative study of human intestinal candidosis, its treatment and prevention when candidoses arise from intestinal sources.

Materials and Methods

Mice

Swiss white mice (Spartan Research Animal, 5735 North Shoeman Road, Haslett, Michigan) weighing approximately 25-30 grams were used.

Candida

Strain "B" of *C. albicans* from the stock cultures of A. L. Rogers, PhD, Department of Microbiology and Public Health, Michigan State University, was used. Inocula were prepared from organisms grown in Sabouraud's glucose broth (Appendix I) for 40 hours at 37 C in 250 ml Erlenmeyer

flasks rotating at 125 rpm on a shaker (New Brunswick Laboratory Rotary Shaker, Model G2, New Brunswick Scientific Co., New Brunswick, N.J.). Part of the yeast suspension was mixed with an equal volume of a 0.5% solution of trypan blue for counting in a haemacytometer and for checking viability. The suspension was then diluted with sterile physiologic saline to obtain the desired cell count.

Inoculation

Volumes of 1.0 ml containing the desired number of organisms (30×10^6 cells for Experiment I and 8×10^6 cells for Experiment II) were administered through a stomach tube. The stomach tube apparatus consisted of a stainless steel 18-gauge hypodermic needle from which the beveled edge had been removed. The needle was inserted 1/4" into a 1" length of Tygon Microbore tubing I.D. 0.040" x O.D. 0.070" and attached to a 3 cc syringe for inoculation of mice.

Drug Administration

Volumes of 1.0 ml of either an aqueous solution of crystal violet (Difco Laboratories, Detroit, Michigan) or nystatin (Mycostatin Oral Suspension, E. R. Squibb & Sons, Princeton, New Jersey) containing the desired amount of drug were administered by the stomach tube. Fresh nystatin solutions were made every 5 days.

Fecal Culture

Presence of *C. albicans* was determined by inoculation of one average-sized fecal pellet freshly collected from each mouse into 5.0 ml of Raulin's medium (Appendix I) which was incubated for 7 days at 24 C. Some broth from all cultures was then transferred to cornmeal plus Tween-80 agar (Appendix I) and incubated for 48 hours to 7 days at 24 C to determine if pseudohyphae and chlamydospores developed.

Kidney Culture

Kidneys were removed, trimmed of connective tissue, minced into pieces approximately 2 mm^3 and plated on Sabouraud's glucose agar (Appendix I). These plates were incubated at 24 C and observed daily for 7 days for presence of yeast-like colonies.

Experiment I

In the first experiment 5 male and 6 female mice were given 30×10^6 cells of *C. albicans* via stomach tube for 4 consecutive days. Fecal specimens were collected from all mice prior to the first inoculation to insure that they were not previously colonized with *C. albicans*. Fecal specimens were collected 1 day after the last inoculum, 3 days after the last inoculum, 1 week after the last inoculum, and weekly thereafter for 10 weeks.

Experiment II

In the second experiment 45 mice were given 8 x 10^6 cells of *C. albicans* via stomach tube for 4 consecutive days. Fecal specimens were obtained prior to the first inoculation to insure that the mice were not already colonized with *C. albicans*. Mice were given a 3-day rest after the last of the 4 inoculations. Fecal specimens were collected 3 days after the last inoculum to insure that the alimentary tracts of all mice were colonized by *C. albicans*. At this time inoculated mice were divided into 5 groups. Four groups of 10 mice each were to receive drug therapy. The fifth group of 5 mice was to act as a *C. albicans* colonization control. Twenty-five uninoculated mice were divided into groups of 5 each to serve as either mouse or drug controls.

Each individual in the group of 10 colonized mice and their respective controls received the specified amount of one of the following drugs in 1-ml volumes per day for 10 consecutive days:

3.2 mg nystatin/mouse
1.6 mg nystatin/mouse
0.1% crystal violet (1 ml)

One milliliter amounts of a 1.0% crystal violet solution were administered to the remaining group of 10 colonized mice and controls for 3 consecutive days, but the drug was terminated at that time due to several deaths in both test and control groups.

Fecal specimens were collected 1 day after the 10th dose of each drug was administered, except in the 1.0% crystal violet group, from which specimens were obtained 1 day after the 3rd (final) dose and weekly thereafter. At this time the mice from each nystatin group and the 0.1% crystal violet group were divided into 2 subgroups of 5 mice each. One subgroup received no further drugs, and their feces were cultured weekly. The other subgroup received an additional 5 days of treatment with the same drug and dose as before for a total of 15 consecutive days treatment. Feces from these mice were cultured 1 day after the last (15th) dose of drug and weekly thereafter for 9 weeks.

Ten weeks after the last inoculation of *C. albicans*, the kidneys from 5 mice of each drug group were placed in culture medium to determine if there was evidence of systemic dissemination.

Results

All mice in Experiment I remained colonized at least 5 weeks after the last inoculation of *C. albicans* as evidenced by this yeast in their feces. After 6 weeks, 2 mice (1 male, 1 female) had negative fecal cultures, while 9 remained positive. In the 7th week, 6 mice were negative (3 males, 3 females) and 5 were positive, while in the 8th week 9 were negative and only 2 remained positive (1 male, 1 female). After 9 weeks all mice were negative for *C. albicans* (Figure 1). All mice appeared healthy throughout the experiment.

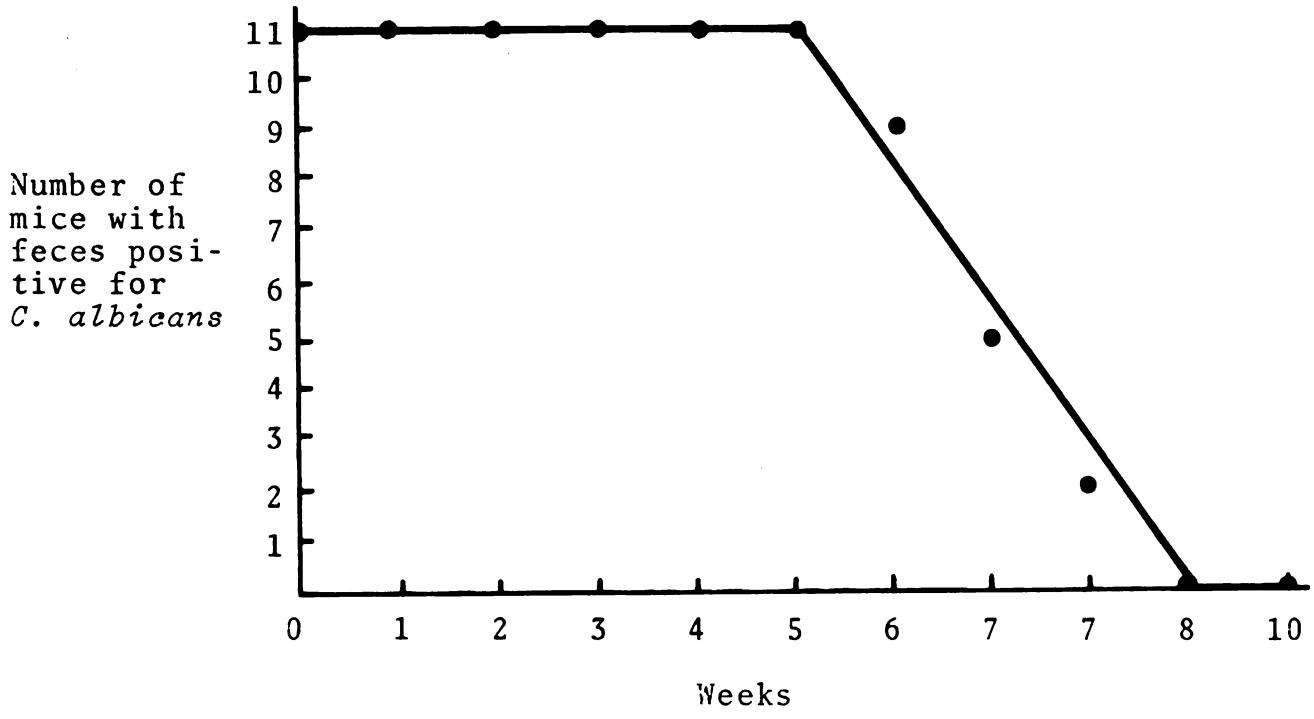

Figure 1. The number of mice with *Candida albicans* in the intestinal tract over a 10-week period. Each of the 11 mice received 30 x 10^6 yeast cells by stomach tube for 4 consecutive days. 0 time = 1 day after the last inoculum.

In Experiment II the groups of mice received drugs for a 10- or 15-day period. Figure 2 shows the results of the individual treatment groups. All of the mice receiving 0.1% crystal violet solution for 15 days became culturally negative and remained negative for *C. albicans* in the feces for at least 7 weeks after treatment. Eighty percent of the mice receiving 0.1% crystal violet solution for 10 days became culturally negative and remained negative at least 4 weeks after treatment. Three days of treatment with 1.0% crystal violet solution reduced the percent colonized to 28% 2 weeks after therapy. Not any were colonized 4 weeks after therapy. Administration of the 1.0% solution was terminated after 3 doses due to several deaths. One mouse in this test group (colonized and treated mice) and 1 mouse in the control group (receiving drug only) died within 24 hours after receiving the second dose. Two mice in the test group and 2 mice in the control group died within 24 hours after receiving the third dose. At this time the viable mice had scruffy coats and no further treatments were given. Dissection of the dead animals revealed perforated ulcerations along the entire intestine.

All of the mice receiving 3.2 mg nystatin per day for 15 days remained intestinally colonized for at least 3 weeks after termination of treatment. Eighty percent were still colonized 4 weeks after treatment. Results were similar in the other groups receiving nystatin. All

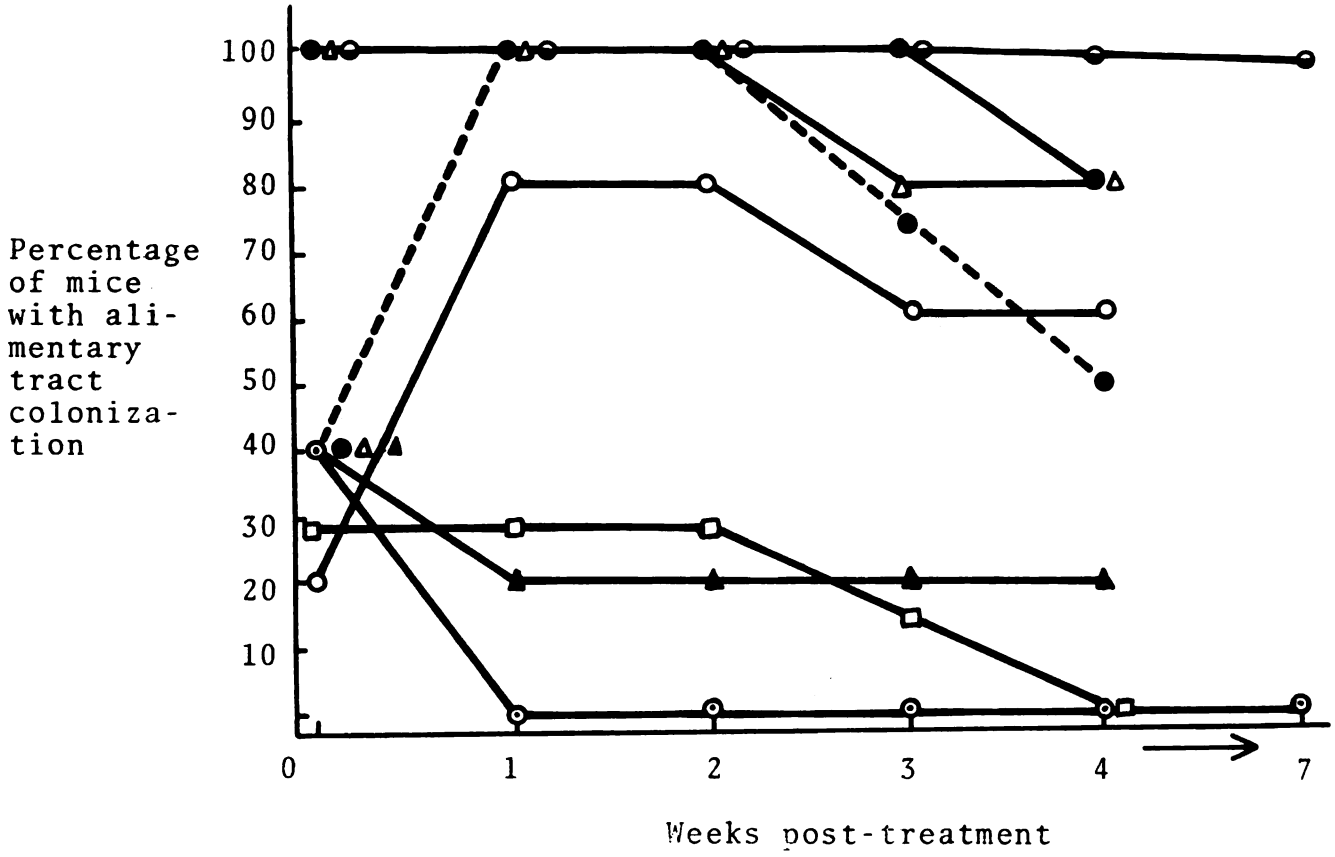

Figure 2. The percentage of mice with *Candida albicans* in the intestinal tract during a 4-week period following treatment of either 10 or 15 days duration with varying amounts of nystatin and crystal violet. Each mouse received 8 x 10^6 yeast cells by stomach tube for 4 consecutive days. 0 time = 1 day after the last day of treatment. Treatment was as follows:

3.2 mg/day nystatin for 15 days (●—●)
3.2 mg/day nystatin for 10 days (●- -●)
1.6 mg/day nystatin for 15 days (△—△)
1.6 mg/day nystatin for 10 days (○—○)
1 ml 0.1% crystal violet for 15 days (⊙—⊙)
1 ml 0.1% crystal violet for 10 days (▲—▲)
1 ml 1.0% crystal violet for 3 days (□—□)
C. albicans colonization control (●—○)

mice in the group receiving 3.2 mg per day for 10 days remained colonized at least 2 weeks after therapy. Seventy-five percent were colonized 3 weeks after treatment and 50% remained colonized 4 weeks after treatment. All of the mice receiving 1.6 mg per day nystatin for 15 days remained colonized 2 weeks after therapy, while 30% remained colonized at least 4 weeks after drug administration. Eighty percent of the mice receiving 1.6 mg per day nystatin for 10 days were culturally positive 2 weeks after therapy, while 60% remained colonized at least 4 weeks after therapy.

The *C. albicans* control mice remained colonized at least 9 weeks after the last inoculum of *C. albicans*.

Figure 3 shows the combined results in Experiment II of all groups treated with nystatin and all groups treated with crystal violet. The majority of mice in the combined nystatin groups remained colonized after treatment; 95% were culturally positive 2 weeks after therapy. However, only 17.6% of the combined crystal violet groups were still colonized 2 weeks after therapy. While 68.4% of the mice in the nystatin groups remained colonized 4 weeks after treatment, only 5.9% of the mice in the crystal violet groups were colonized at that time.

Discussion

The mouse model for intestinal colonization by *C. albicans* described in this paper is easy to establish and

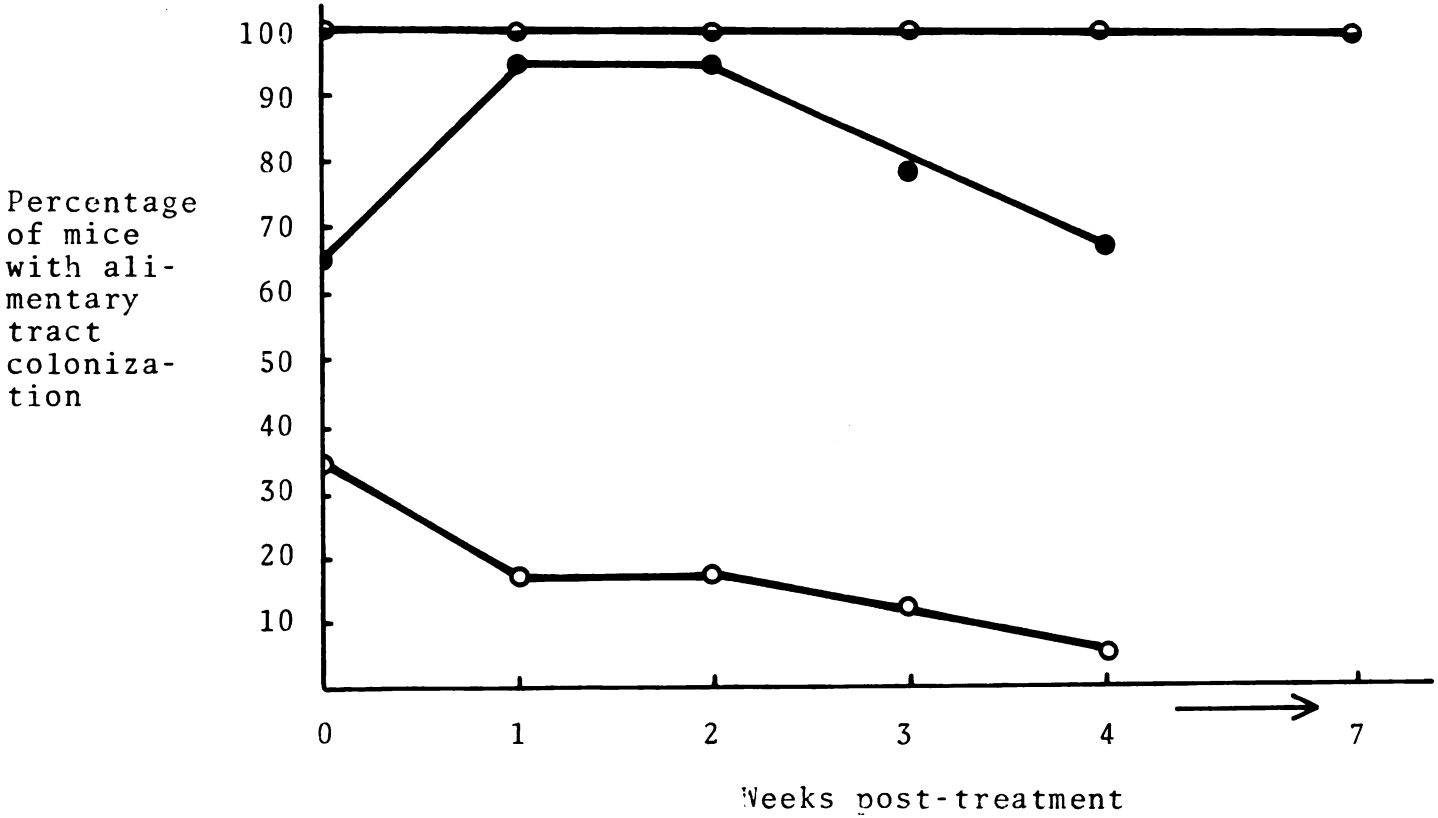

Figure 3. The percentage of mice with *Candida albicans* in feces after treatment with nystatin and crystal violet. The results are combined for each agent. 0 time = 1 day after last day of treatment. *Candida albicans* colonization control (O—O), nystatin treated groups (20 mice) (●—●), crystal violet treated groups (17 mice) (O—O).

the duration of infection is sufficient to be useful in the study of effects of drugs used in therapy of candidosis of the alimentary tract. It appears, from the data obtained, that the smaller inoculum (8×10^6 cells) results in a longer-lasting colonization than does the larger inoculum (30×10^6 cells). Further work is necessary to determine the size of inoculum and number of inocula which would result in the longest period of colonization. At present, this procedure was established for short-term (9 weeks or less) studies.

In a similar experiment using only nystatin, Sternberg *et al.* [22] showed that while nystatin reduced the number of *C. albicans* in feces during therapy, colony counts in both humans and mice rose after cessation of nystatin therapy. In the present study, nystatin and crystal violet were used to determine if either of these drugs could eradicate the intestinal population of *C. albicans*. The dosages selected were comparable to those used in human therapy.

Nystatin was ineffective in removal of the *C. albicans* population in the alimentary tract. Raising the dose, lengthening the treatment time, or both, had no effect on the number of mice that remained colonized. The groups of mice receiving crystal violet, however, became and remained culturally negative with degrees of success varying with drug concentration and length of treatment. While all

groups receiving crystal violet experienced a marked reduction in percent colonization, the group receiving a 0.1% solution for 15 consecutive days showed total eradication of the intestinal population. This is the concentration that is recommended for use in closed instillations in humans. These mice remained culturally negative at least 7 weeks after treatment (9 weeks after last inoculation of *C. albicans*).

The mice receiving a 1.0% solution of crystal violet did not tolerate this high concentration well. This dose is 10 times more concentrated than that recommended for analogous use in humans; it is, however, the recommended concentration for topical use in humans.

The effect on the intestinal yeast population obtained with nystatin administration is comparable in mice, other laboratory animals, and humans [13,22]. Presumably the results of crystal violet treatment would also be comparable. If so, it would be possible to eradicate the carrier state for *C. albicans* in humans. This has many ramifications. Since candidosis is a disease of endogenous nature, probably originating from intestinal foci, eradication of the intestinal population coupled with topical therapy to eliminate any extraintestinal infection would be of utmost benefit to the patient in prevention of recurrent infections.

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ARTICLE II

Eradication of *Candida albicans* from the Gastrointestinal Tract of Women

By

Olsen, Linda S., Rogers, Alvin L., Ryan, Mary H.

(to be submitted to the New England Journal of Medicine)

ARTICLE II

Abstract

Candidosis, most commonly caused by *Candida albicans*, is an infection of endogenous nature, probably originating from an intestinal focus. Oral gentian violet therapy successfully eradicated the intestinal population of *C. albicans* in women with recurrent vaginitis due to this organism. Patients treated with the prescribed regimen who submitted appropriate specimens remained culturally negative for intestinal and vaginal *C. albicans* for at least 3 months. Since *C. albicans* is rarely found in non-animal environments, eradication should be long-lasting. These findings have implications concerning treatment and management of debilitated patients as well as normal patients.

Introduction

Candida albicans is a commensal yeast in the intestine of 25-50% of healthy persons [2,18]. It is passed from mother to neonate at birth during passage through the birth canal [8,16]. The incidence of *Candida* and the change from commensal to pathogen are increased in debilitated persons as well as in those with altered physiological

states. Examples of conditions which predispose to candidosis are: oral contraception [7,11], pregnancy, infancy, diabetes, immunosuppressive therapy, immune deficiencies, and antibiotic therapy [13,18].

It is thought that the intestine is a reservoir for infection of other parts of the body by *C. albicans* [1,9, 16]. In debilitated persons, systemic dissemination may occur. More commonly, the gut may seed the perianal area causing cutaneous candidosis of both infant and adult. The intestine is also a reservoir for the infection of the vagina and reinfection after topical vaginal treatment [17].

To eliminate the reservoir, *C. albicans* must be eradicated from the alimentary tract. Since *C. albicans* is most frequently acquired at birth [8,16] and is not normally found in non-animal environments [13], eradication should be long lasting. Sternberg *et al.* [14], as well as other investigators, have suppressed the intestinal *Candida* population by administration of nystatin. However, they failed to completely eradicate the *C. albicans* population of the alimentary tract as evidenced by reappearance of the yeast within 2-5 days after treatment ended [14].

Candida albicans can be demonstrated in the normal oral cavity of 25-50% of healthy persons (2,18] and is frequently found in the estophagus at autopsy [3]. Thus, to clear *C. albicans* from the alimentary tract, foci of colonization both in and above the intestine must be

eliminated. It is possible that this could be done by administration of gentian violet solution in conjunction with gentian violet enteric-coated tablets which, together, would reach *C. albicans* in the oral, pharyngeal, and esophageal as well as intestinal areas. *Candida albicans*, unlike other *Candida* species, is rarely found outside the natural animal hosts [13], so eradication of the intestinal reservoir should prevent most relapses.

Gentian violet is an old drug which is currently marketed and used for therapy of candidosis in infants and adults. It is effective against organisms with gram positive cell walls. The mechanism of action is unknown [4,5,6,12,15]. Not much is known about its metabolism, but little is absorbed when given by mouth. In humans it is nontoxic and nonirritating when used topically or orally. Up to 5 mg/kg body weight has been administered intravenously without toxicity [16,19]. Oral tablets are sold as an over-the-counter drug for the treatment of enterobiasis. These tablets have been used in treatment of enterobiasis and strongyloidiasis without untoward reactions. Hypersensitivities to this drug have not been reported [5,12,15].

The object of this study was to determine if administration of gentian violet solution in conjunction with gentian violet oral tablets would clear the adult alimentary tract of *C. albicans*. This has implications concerning the management and prophylaxis of candidosis

in the debilitated patient as well as in the healthy patient with pruritus ani, diaper rash, or vaginitis due to *C. albicans*.

Materials and Methods

Patients

Healthy non-pregnant female patients 18-30 years of age who presented themselves for treatment at Olin Health Center (Michigan State University, East Lansing, Michigan) with recurrent vaginitis due to *C. albicans* alone were chosen on a volunteer basis. The cause of vaginitis was determined by physicians at Olin Health Center, supported by laboratory examinations by personnel at Olin Health Center Laboratory. In those patients in which *C. albicans* was the sole cause of vaginitis, an oral swab and rectal swab or fecal specimen were also collected to determine presence of oral and intestinal colonization. These and further specimens were processed in E-216 Fee Hall, Michigan State University. Only those patients exhibiting both vaginal and intestinal colonization were accepted for participation in this study. Informed consent (Appendix II) was obtained from each volunteer after the nature of the procedure had been fully explained. Patients were free to withdraw from the study at any time without penalty.

Cultures

Raulin's medium (Appendix I) was used for primary isolation of *C. albicans*. Chlamydospore formation on cornmeal plus Tween-80 agar (Appendix I) was used to identify *C. albicans*. All cultures were incubated at 24 C for 7 days to determine presence of *C. albicans*. The swabs used in collection of oral and vaginal specimens were inoculated directly into 5 ml of Raulin's medium. Either fecal specimens or rectal swabs were obtained before treatment. Only fecal specimens were obtained after medication was taken. Approximately 1 gram of fecal material was inoculated into 5 ml of Raulin's medium. Oral swabs, fecal specimens or rectal swabs, and vaginal swabs were placed into culture media for *C. albicans* before therapy, 2 days, 1 week, and 1 month after oral therapy was completed. Specimens were obtained from 5 patients 3 months after completion of therapy.

Medication

All patients admitted to the study received the following:

(1) Gentian violet oral tablets (Jayne's P-W Vermifuge, Glenbrook Laboratories, Division of Sterling Drug, Inc., 90 Park Avenue, New York, N.Y. 10016). Each tablet contains 25 mg gentian violet. Patients were instructed to follow the recommended adult dosage of 2 tablets 3

times daily during meals for 10 days. Patients were advised to refrain from alcohol consumption during treatment, as recommended in the *Physician's Desk Reference* [12].

(2) Gentian violet (Merck, Sharp and Dohme, Division of Merck Co., Inc., West Point, Pa. 19486) in a solution with a final concentration of 0.1%. Patients were instructed to dilute 30 cc of a 1.6% solution with 450 cc of grape juice or another strongly flavored non-alcoholic beverage to produce a final volume of 480 cc, giving a final concentration of 0.1%. Twice daily for 4 consecutive days, 60 cc of this solution was to be swished around in the mouth, then swallowed. Patients were instructed to refrain from eating or drinking for 2-3 hours after administration of the solution.

(3) One course of treatment with gentian violet tampons (Genapax, Lakeside Laboratories, Inc., 1707 E. North Avenue, Milwaukee, Wis. 53201). Each tampon contains 5 mg gentian violet. Patients were instructed to insert 1 tampon for 3-4 hours each day for 12 consecutive days. Patients were advised to have their partner use a condom during sexual intercourse, as recommended in the *Physicians' Desk Reference* [12].

Results

Twenty-two patients were involved in the study. Four were unwilling to complete the major portion of the

treatment due to nausea, vomiting, diarrhea, or a combination of these. All patients completing 6 days or more of therapy became culturally negative for oral and intestinal *C. albicans*. Table I shows the results of all patients studied.

Stool Specimens

Of the patients who carefully followed the prescribed course of treatment (i.e., those who took all the tablets, at least one dose of the liquid solution, and used all the tampons), 100% (9) had stool cultures which were negative for *C. albicans* 1 month after completion of therapy. Seven specimens were obtained 2 days and 1 week after therapy, of which 85.7% (6) were negative each time. Three of these patients also submitted 2- and 3-month post-treatment specimens which were also negative.

Nine patients did not follow the oral treatment exactly, but did report completion of 6 days or more of tablet therapy, used all the tampons, and took at least 1 dose of the liquid solution. All of these patients were culturally negative 1 month after therapy. Of the 6 specimens obtained 2 days after therapy, 16.6% (1) were negative. Seven specimens were received 1 week after therapy, of which 57% (4) were negative. Two of these patients also submitted specimens which were negative for *C. albicans* 2 months after therapy.

Table I. Results of gentian violet treatment in patients with *Candida albicans* in the gastrointestinal tract and vagina. Patients 1-9 took all medication as directed; patients 10-18 took 6 to 10 days of therapy; patients 19-22 took 5 days or less of therapy. Specimens from the stool (S), vagina (V), and oral cavity (O) were collected before and after treatment.

Patient number	Before treatment			2 days after treatment			1 week after treatment			2-3 weeks after treatment			1 month after treatment			2 months after treatment			3 months after treatment		
	S	V	O	S	V	O	S	V	O	S	V	O	S	V	O	S	V	O	S	V	O
1	+	+					+*	+*											-	-	-
2	+	+					-	-	-							-	-	-	-	-	-
3	+	+	-	+	+	-	-	-	-				-	-	-						
4	+	+	-	-	-	-	-	-	-				-	-	-						
5	+	+	-	-	-	-	-	-	-				-	-	-						
6	+	+	-	-	+	-	-	+	-				-	+	-	-	+	-			
7	+	+	-	-	+	-	-	+	-				-	-	-						
8	+	+	+	-	+	-	+	+	-				-	+	-						
9	+	+	-	-				-					-	+	-						
10	+	+	-				+	-	-				-	+	-	-	-Δ	-			
11	+	+	+	+	-	-				+	+	-				-	-	-			
12	+	+	-	+	-	+	-	-	-				-	-	-						
13	+	+	+	+	-	-	-	-	-				-	-	-						
14	+	+	-				-	+					-	+	-						
15	+	+	+	-	-	+	+	-	-				-	-	-						
16	+	+	+	+	+	+	+	+	+				-	-	-						
17	+	+		+	+	-	-						-	+	-						
18	+	+	-							-	-	-									
19	+	+	-	-	+	-	-	+	-				-	+	-						
20	+	+	+	+	+	+							+	+	-						
21	+	+	-	-	+	-							+	+	-						
22	+	+	+	-	-	-	+∫	-∫	-∫				+	-	-						

*1 day after 4-day tetracycline challenge; Δ2 weeks after additional course of vaginal therapy; ∫10 days after treatment.

Four patients took 5 days or less of the oral therapy. One of these patients became culturally negative 2 days after completion of therapy and remained negative for at least 1 month after therapy. The others remained culturally positive for intestinal *C. albicans*.

Oral Specimens

Oral specimens from 19 patients were collected before administration of medication. At this time 36.84% (7) of these patients had oral cultures positive for *C. albicans*. Twenty-five percent (4) of the 16 specimens obtained 2 days after completion of total oral medication were culturally positive at this time. Of the 13 specimens obtained 1 week after therapy, 7.69% (1) were positive. All 22 patients, even those who took only 1 dose of liquid medication (2 patients), had negative oral cultures 1 month after therapy.

Vaginal Specimens

Of the patients who completed vaginal therapy, but not necessarily oral therapy (18 patients), 55.5% (10) had vaginal cultures which were negative for *C. albicans* 1 month after therapy.

Of the 17 patients who both became negative for oral and intestinal *C. albicans* and submitted vaginal specimens, 64.7% (11) were culturally negative for vaginal *C. albicans* 1 month after therapy.

All patients who had both negative vaginal and negative stool cultures 1 week after therapy (4 patients) remained negative at both sites at least 1 month after therapy. One of these patients submitted specimens 3 months after completion of therapy which were negative for *C. albicans*. Of the 8 patients who had negative stool cultures 1 week after therapy and who submitted the appropriate specimens, 75% (6) had negative vaginal cultures both 1 week and 1 month after therapy.

Discussion

Current therapy for candidosis is aimed at the site of infection rather than the intestinal source of the infection. Even when oral intestinal therapy with nystatin, the current drug of choice, is combined with topical therapy to the extra-intestinal site, the goal and result is merely to return the population of *C. albicans* to commensal proportions. Sternberg *et al.* [14] showed that, although nystatin reduced the intestinal yeast population during therapy, within 2-5 days after cessation of nystatin the *C. albicans* population had returned to numbers as high or higher than those observed before treatment. The purpose of the present study was to determine if the alimentary population of *C. albicans* could be totally eradicated through use of gentian violet.

In mouse experiments comparing crystal violet (a more refined form of gentian violet) and nystatin with regard

to the ability to remove the intestinal population of *C. albicans*, it was shown that nystatin was ineffective but that crystal violet eradicated the yeast. In the present study gentian violet was found to be effective in eradication of the carrier state of *C. albicans* in humans. The data indicate that at least 1 dose of a 0.1% gentian violet solution coupled with 6 to 10 days of gentian violet oral tablet therapy results in long-lasting removal of the intestinal reservoir of *C. albicans*.

In some patients, nausea and at times vomiting and/or diarrhea accompanied the treatment. These discomforts were most often associated with the administration of the liquid solution of gentian violet. Most patients noting these transitory side effects were willing to endure the discomfort in light of the benefits they hoped to obtain. Since the number of doses of the liquid did not seem to alter removal of intestinal carriage as long as most of the tablets were taken, only 1 dose of liquid may be sufficient, or the liquid may not be necessary at all.

The 35-45% failure rate experienced after 1 course of the vaginal tampons compares with the failure rates observed with other vaginal antimycotic agents [10]. However, the failure rate was reduced to 25% (2 of 8) in those patients whose intestines were negative for *C.*

albicans within a few days of completion of vaginal therapy.

Since the intestinal source was removed and the vaginal site of infection treated, these patients would not be expected to develop recurrent vaginal candidosis in the future. Administration of another course of vaginal tampons would be expected to remove the vaginal population of *C. albicans* in the vaginal failures. This removal would be long lasting if the intestinal source were also removed.

It can be concluded, then, that gentian violet may be effective in eradicating the intestinal reservoir of *C. albicans*. Candidosis is usually a disease of endogenous origin, spread from gastrointestinal foci; thus, elimination of the intestinal population coupled with topical therapy to any extra-intestinal infection would be of utmost benefit to the patient. Since *C. albicans* (1) unlike other *Candida* species, is rarely found in non-animal environments [13], (2) is most commonly acquired at birth by swallowing a portion of the vaginal contents on passage through the vaginal canal with resultant intestinal colonization [8,16], and (3) infections are usually of an endogenous nature, originating from gastrointestinal carriage [16,17], eradication of the alimentary population of *C. albicans* should result in a long-lasting cure for such diseases as recurrent vaginitis, pruritus ani, and diaper rash due to *C. albicans*. This also has

implications concerning treatment and management of the debilitated patient. *Candida albicans* is able to spread by persorption from the intestine to internal organs, where, in the debilitated person, it may cause a serious infection [3]. Removal of the intestinal source of infection could prevent a potentially lethal candidosis.

Gentian violet may be tolerated intravenously in doses of up to 5 mg/kg body weight without toxicity [15, 19]. In cases of systemic dissemination, intravenous therapy combined with oral therapy could prove to be a less toxic and longer-lasting treatment than the current nephrotoxic drug of choice, amphotericin B.

This study has shown that it is possible to remove the intestinal reservoir of *C. albicans* by oral administration of gentian violet, a nontoxic, inexpensive drug.

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APPENDICES

APPENDIX I

CULTURE MEDIA

Sabouraud's Glucose Broth

Glucose	40 g
Neopeptone	10 g
Distilled water	1000 ml

Adjust to pH 5.6 and autoclave at 121 C for 15 min.

Raulin's Medium

Brown sugar	70 g
Tartaric acid	4 g
Ammonium nitrate	4 g
Potassium carbonate	0.5 g
Magnesium carbonate	0.4 g
Ammonium phosphate	0.25 g
Zinc sulfate (crystals)	0.07 g
Ferrous sulfate	0.07 g
Manganese sulfate	0.07 g
Distilled water	1500 ml

Adjust to pH 2-3 and autoclave at 121 C for 15 min.

Cornmeal Agar plus Tween-80

Cornmeal	40 g
Water	1000 ml
Agar	20 g
Tween-80	10 g

Simmer cornmeal and water for one hour. Filter or decant, bring volume up to 1000 ml and add agar; melt and filter again if necessary. Add Tween-80 and autoclave at 121 C for 15 min.

APPENDIX II

CONSENT FORM

I understand that my physician has decided that the use of the antifungal agent gentian violet is indicated for the treatment of my disease.

In order to better understand the treatment of candidosis (yeast infections) with gentian violet, Doctors Mary Ryan, Alvin L. Rogers, and their associates at Michigan State University wish to run special studies on oral, fecal, and vaginal specimens. This will consist of approximately four oral, fecal, and vaginal specimens obtained before and at various times after treatment. I understand and agree that any data collected may be used for research, teaching, and publication.

I further understand that the medical treatment I receive will be the same whether or not I participate in the study. I understand that all records will remain confidential and that I am free to withdraw from this study at any time without penalty.

_____

The purpose of this study has been fully explained to me and I have read and understood the above statement. My participation in this study is freely given.

Signed_____

Witness_____

Date_____

APPENDIX III

PATIENT INSTRUCTIONS

1) Mix the entire bottle of gentian violet solution with 15 oz. of grape juice. We recommend a frozen concentrate. When mixing the frozen concentrate, use as little water as possible. The gentian violet is extremely distasteful, so the stronger the grape juice taste, the more the gentian violet taste will be disguised. The gentian violet will stain clothing and possibly the container you put the solution in, so be careful.

The total volume of the final solution (gentian violet plus grape juice) will be 16 oz. (2 cups). This will last four days. Take 2 oz. two times per day for 4 days. Stir or shake the solution to make sure it is evenly mixed before taking each dose. However distasteful, swish it around your mouth and swallow *all* of it each time. It will turn the inside of your mouth purple.

Do not eat or drink anything for 2-3 hours after swallowing this liquid. The bad taste will go away after a few minutes.

You may keep the solution at room temperature or you may refrigerate it. As long as you keep it covered it shouldn't spoil.

2) Take the gentian violet tablets (Jayne's P-W Vermifuge) as directed on the bottle under "adult dosage"; that is, 2 tablets three times per day for 10 days. Take these during a meal. They may cause purple feces.

3) Do not drink any alcoholic beverages during the 10 days of oral treatment. The alcohol will wash the gentian violet out of your system so the medication will not have a chance to work.

4) Insert one Genapax tampon into the vagina and leave it in for 3-4 hours once each day for 12 days. Try to do this at about the same time each day. Any vaginal discharge produced after the tampon is removed may be purple and may stain your clothing. A condom may be used

during intercourse to avoid staining your partner purple.

5) Start all medication on the same day.

6) Follow the diet for the 10 days you take the tablets. In particular, don't eat any fruit for these 10 days. This helps to cut down on the yeast population of the intestine. After the 10 days you may resume your regular eating and drinking habits.

7) Bring a stool specimen to me (Linda Olsen) on April 21, April 28, and May 19. I will be in room 294 in Giltner Hall on the above days from 3-5 p.m. You may collect the stool specimens in the containers provided or any other suitable container (e.g., a small, plastic margarine tub). When you give me the stool specimen I will give you a sterile swab so you can collect a vaginal specimen in the rest room across from my office. Also I will collect a mouth swab.

It is very important that you bring these stool specimens to me on the assigned days. Otherwise, we will not be able to follow your progress as closely as we would like.

If, for any reason, you cannot deliver a stool specimen to me on the appropriate day or time (3-5 p.m.), please call me at home in the evening so we can make other arrangements. My phone number is 351-3337.

8) A suggested regimen is as follows:

Morning - 2 tablets during meal
2 oz. solution after meal

Afternoon - 2 tablets during meal

Evening - 2 tablets during meal
Insert Genapax tampon for 3-4 hours
2 oz. solution before bedtime

Be sure to eat some sort of food when taking the tablets and leave at least a 5-hour interval between doses of tablets.