Mechanisms by Which Antibiotics Increase the Incidence and Severity of Candidiasis and Alter the Immunological Defenses

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INTRODUCTION

Substantial clinical evidence has been accumulated, showing that candidiasis is a medical problem of increasing magnitude (31–33, 35, 37–40, 44, 45, 53, 54, 58, 64, 74, 75, 80, 82, 88, 91, 127, 136, 150, 153, 155, 156, 162). That patients on antibiotics experience proliferation of *Candida albicans* in the alimentary canal is no longer a point for dispute. That the increased incidence of severe fungus infections is associated with antibiotic therapy, particularly in patients with subnormal defense mechanisms, is being increasingly accepted. A recent report (136) has presented an analysis of published experimental and clinical data which indicate that the two clinically disparate conditions—the ostensibly benign proliferation of *C. albicans*, and the invasion and spread of the organism in debilitated patients—are interrelated. This report presents evidence that the antibiotics enhance the invasiveness of the *C. albicans*, not only by a direct effect on the intestinal flora, and on the *Candida* itself, but also by depressing the host defense mechanisms.

EFFECT OF ANTIBIOTICS ON C. *ALBICANS*

Growth of C. albicans

There have been several explanations of the increased candidal growth in patients on antibiotics. Whether the broad-spectrum antibiotics directly stimulate the growth of *Candida* in vitro is a moot point, on which contradictory evidence has been presented. Some of the studies on the effect of antibiotics on pure cultures of *Candida* have shown pure chlortetracycline to increase the growth of *Candida* (23, 72, 105), others showed no effect (93, 96), and still others attributed the enhancing effect to a substance other than the antibiotic (i.e., dicalcium phosphate) in the capsule preparation (84, 117). Although penicillin, streptomycin, or broad-spectrum antibiotics enhanced *Candida* growth in some studies (21, 22, 67, 69) they had no effect in others (23, 67, 96). The explanations of antibiotic enhancement of growth of yeast include direct stimulation (72, 105, 117), removal of organisms competing for nutrients (14, 70, 78, 144), and removal of organisms that secrete antifungal substances (8, 51, 52, 78, 116, 126, 165). It is provocative that, despite the conflicting results from in vitro studies, almost all the in

vivo studies showed outgrowth of fungi or increased lethality of fungi, or both, in the treated animals (5, 14, 34, 39, 49, 55, 61–63, 68, 121, 129, 131, 137, 138, 163).

C. albicans Endotoxin

Since *C. albicans* proliferates in the alimentary canal of patients on antibacterial therapy, the demonstration of a *C. albicans* endotoxin assumes some importance, as a potentially pathogenic factor. With increasing numbers of the organisms, the endotoxin released during their reproduction and death may cause local irritation, and participate in the damage of the surface tissues that allows for penetration of the *Monilia* into deeper tissues and blood.

The first to call attention to the fact that mice could be killed by an endotoxin obtained from *Candida* cells (by mechanical disintegration of acetone-dried cells) was Salvin (132). In 1952, he attributed the virulence of *C. albicans* to the tissue-damaging effects of the toxin. Four years later, Winner (160) postulated that an endotoxin, produced by *C. albicans*, might be responsible for many of the pathological effects of the organism. He reached this conclusion in an effort to explain why, although rabbits possessed some natural immunity to infection with *Candida*, induction of agglutinin formation did not prevent or minimize the lesions. [Note: *Candida*-cidal, or inhibiting, antibodies distinct from agglutinating antibodies which appear to exert no protective effect, have been identified in normal human serum (85, 128, 130).] Roth and Murphy (131), in 1957, found that an endotoxin, extracted from *C. albicans* by supersonic vibration of viable yeast cells, was not harmful to mice when injected alone, but it was lethal to mice treated with chlortetracycline. The combination of the extract with oxytetracycline was less toxic than the combination with chlortetracycline; that with chloramphenicol or streptomycin was inactive. In 1958, Winner (159) found that the filtrate obtained from a several-day-old culture of *C. albicans* produced erythema in guinea pigs and rabbits, and suggested that some of the lesions of experimental candidiasis might be caused by a diffusible toxin. Further verification of the tissue-damaging effects of *C. albicans* endotoxin has been reported from other laboratories. Dobias commented briefly, in his 1957 review of moniliasis in pediatrics (37), that mice developed toxic symptoms and died within 2 hr after intravenous injection of a soluble, filterable extract which he prepared by mechanical disintegration of *C. albicans* cells.

In 1961, Mourad and Friedman (107) showed that a soluble extract obtained by sonic vibrations of *C. albicans* could cause death in mice, when given intravenously alone, in sufficiently large doses. They found that both the sonically broken cells and the supernatant fluid therefrom were lethal. A recent brief report by Dennis and Peterson (36) described production of shock, and sometimes death, in dogs injected intravenously with a soluble extract of *C. albicans*, obtained by sonic rupture of the yeast cells. They suggested that the shock seen clinically in *Candida* septicemia might be caused by *C. albicans* endotoxin. The possibility that *C. albicans* endotoxin is responsible for the cardiovascular collapse, or shock, seen in patients with *Candida* septicemia, had been suggested earlier by Braude and Rock (18).

Maibach and Kligman (92) have presented evidence that a soluble lyophilized cell-free extract of *C. albicans*, or the sterile sediment of ground and ruptured yeast cells, was capable of producing dermatitis in about three-fourths of 30 volunteers, when applied to the skin under occlusive tape. The skin lesions ranged from a simple erythema to a pustular dermatitis, which mimicked the dermatological disease caused by viable *C. albicans*, applied similarly. They concluded that the irritation and damage to the skin produced by *C. albicans* in clinical infections was caused by the endotoxin released by the death of the cells which were multiplying on the surface and in the stratum corneum. Their work indicated that the *C. albicans* did not penetrate the intact living tissue; the conspicuous epidermal damage seemed to be due to irritants (endotoxin), which filtered into the skin. They emphasized that the endotoxin of *C. albicans* was extremely potent; few irritant chemicals could match the angry dermatitis provoked by the *Candida*.

Another mechanism by which the *Candida* organisms may cause tissue damage is by proteolytic activity. Kim et al. (76) have demonstrated that leucine aminopeptidase is produced by actively growing *C. albicans* cultures. It is possible that when this enzyme is present in large concentrations, as would be the case where the *Candida* was flourishing, it might cause damage to the mucosa, facilitating penetration by mycelia.

Mycelial Phase of C. albicans

The mycelial phase of *Candida* has been incriminated by several investigators as the form that is primarily responsible for its invasion of tissues. Hill and Gebhardt (65), in 1956, observed that, within 60 min after subcutaneous injection of yeastlike *C. albicans* cells into mice, almost all had formed a short rudimentary pseudomycelium; cells suspended in the saline diluent

retained their yeastlike morphology. Slides prepared 2 to 24 hr after the injection showed a progressive increase in length of the hyphae. Except for *C. stellatoidea*, the only species to develop these changes in morphology in vivo was *C. albicans*. The following year, Taschdjian and Kozinn (152) and Rogers (125) observed that the course of clinical thrush could be correlated with the morphological appearance of the *Candida* cells obtained from the mucosa. In the preclinical stage, variable numbers of blastospores were found exclusively, singly or in short chains. The next stage was represented by invasion of the oral epithelium and the formation of small intracellular colonies of *C. albicans*. It was at this stage that filaments became noticeable. During the course of clinical thrush, oral smears showed both spores and hyphae. Taschdjian and Kozinn (152) commented that clinical thrush coincided with the first formation of hyphae. In the saprophytic, nonpathogenic stage, only spores were seen. In a more recent publication (79), they observed that all reports of fatal enteric candidiasis stressed massive mycelial invasion of the esophageal or intestinal wall as a typical autopsy finding. They concluded that invasion of mucocutaneous surfaces by *C. albicans* is apparently accompanied by the formation of mycelia. Not only in the enteric and oral forms of *Candida* infections are mycelia seen, but also in the systemic form of the disease. Identification of the organs invaded by *Candida* has been made by detection of the hyphae in the affected tissue, as well as by growth of *C. albicans* in cultures made from fresh autopsy material.

The work of Gresham and Burns (56) has provided additional experimental verification of the role of mycelia in tissue invasion by *Candida*. They have shown that *C. albicans*, alone of the *Candida* species, produced abundant mycelia in animal tissues, and that the mycelial phase enables the fungus protoplasm to penetrate into the tissues as a syncytium. Their preparations from recent candidal lesions revealed that the mycelial form of *C. albicans* is encapsulated. They postulated that the capsule might possibly be the source of the endotoxin described by other investigators. Because they found mycelial elements in the blood stream of infected animals, they concluded that the mycelial phase, as well as the blastospores, are of importance in dissemination of the organism through the blood. In a study of an agent capable of inhibiting mycelial production (iso-nicotinic acid hydrazide), Gresham and Whittle (57) proved the mycelial form of *C. albicans* to be an actively growing stage, by demonstrating ribonucleic acid (RNA) within the hyphae. They found the RNA not only at the hyphal tip but also at the septa, which indicates that *C. albicans* produces a true mycelium, and not a pseudomycelium, as has been reiterated. They emphasized this point, since the hyphal thread implies functional or metabolic continuity along the entire length, whereas a pseudomycelium implies functionally disconnected units. Such functional continuity is advantageous in colonization of tissue remote from the point of fungal entry.

The first suggestion that antibacterial treatment might result in increased mycelial penetration of tissues by *Candida* was made in 1954 by Beemer et al. (11). On histological examination of the monilial lesions in the mouth, esophagus, larynx, and intestines of a woman who died subsequent to post-traumatic infection and granulocytopenia possibly secondary to sulfathiazole treatment, they found abundant mycelia extending into submucosal and deeper tissues. They then demonstrated that the *Candida* on the gut, obtained postmortem, proliferated and invaded the deeper tissues when segments of ileum were immersed in penicillin solution, in streptomycin solution, or in chloramphenicol solution, but not when the segments were immersed in solutions free from antibiotics.

One possible explanation for the invasion of tissues by mycelia in antibiotic-treated patients was provided by Campbell and Heseltine (22), who observed an increase in mycelial elements in the presence of filtrates from tetracycline-treated throat and fecal cultures, and speculated that this might reflect a change from a relatively saprophytic to a more pathogenic form. The observation of increased invasion of peritoneal surfaces by *C. albicans*, when given intraperitoneally with the tetracyclines (34, 63, 121, 137, 138, 163) provides in vivo evidence that the broad-spectrum antibiotics favor the invasive phase of the *Candida*.

EFFECT OF ANTIBIOTICS ON HOST RESISTANCE
TO *C. ALBICANS*

Antibiotic-Increased Lethality of Candida

The early work by Seligman indicated that the increased lethality of *Candida*, given to animals with tetracyclines, was not mediated by antibiotic induction of increased virulence of the yeast, but seemed, rather, to be referable to an effect of the tetracycline on the host (137, 138). He observed that mixing chlortetracycline with *C. albicans*, and then injecting the fungus, which had been separated from the antibiotic, resulted in no increase in lethality for mice over the controls given untreated *Candida* (137). On the other hand, injection of *Candida* mixed with chlortetra-

cycline resulted in the death of 16 of the 17 test mice. Injection of Candida 4 to 24 hr after chlortetracycline resulted in considerable mortality, the toll rising as the antibiotic and Candida injections were administered at closer intervals. If, however, Candida cells were injected 8 to 24 hr before the antibiotic, there was no increase in pathogenicity; the host was then able to withstand the infection. He showed that oxytetracycline simulated the effect of chlortetracycline on intraperitoneal injection with Candida, whereas penicillin, streptomycin, and chloramphenicol did not (138). His work indicated, however, that there was little or no increase in lethality if the drug and the fungus were not introduced by the same route. In fact, chlortetracycline seemed to protect somewhat against Candida lethality when both were introduced intravenously. Roth and Eylar (129) and De Mello and Kiser (34) verified Seligman's observation that intraperitoneal injection of chlortetracycline with Candida greatly enhanced lethality, and that this enhancement did not seem to have been caused by the effect of the antibiotic on the yeast. Roth and Eylar showed that Monilia grown in cultures containing chlortetracycline or strains isolated from patients on antibiotic therapy were no more virulent than were control cultures. Unlike Seligman, however, this group showed that oral administration of the tetracycline resulted in markedly increased mortality of mice injected intraperitoneally with Candida. De Mello and Kiser confirmed the failure of intravenously administered chlortetracycline to accelerate the lethality of intravenous injections of Candida, whereas preceding intraperitoneal Candida injections by intraperitoneal tetracycline produced an acute lethal infection. In a later study, Roth and Murphy (131) showed that chlortetracycline potentiated lethality of intraperitoneally injected Candida in additional species: guinea pigs, rabbits, and monkeys. Henry and Fahlberg (63) showed that half of the mice infected with a sublethal dose of C. albicans, died when given 3- and 4-mg daily doses of tetracycline the day before infection, and 13 days thereafter. There was no significant increase in mortality over that seen in infected untreated groups on 1- and 2-mg daily dosage of tetracycline. Hydrocortisone also increased mortality, and had an additive effect when given with tetracycline, increasing mortality of mice on the 1- and 2-mg daily doses of the antibiotic. The authors postulated that the enhancing effect of cortisone or tetracycline on the infectivity of C. albicans probably resulted from the action of the drug on the host. Dukes and Tettenbaum (46) reported that concentrations of C. albicans incapable of producing fatal

infections in mice (intraperitoneal, intravenous) evoked fatality in 33 of 40 when the yeast cells were injected suspended in tetracycline (2 mg), chlortetracycline (2 mg), or oxytetracycline (3 mg), but not with penicillin (5 mg), streptomycin (2 mg), or chloramphenicol (0.5 mg). They found that enhancement of the fungus infection occurred only when the same route of entry was used for fungus and antibiotic.

The effect of dosage of the broad-spectrum antibiotics on the lethality of C. albicans in the mouse was explored by Andreoni et al. (5). They found that intraperitoneal injection of 1,000 µg of tetracycline raised the mortality rate of mice given an LD_{50} dose of C. albicans to 100%. At doses of 300 to 500 µg the mortality was 75%, but at 100 µg there was no change in mortality (from the 50% of the control infected group). Findings with oleandomycin were similar. With novobiocin, however, the low dosages of 100 and 300 µg exerted a protective effect, the mortality rates being 0 and 25%, respectively. At 500 and 1,000 µg, the mortality rates were the same as those provoked by the other tested antibiotics, namely, 75 and 100%.

Much of the foregoing evidence seems to implicate alteration of the host by antibiotics as a major factor in the increased pathogenicity of Candida, particularly when injected together, but whether the antibiotics exerted only a local effect, or whether they also influenced the immunological defense mechanisms, was not established.

Local Irritant Effect of Antibiotics

The studies of the antibiotic enhancement of the lethality of Candida infections suggested that the antibiotics caused damage to the host tissues at the site of infection, thereby increasing local invasion by the fungal organisms. Seligman (137, 138) attributed this effect to antibiotic damage of host tissues. De Mello and Kiser (34) attributed the antibiotic increase of lethality of intraperitoneally injected Candida to an increase in permeability of peritoneal membranes, caused by the tetracyclines. Hazen et al. (61) demonstrated that oral mucosal moniliasis could not be produced in rabbits, unless they had been treated with tetracycline 2 days before and after having the yeast cells instilled. Similarly, Felisati et al. (48) showed that bronchial candidiasis resulted from instilling Candida into bronchi of rabbits that were simultaneously administered tetracyclines by the same route. This was not seen with penicillin, streptomycin, or chloramphenicol. That local irritation of the tissues exposed to the tetracyclines might be responsible for decreased resistance to Candida was also

demonstrated by Messer and Freter (100), who reported that chlortetracycline, streptomycin, and chloramphenicol, alone or in combination, lowered the 50% infective dose of orally administered *Candida* (to mice or chicks) by a factor of 3 to 54 as compared with antibiotic-free controls. In this study, elimination of antagonistic bacteria did not appear to have been the major factor predisposing to *Candida* overgrowth during oral administration of antibiotics. The increased susceptibility of the mice to *Candida* infection was unaffected by replacement of aerobic or anaerobic enteric flora, or both, with antibiotic-resistant flora 24 hr before challenge with *Candida*. On the basis of their work with gastrointestinal irritants in place of antibiotics, these investigators suggested that the antibiotics may enhance the growth of *Candida* as a result of direct action on the tissues of the host. De Mello and Kiser (34) similarly found that irritating organic substances and tetracycline derivatives devoid of antibacterial activity caused potentiation of intraperitoneally injected *Candida*.

Immunological Responses to Candida

Before considering the mechanisms by which antibiotics may interfere with the immunological defenses against *C. albicans*, it is necessary to evaluate the evidence regarding the response of the host to this organism. There has not been uniformity in the literature, as to the degree to which host defenses protect against infection by *Candida albicans*, which has commonly been considered a saprophyte, and thus not normally invasive.

Candida antibodies. Investigators who have explored the development of antibodies to *Candida* have come to conflicting conclusions. Winner (159, 160) found that increased titers of agglutinating antibodies in rabbits, developed in response to *C. albicans*, failed to increase their resistance to infection. Because of the size and complexity of the *Candida* cells and their tough polysaccharide wall, he suggested that the yeasts may be relatively immune to the action of host enzymes, to surface-acting antibodies, and to phagocytosis (159). Nonetheless, he commented that rabbits seemed to possess some natural defenses against infection with *C. albicans*, in view of the fact that there is a definite threshold dose, below which the animal recovers from a transient illness, and above which it dies (159, 160). Donomae et al. (41), Mourad and Friedman (106), and Hasenclever and Mitchell (60), however, were able to demonstrate development of anti-*Candida* activity in the sera of rabbits (41) and mice (60, 106) that had been immunized against *C. albicans*.

Clinical investigators have confirmed the failure of agglutinating antibodies to *Candida* to protect against *Candida*, high titers of agglutinins or positive complement-fixing antibodies, or both, having been reported, not only in normal subjects, but in patients with candidiasis, who manifestly have poor resistance (19, 43, 54, 64, 123, 161, 162). However, the demonstration of *Candida*-inhibiting, or *Candida*-cidal substances in the serum of normal subjects by Roth et al. (128, 130) and by Louria and Brayton (85; in the α- and β-globulin fractions, but not in the γ-globulin), and the observation of low levels of this factor(s) in the blood of infants and of patients with acute blood dyscrasias (128, 130), or in cirrhotics, azotemic diabetics, and candidiasis patients (85, 128) indicate that one or more protective antibodies to *Candida* do exist.

Phagocytosis of Candida. The cellular response to *C. albicans* is also subject to some dispute. Relative resistance to phagocytosis has been observed by Winner (159, 162), who reported that the cellular response to *C. albicans*, in tissues taken from animals with experimental candidiasis, was not consistent. There was often a relatively poor cellular response, but occasionally a well-marked inflammatory exudate was seen. In other studies of the course of experimental *Candida* infection, however, the importance of macrophages in preventing local extension of the candidal lesions has been stressed. Masshoff and Adam emphasized the importance of histiocytes in the elimination of *Candida* from the tissues (99); Gresham and Burns (56) attributed the relative resistance to *Candida* of organs, such as liver, lungs, and spleen, to their ample supplies of macrophages; and Louria et al. (87) attributed the rapid growth of candidal elements in the tubular and glomerular lumina to their isolation from the host defense mechanisms in that locus. Louria and Brayton (86) demonstrated, in vitro, that normal human blood leukocytes in normal serum, can phagocytose *Candida* cells; Hill and Gebhardt (65) demonstrated various stages of degeneration of yeast cells that had been engulfed by phagocytes in the tissues of mice that had received *C. albicans* subcutaneously.

It is possible that impairment of phagocytic activity may be responsible for the increased susceptibility to *C. albicans*, caused by corticosteroids. Mankowski (95) demonstrated that cortisone, given to mice in dosages of 0.95 to 3.75 mg per day for 3 days, preceding infection with *C. albicans* or other fungi, depressed the leukocytic response of mice to the *C. albicans* infection, and increased the mortality rate. Louria et al. (87) demonstrated that cortisone caused maximal enhancement of *Candida* in-

fection only when given not later than 24 hr after the infecting dose. A delay of 24 hr decreased mortality from 90 to 70%; delay until 7 days after infection markedly reduced the enhancing effect. The authors concluded that by that time the host defenses had been well established. O'Grady et al. (112) found that histiocytes of cortisone-treated, *Candida*-infected mice often contained *Candida* cells, whereas in untreated animals such phagocytosis was not seen. In the treated animals, active budding of the yeast cells within the phagocytes was noted, indicating that the cortisone may have interfered with intracellular destruction of the engulfed organism, a characteristic of corticosteroid activity reported with other microorganisms (3, 89, 90).

Direct evidence that impaired phagocytosis may play a part in increased susceptibility of patients to *Candida*, is provided by the reports of low phagocytic activity of polymorphonuclear leucocytes in patients with diabetes mellitus (20, 119), hepatic disease (122), or reticuloendothelial neoplasms (17, 71, 101, 113), all diseases characterized by a propensity towards serious *Candida* infections. The possibility that there may be a specific inhibitor of phagocytosis in the serum or urine of patients with reticuloendothelial neoplasms was considered by Kimball and Brody (77). They demonstrated that *C. albicans* or starch particles, suspended in the sera of patients with reticuloendothelial neoplasms and placed on rabbit ear abrasions (skin window-technique), were not phagocytosed by the rabbit phagocytes; *Candida* cells or starch particles suspended in sera from normal subjects were.

A recent paper by Mitchell and Sbarra (102), describing reduced phagocytic activity of leukocytes (against *Escherichia coli* and *Pseudomonas aeruginosa*) of approximately one-fourth of a small group of pregnant women is of interest in view of the increase in incidence of vaginal candidiasis during pregnancy (115, 136). Whether the reported increased susceptibility to candidiasis of experimental animals on estrogens (94, 95, 135), however, is referable to altered phagocytic activity remains unresolved. Mankowski (95) reported that estradiol (0.025 to 0.2 mg), like cortisone, inhibited the leukocytic response of mice to inoculations with *C. albicans*. Nicol et al. (110), on the other hand, have characterized estrogen as the natural stimulant of body defense, since they demonstrated that estrogens stimulated both γ-globulin synthesis and phagocytosis of inert particles and microorganisms.

Phagocytosis and serum factors. Comparatively little attention has been paid *Candida* by immu-

nologists, who are elucidating the patterns of phagocytic response to other foreign antigens. It is necessary, therefore, to draw upon information derived from studies of the host responses to bacteria and nonliving particles, in an attempt to clarify the host responses to *Candida*.

The interrelationships of phagocytosis and serum factors were discussed by Austen and Cohn (7, 25) in a 1963 review. Phagocytosis is divided into two phases: the ingestion of the particle, and the intracellular fate of the particle. Both specific antibodies (heat-stable globulins) and a heat-labile component, which may be complement, have been shown to enhance the phagocytic capacity of the blood and tissue phagocytes (7, 12, 27, 120, 124, 145, 148). In a 1964 review, Halpern (59) observed that phagocytosis of microorganisms is subject to essentially the same kinetic laws as the phagocytosis of inert particles, and reiterated that the rate of bacterial phagocytosis is strongly influenced by humoral factors, such as antibodies. The destruction of the phagocytosed cell is dependent on the enzymes (i.e., in lysosomes) of the phagocytes. Li, Mudd, and Kapral (83) found a thermostable factor to be essential for phagocytosis of *Staphylococcus aureus* by human leukocytes; killing required a thermolabile factor. Shayegani et al. (140) later demonstrated this dissociation in systems by use of leukocytes and sera of rabbits immunized with *S. aureus*; leukocytes and sera of nonimmunized rabbits did not show this dissociation. Leukocytes and sera of infants, during the first few months of life and toward the end of the first year, also showed dissociability of phagocytosis and intracellular killing of *S. aureus* by heat inactivation of their sera; the few infants 5 to 6 months old that were studied did not (140). As the authors pointed out, it is in the middle of the first year of life that antibody levels tend to be low.

In the case of *Candida*, the lowest titers of an inhibiting antibody were seen in sera from infants 4 to 6 weeks old (128). By 6 months, titers had reached significantly higher levels, and by 9 months titers were at levels approximating the low limits of maternal serum levels. The low titers during early infancy can be correlated with the severity of candidiasis at that age (31, 32, 35, 37, 53, 82, 136). The serum factors reported by Roth et al. (128) and by Louria and Brayton (85), were shown to be relatively heat-stable, so far as their activity against *Candida* is concerned. Their activity in a phagocytic system, however, was not tested. A heat-labile substance was reported by Miya and Marcus (103) and by Wu and Marcus (164) to enhance the phagocytosis and intracellular digestion of

another fungus, *Histoplasma capsulatum*, by leukocytes from immunized and control mice.

Many pathogens apparently possess surface components that interfere with phagocytosis in the absence of specific antibodies (7, 25). Such surface components, which contribute to the virulence of the organisms, have been defined in the case of the capsular polysaccharides of the pneumococcus or of Friedlander's bacillus, and of the M protein or capsular hyaluronic acid, or both, of group A streptococcus (7, 50, 66, 81, 148, 149). For example, Hirsch and Church (66) postulated that for phagocytosis of encapsulated strains of streptococci, a "capsule-neutralizing" thermolabile factor is required. Stollerman et al. (149) provided evidence supporting the requirement for a specific serum factor (opsonin) for phagocytosis of encapsulated streptococci. They showed that there were marked differences in engulfment of group A organisms by human bloods, only with encapsulated strains. Human bloods deficient in the factor required for opsonization of encapsulated streptococci showed a normal rate of phagocytosis against all other organisms and particles studied. Mudd et al. (108) demonstrated that there is at least one antigen of the staphylococcal cell wall that is critical for phagocytosis by human blood leukocytes. Removal from the phagocytic system of antibody to the teichoic acid component of the cell wall prevented phagocytosis (108); removal of antibody against the non-type specific agglutinogen did not (83).

Since *C. albicans* is an encapsulated organism (56, 159, 162), in response to which both agglutinating and inhibiting antibodies are formed, it seems likely that the observed differences in resistance may be explicable on the basis of the interaction between specific antibodies and phagocytes. To study the intracellular survival and outgrowth of the yeast cells from the leukocytes that had engulfed them, Louria and Brayton (86) found it necessary to use strains of *Candida* to which normal sera have no *Candida*-cidal antibody. Incubation of *C. albicans* strains with leukocytes and sera obtained from normal subjects showed that the most virulent strains grew out of viable leukocytes, 38 to 64% of phagocytosing leukocytes having one or more mycelia penetrating the cell wall after 4 hr of incubation. The less virulent strains showed outgrowth of mycelia from 16 to 28% of the leukocytes that had engulfed them. There was no evidence that leukocytes were able to kill any of the five strains of *C. tropicalis* or of one strain of *C. guilliermondi*, strains against which the *Candida*-cidal factor of normal serum is ineffective.

The demonstration of lower than normal levels of a *Candida*-cidal substance in the sera of patients with cirrhosis, hepatitis, diabetic azotemia, renal disease, or candidiasis (85), and of low levels of a *Candida*-inhibiting substance in infants (128), thus may bear a direct relationship to the high rate of candidiasis in such population groups. It seems probable that a deficiency of a specific anti-*Candida* factor impairs the ability of phagocytes to destroy the *Candida* cells.

Abnormal metabolism of phagocytes with impaired function. The recent experiments of Sbarra and Karnovsky et al. (114, 133) and Cohn and Morse (26), who have demonstrated that in vitro phagocytic ingestion of particles is almost entirely dependent on energy from glycolysis, provide a new basis for understanding the impaired phagocytosis seen in diabetes, and, possibly, in glucocorticoid-treated animals and patients. Following Beck's (10) demonstration that leukocytes from human blood show a very high aerobic glycolysis, Sbarra and Karnovsky (133) observed that active glycolysis is essential during phagocytic uptake of inert particles by guinea pig granulocytes and that increased metabolism is involved in particle uptake. Cohn and Morse (26) then demonstrated that rabbit polymorphonuclear leukocytes are dependent on an exogenous supply of glucose for continuance of the phagocytic process. Endogenous glucose, in the form of glycogen, is insufficient to maintain the phagocytic process for prolonged periods. Metabolic inhibitors which blocked glycolysis produced a marked inhibition in ingestion of particles. Oren et al. (114) have shown that guinea pig polymorphonuclear leukocytes and peritoneal monocytes are dependent solely on glycolysis for metabolic energy for phagocytosis; alveolar macrophages depend also on oxidative phosphorylation.

These findings certainly bear some relevance to the high susceptibility to bacterial and fungal infections in a disease with impaired glucose utilization such as diabetes mellitus. The production of lactate from glucose by leukocytes was shown by Martin et al. (98) to be greater in cells taken from healthy subjects than in those from patients with moderately severe, untreated diabetes. Addition of insulin to the leukocyte preparation resulted in a significant increase in the lactate production by the cells from the diabetic patients, but produced no change in the metabolism of the normal cells. Esmann (47) found that the glycogen content of leukocytes from poorly controlled diabetic patients and its synthesis by leukocytes from such patients were below normal. Leukocytes obtained after im-

provement of diabetic control exhibited normal glycogen content and synthesis.

The effects of diabetes, in the presence or absence of acidosis, on leukocytic behavior, have been studied in several species of animals, and in man. Cruickshank and Payne (30) assumed impairment of leukocytic capacity to destroy engulfed bacteria, in his demonstration of impaired antipneumococcal capacity of the blood of rabbits, with acute alloxan diabetes and acidosis, infected with pneumococci. This observation was confirmed some years later by Wertman and Henney (158), using leukocytes from alloxan diabetic rats, and staphylococci. They reported that infected rats with hyperglycemia had a lower percentage of active neutrophils (42 to 45%, as compared with 87 to 91% in nondiabetic infected rats) and that the phagocytic activity of these cells was reduced. Cohn (24), however, found that mice with alloxan diabetes exhibited a normal intraperitoneal response to staphylococcal infection and that there was normal ingestion and destruction of these bacteria. No differences between normal, chronic alloxan diabetic, and acute acidotic diabetic rabbits were reported by Schauble and Baker (134) in the migration of polymorphonuclear leukocytes to the site of injected *Micrococcus pyogenes*, or *Rhizopus oryzae* (isolated from a fatal case of mucormycosis). However, there was greater proliferation of both pathogens in the cutaneous lesions in the acutely toxic alloxan-diabetic rabbits than in the normal or chronic diabetic animals. Cruickshank (29) observed no difference from normal animals in the rate of removal of bacteria from the circulation in animals with chronic nonketotic diabetes, but he reported a failure of the inflammatory reaction in staphylococcal skin lesions of rabbits with acute ketotic alloxan-diabetes.

Sheldon and Bauer (141) observed that there was a delay of several hours in the onsets of granulocytic responses to experimental cutaneous mucormycosis in rabbits with acute alloxan diabetes and acidosis, as compared with normal rabbits. The authors attributed the increased growth of fungus in the tissues of the diabetic rabbits to the delay in polymorphonuclear migration to the site, to the decrease in intensity of the leukocytic response, and to the apparent impairment of its efficacy. Drachman (42) recently demonstrated the suppression of phagocytosis of pneumococci injected intraperitoneally into chronically diabetic rats. In an in vitro system, he has shown that the phagocytic capacity of leukocytes from peritoneal exudates of normal or diabetic rats could be depressed, not only in diabetic serum, but also in normal serum made hypertonic

by hyperglycemic levels of added glucose, or of other hexoses or pentoses. He found that phagocytosis by granulocytes in whole blood was unaffected. He postulated that the impaired phagocytic defense of the nonketotic diabetic host may be due to the osmotic effects of the high glucose levels on the granulocytes operating in inflammatory exudates.

Correlation of abnormalities of phagocytic metabolism with impaired phagocytic capacity is possible with diabetes, leukemia, and corticosteroid therapy—all conditions associated with depressed resistance to bacterial and fungal infections. Direct evidence that the phagocytic function of leukocytes from diabetic patients is impaired has been provided by Perillie et al. (119) and Bybee and Rogers (20). Perillie, Nolan, and Finch (119) compared the local exudative cellular response to inflammation in uncontrolled diabetic patients with that evoked in normal subjects, in controlled diabetics, and in nondiabetic uremic patients with acidosis. They reported that the early granulocytic phase of the cellular response was significantly delayed in the patients with acidosis, whether subsequent to diabetes or to renal failure, as compared with normal patients and nonacidotic, diabetic patients. After correction of the acidosis in the diabetic group, there was a completely normal local cellular response. Bybee and Rogers (20) employed an in vitro system to compare the phagocytic activities of polymorphonuclear leukocytes of acidotic and controlled diabetic patients, and of normal subjects. They found that only the leukocytes from the acidotic patients exhibited an impaired capacity to ingest and destroy pathogenic and nonpathogenic strains of staphylococci. The defect disappeared when the acidosis had been corrected. Suspension of cells from acidotic patients in normal serum did not correct the abnormality, nor could it be induced in normal cells suspended in acidotic serum.

Martin et al. (97) demonstrated that addition of 0.1 to 1.0 μg/ml of cortisone or hydrocortisone to leukocytes from normal subjects caused a diminution of lactate production and glucose utilization. Allison and Adcock (3) suggested that the impaired ability of leukocytes from corticosteroid-treated animals to destroy engulfed microorganisms (3, 89, 90, 104) may be referable to the corticosteroid-induced derangement of the metabolic activity of phagocytes. Beck (10) observed that leukocytes from patients with chronic myelocytic leukemia and chronic lymphatic leukemia showed a significantly lower rate of aerobic glycolysis than did normal leukocytes. Perillie and Finch (118) observed that the early exudative cellular inflammatory response was both delayed

and diminished in comparison with the normal, in acute and chronic leukemic patients. Reduced phagocytic activity of neutrophiles in chronic granulocytic leukemia has been reported by Jersild (71) and Braude et al. (17).

Resistance to Candida albicans. In view of the existence of antibiodies to *C. albicans*, the demonstration that phagocytosis of yeast cells and mycelia is possible, and the development of systemic or fatal candidiasis only in patients with impaired or immature immunological mechanisms (6, 9, 13, 16, 28, 31–33, 35–40, 53, 54, 58, 64, 74, 75, 80, 82, 88, 91, 127, 136, 153, 157, 162, 167), it is reasonable to assume that the normal subject has immunological defenses against *C. albicans*. The presence of anti-*Candida* substances in the normal blood serum is evidence of penetration into the tissues by the organism. Winner (161), Rimbaud et al. (123), and Giunchi (54), who found agglutinating antibodies to *Candida* in normal serum, concluded that symptomless infection is probably more common than suspected. The observation of low titers, during infancy, of agglutinating antibodies (19, 54) and of *Candida*-inhibiting antibodies (128), and the increasing titers of both types of antibody with age (54, 128), provide additional evidence that candidal invasion of tissues is not a rare phenomenon. Since antibiotics are commonly administered and are known to give rise to proliferation of *Candida*, because antibiotics almost always constitute part of the therapeutic regimen of patients with impaired defense mechanisms, and because the incidence and severity of candidiasis have increased significantly during the antibiotic era (136), it is important to evaluate the available data on the effect of these agents on the defense mechanisms.

Antibiotic Depression of Immunological Defenses

Effect of antibiotics on antibody production. Relatively little work has been done on the effect of the antibiotics on synthesis of antibodies, other than the suppression of antibody production which results from "removal" of the bacterial antigenic stimulus. Slanetz (143) provided evidence, in 1953, that the suppression of antibody response after chlor- or oxytetracycline could not be attributed entirely to antibiotic elimination of the causative organism. He found that either of those broad-spectrum antibiotics, given to rats and mice for 2 days, or for 2 weeks prior to injection of noninfectious antigen derived from killed *Salmonella* organisms, caused changes in antibody production. In general, after the antibiotics had been fed to the animals for 2 days, there were increases in antibody titers. When the animals had been fed the tetracyclines

for 2 weeks before injecting the antigen, they showed depression of total protein and β-1 globulin titers, as compared with controls. Stevens (147), in the same year, demonstrated that the antibody response to labeled antigen (I^{131} bovine γ-globulin) was significantly suppressed in rabbits given chlortetracycline, tetracycline, dihydrostreptomycin, or penicillin, in dosages comparable to those in clinical usage. Allen and Cooper (2) did not find chlortetracycline to interfere with development of immunity to a nonliving vaccine (erysipelas bacterin), when it was injected intraperitoneally to mice at a level of 20 mg/kg for 2 days before and 4 days after the vaccination, a study comparable to the short phase of the Slanetz (143) study.

The effects of antibiotics on the antibody and other serum proteins of animals infected with *C. albicans* was reported by Gorczyca and McCarty (55). They demonstrated that goats given oxytetracycline (60 mg per day) intramuscularly, and goats given benzathine penicillin (300,000 units intramuscularly) every other day, developed changes in their serum proteins. In addition, the alterations in serum proteins caused by *C. albicans* infection were influenced by the antibiotics. The *C. albicans* infection, given by intranasal insufflation to otherwise untreated goats, caused a drop in albumin (from 65 to 54% of total serum protein), a slight rise in the α- and β-globulins (an approximately 1% increase in percentage of total protein for each moiety as compared with the base control), and an increase of 4.7% in the γ-globulin fraction (from 15.1 to 19.8% of the total protein). The goats given oxytetracycline for 3 weeks prior to the infection showed rises in albumin and in β-2 globulin, no significant effect on α-globulin, a slight fall in γ-globulin, and a more pronounced fall in β-1 globulin. With penicillin, albumin decreased, and all of the globulins rose, or in the case of β-1 globulin remained essentially unaffected. After the *Candida* infection, the antibiotics were continued for another 21 days, and the serum protein responses were recorded at weekly intervals. Both the penicillin and the oxytetracycline-treated goats showed sharp drops in albumin. Both groups had rises in γ-globulin, the rises being somewhat greater in the penicillin group than in those on oxytetracycline. The rises in α- and γ-globulins in the oxytetracycline group were less than in the *Candida*-infected goats on no antibiotic. The β-1 globulin level remained significantly lower in the goats on oxytetracycline than in the penicillin-treated, or infected untreated goats; the β-2 globulin level rose sharply. Neither the β-1 nor the β-2 globulin showed a significant change in the penicillin-treated group. None of the *Candida*-

infected goats given no antibiotic or given penicillin died, whereas 80% of those on oxytetracycline died.

The observation that prolonged tetracycline or oxytetracycline treatment caused a drop in β-1 globulin (55, 143) is of particular interest in view of Louria and Brayton's (85) recent observation that the *Candida*-cidal substance found in the sera of normal subjects migrates with the α- and β-globulin fractions. It seems possible that the drop in β-1 globulin, and the relatively lower level in α-globulin, seen in the oxytetracycline-treated goats, as compared with the higher levels in the penicillin-treated, and infected, nontreated goats (55), may be related to the high mortality rate due to *Candida* in the oxytetracycline-treated animals.

The study by Roth and Goldstein (130), which also revealed inhibition of *C. albicans* by normal human sera showed that short-term (up to 12 days) administration of tetracyclines had no detectable effect on the capacity of their sera to retard the in vitro growth and reproduction of *C. albicans*. The effect of long-term administration of the antibiotics on the anti-*Candida* factor was not studied.

That chloramphenicol, at the same low levels as those that suppress bacterial protein synthesis (50 μg/ml), inhibits the synthesis of antibody in tissue culture has been demonstrated by Ambrose and Coon (4). They showed that the continuous presence of chloramphenicol in the medium during the entire 15- to 21-day incubation of lymph node fragments (from immunized rabbits) produced nearly complete suppression of secondary antibody response. When the antibiotic was present only during the first 6 days of the culture, the antibody response was reduced 90%.

Additional evidence that the host response to infection is altered by antibiotics has been provided by investigators exploring the effects of antibiotics on the susceptibility of the host to specific antibiotic-resistant strains of microorganisms. Simon (142) showed that oral doses of 50 mg of tetracycline, given twice daily to guinea pigs, increased their susceptibility to aerosol-administered tetracycline-resistant staphylococci. Bohnhoff and Miller (15) reported that introduction of a large dose (50 mg) of streptomycin or penicillin into the gastrointestinal tract of mice increased their susceptibility to a streptomycin-resistant strain of *Salmonella* enteritis. They observed some increase in susceptibility to *Salmonella* after treatment with the same dose of oxytetracycline or bacitracin, but not nearly to the same degree as that caused by streptomycin or penicillin.

Effect of antibiotics on phagocytosis. The nature of the antibiotic depression of host resistance in the last two studies (15, 142) was not indicated. It is possible that it may have been suppression of antibody synthesis. On the other hand, antibiotic depression of phagocytosis is also a possibility.

In 1950, Munoz and Geister (109) showed that chlortetracyline depressed the in vitro phagocytosis of *S. aureus* by normal human leukocytes, the percentage of cells having phagocytosed the microorganism decreasing with increasing concentrations of the antibiotic. Most marked inhibition was seen at a concentration of 1,000 μg/ml. There was also significant inhibition at concentrations of the antibiotic even lower than the therapeutic blood levels. Snell (146) reported that, under certain conditions, oxytetracycline reduces the relative rate of phagocytosis when administered concurrently with lipopolysaccharides, which are stimulants of phagocytosis.

In vivo demonstration of antibiotic depression of phagocytosis of *Candida* has been provided by two groups of investigators. Donomae and Kawamori (39) compared the phagocytic rates of cells obtained from the ascitic fluid of mice injected intraperitoneally with *Candida*, with and without simultaneous injection of tetracycline. The phagocytic rates of the tetracycline-treated mice were lower than those of the control infected group at 12, 24, and 48 hr after injection. Studies of monocytes from the subcutaneous tissue of rabbits, at 2 hr to 7 days after injection with *Candida*, revealed that oral treatment with chlortetracycline (250 mg; 50 mg) for 1 week before the infection markedly decreased the rate of phagocytosis. Takahashi et al. (151) verified Donomae's findings. They found that the phagocytosis by histiocytes and monocytes (supravital staining) from the subcutaneous tissues of rabbits given tetracycline orally was markedly decreased, as compared with that by cells from control groups. The tetracycline depression of phagocytosis could be partially inhibited by administration of vitamin B_1 or of co-carboxylase.

Nicol and Sewell, using in vivo phagocytosis of particulate carbon as the test, commented that neither sulfonamides (111) nor the antibiotics (139) exerted a significant effect on the rate of removal of carbon particles from the circulation of white mice. However, some difference was seen between the effect of penicillin and that of tetracyclines. The control phagocytic index was given as 18 $\pm$ 5.8. The penicillin-treated mice had a phagocytic index of 27 $\pm$ 2.6 after subcutaneous administration and 21 $\pm$ 1.6 after oral dosage; mice orally treated with chlortetracycline had a phagocytic index of 12 $\pm$ 2.8;

the mice orally treated with oxytetracycline, 14 ± 5.1; and the tetracycline orally treated mice, 17 ± 3.2. Subcutaneous injection of the tetracyclines caused no change from control values. Chloramphenicol, neomycin, streptomycin, and dihydrostreptomycin showed some lowering of phagocytic ratios (12 to 14), as compared with the control values.

Zalman et al. (166) found penicillin to have stimulated phagocytosis of staphylococci by guinea pig leukocytes, to the same extent as did antistaphylococcus antiserum. In their study of the effects of erythromycin or of chloramphenicol, however, they found that the antibacterial agents "prepared the bacteria" for phagocytosis, whereas addition of the antibiotics to the leukocytes reduced their phagocytic capacity. When the antibiotics had been added to the leukocytes 15 min before the bacteria, the phagocytosis enhancement was greatly reduced, as compared with the phagocytosis seen when the antibiotic-treated bacteria were added to the leukocyte preparation.

The effect of the administration of tetracyclines on the phagocytic activity of the reticuloendothelial system of mice was recently reported by Kalinina and Kinman (73). They had found, earlier, that a single dose of chlor- or oxytetracycline sometimes stimulated phagocytosis, depending on the dose employed. In their confirmatory study, they found that a single dose of 0.5, 1.0, or 2.0 mg of chlortetracycline stimulated phagocytosis of intravenously injected *S. aureus*; a single dose of 0.2 mg had no effect. Administration of 2.0 mg of tetracycline stimulated phagocytosis; doses of 0.5 or 1.0 mg exerted no significant effect. Oxytetracycline, in a single dose of 1.0 or 2.0 mg, stimulated phagocytosis; 0.2 or 0.5 mg showed no effect. Repeated administration of the tetracyclines never caused enhancement of phagocytosis. The repeated administration of chlortetracycline, in high therapeutic dosage, caused depression of phagocytosis; no such suppression was produced by tetracycline.

The comparative effects of the different antibiotics on phagocytosis cannot be deduced from the foregoing data, the investigative procedures having differed in each study and the work being of too preliminary a nature. That antibiotics have been shown both in vitro and in vivo to depress phagocytic activity suggests a direct effect on the leukocytes. That there may also have been an indirect effect as a result of suppression of the enteric flora, is suggested by the recent work of Abrams and Bishop (1, 154). They have shown that the normal intestinal flora enhanced the ability of the host to cope with infection by enhancing leukocytic mobilization.

Perhaps this activity may partially explain the suppression of phagocytosis in mice given chlortetracycline orally, but not when it had been given subcutaneously, as reported by Sewell and Nicol (139).

DISCUSSION

The conversion by antibacterial therapy of *C. albicans*, an organism considered saprophytic, to one with marked pathogenic potential was demonstrated early in the course of laboratory investigations of antibiotics. In the clinic, the alteration in ecological balance in the alimentary canal, caused by antibiotics, was deemed essentially benign for many years. The outgrowth of *C. albicans* in the stools or on the mucosae of patients on antibacterial therapy has not been considered a problem, with the possible exception of its occurrence in patients with debilitating diseases, or on simultaneous corticosteroid or antineoplastic therapy, or both. In such patients, the hazard of dissemination of *Candida*, with development of systemic candidiases, or of serious visceral involvement, has been recognized for some time. The use of antibiotics in such patients is known to increase the incidence and severity of fungal infections. In fact, many of the clinicians who reported fatalities caused by *C. albicans* considered the hematogenous spread of the organism to have been secondary to its penetration of the mucosa of the alimentary tract (18, 28, 32, 35, 53, 82, 88, 157, 167), where the *Candida* flourishes in the presence of antibacterial antibiotics. Pediatricians have voiced alarm about the increasing incidence and severity of candidiasis during infancy (32, 37, 45, 80, 82, 115). *C. albicans* has been detected as the primary etiological factor in an increasingly large percentage of infant mortalities. In infancy, the source of the *Candida* infection has been considered to have been the birth passage. Although antibiotic therapy has intensified the disease, its onset has been reported in infants before they had been given antibiotics. Thus, the relationship of infantile candidiasis to antibiotics is more likely to be indirect, possibly a consequence of the increased incidence of vaginal moniliasis in pregnant women on antibiotic therapy.

The degree to which antibiotic enhancement of the growth of *Candida* may contribute to nonfatal diseases remains to be determined. It has been reported that the incidence of fungal pyelonephritis and of cortical renal infection has increased since the advent of antibiotics (54, 64, 74, 91, 136, 162). In fact, a 1960 editorial (155) warned that renal candidal infection is a risk, even in some otherwise healthy people on anti-

biotic therapy. A recent analysis (136) of the published literature on candidiasis discusses the similarity in pathological changes in the kidneys of normal animals infected with sublethal doses of *C. albicans* to those seen in the kidneys of patients with renal candidiasis. Winner and Hurley (162), who commented on the similarity of the experimental renal disease to human renal candidiasis, found that only the kidneys were involved when small doses of *C. albicans* had been injected into mice. That minimal renal infection by *C. albicans*, characterized by cortical micro-abscesses, can be eliminated by normal animals, was demonstrated by Winter and Foley (163) and by Louria et al. (87). Both groups of investigators showed that suppression of the host defenses (by corticosteroids) resulted in markedly increased invasion by *Candida* hyphae and an increase in mortality. In the infected animals on no therapy, the cortical lesions caused by *C. albicans* were localized by the accumulation of macrophages and proliferating fibrous tissue at their peripheries; 14 days after infection, only cellular scars remained (87).

The source of *C. albicans* in human renal candidiasis has remained something of an enigma, in view of the belief that *Candida* is rarely invasive. Evidence that *Candida* has been detected with increasing frequency in catheterized urines of patients on antibiotics, but without overt kidney disease, reviewed elsewhere (136), implies that the kidneys must have filtered out the organism from the circulating blood (44, 54, 91, 136). It seems plausible that the derivation of the circulating *Candida* is from the site of its proliferation, usually in the alimentary canal, and particularly in patients on antibiotics lacking antifungal activity. The low frequency of severe renal candidiasis in such patients is probably a consequence of the ability of normal host defenses to eliminate the organism before irreversible renal damage has taken place.

This report has provided evidence that antibacterial agents can increase the likelihood of candidal invasion and complications. The mechanisms by which the antibiotics increase the hazard of candidiasis entail more than simple overgrowth by *Candida* in the absence of competing organisms. They may include tissue damage by the antibiotic or by endotoxin released by proliferating *Candida*, conversion of the *Candida* to a more invasive form, and direct depression of host-defense mechanisms, such as antibody synthesis and phagocytosis. Such suppression is probably a serious problem only for patients with already depressed immunological mechanisms. It is not generally realized, however, that candidal penetration of the mucosa at the site of

its proliferation is probably a frequent occurrence. Certainly the increasing titers of antibodies to *Candida* with increasing age indicate that the organism can penetrate into the tissues and blood, where the defense mechanisms normally limit its multiplication and spread. The presence of *Candida* in the urine is evidence that it can reach loci remote from the site of its primary outgrowth, probably when its numbers in the gut are excessive, or when there is some interference with defense mechanisms.

The degree to which immunological factors may protect against an agent that was considered only saprophytic under normal circumstances has not been generally appreciated. Therefore, this report has presented a brief survey of the literature that provides evidence of host defenses against *C. albicans*, as well as data on immunological responses to other microorganisms, which are relevant to the host response to *Candida*.

The first line of defense is provided by the phagocytes, which have been shown to engulf and destroy the *Candida* cells in the tissues of the host in experimental candidiasis. Support for the role of phagocytes in limiting candidal spread is provided by evidence that patients with diseases characterized by a high incidence of *Candida* infections have leukocytes with low phagocytic activity. The demonstration of impaired glucose metabolism in leukocytes of diabetic patients, of leukemic patients, and of animals on corticosteroids, allows for the correlation of impairment of phagocytic function with the metabolic impairment.

Demonstration that enteric flora enhance leukocyte mobilization suggests that antibiotic suppression of the enteric flora may interfere with the ability of the host's leukocytes to destroy the invading organism. The invasion, itself, may be mediated by local damage caused directly by the antibiotics, or indirectly by the endotoxin released from the proliferating *C. albicans*. Direct evidence of suppression by the tetracyclines of phagocytosis of *Candida* injected into the subcutaneous tissues of experimental animals, and in vitro demonstration of impairment of phagocytosis by chlortetracycline, further elucidate the apparent increased susceptibility to *Candida* infections caused by the tetracyclines.

Production of antibodies to *C. albicans* in normal subjects provides evidence both of symptomless tissue invasion, and of building up of defenses. Evidence has been presented that broad-spectrum antibiotics inhibit antibody synthesis, and that tetracyclines depress the levels of the globulin fractions which have been shown to possess *Candida*-cidal activity, and to be below normal in infants, and in patients with

diseases known to be associated with a high incidence of candidiasis.

It is conceivable that a deficiency of a specific anti-*Candida* factor, however caused, may impair the ability of phagocytes to destroy the *Candida* cells. Inter-relationships of serum and cell factors in the phagocytic process have been conclusively demonstrated with other microorganisms. It has been found that specific antibody is of particular importance in the phagocytosis of encapsulated bacteria. Since *C. albicans* is also encapsulated, the deficiency of a specific anticandidal factor in patients with clinical candidiasis, and in patients with diseases subject to candidal complications, may contribute significantly to their susceptibility to dissemination of *C. albicans*. In such patients, even a slight additional compromise of the body's defenses, or an increase in the population of the potential pathogen, may be sufficient to allow for its spread throughout the body.

SUMMARY

Antibiotic therapy has contributed to the increasing incidence and severity of clinical candidal infections. The proliferation of *C. albicans* in the intestinal contents, and on the mucosal surfaces, heretofore considered of little clinical importance, has been shown to have pathogenic significance.

The mechanisms by which antibiotics increase the hazard of candidiasis by affecting the flora directly include overgrowth of *Candida* in the absence of competing organisms, local tissue damage, and increased invasion by the *Candida*, either as a consequence of the local tissue damage, or possibly, due to conversion of the *Candida* to a more invasive form.

Evidence has been presented that the normal immunological defenses to *C. albicans* include anti-candidal antibodies, as well as phagocytic ingestion and destruction of the *Candida* cells and mycelia. Antibiotics have been shown to inhibit both antibody synthesis and phagocytic activity, and thus may reduce the host resistance to invasion by *C. albicans*. The degree to which antibiotics depress host factors probably constitutes a serious problem only for patients with already poor immunological defenses. The combination of both irritating products of candidal outgrowth, and antibiotic effects on immunological responses, however, probably contribute to the increasing incidence of sublethal forms of candidal infections, in otherwise normal subjects.

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