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Human Sex Hormones Stimulate the Growth and Maturation of *Coccidioides immitis*

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Because men and pregnant women show increased susceptibility to extrapulmonary dissemination of coccidioidomycosis, studies were conducted to determine the direct effect of human sex hormones and related compounds on the growth and maturation of *Coccidioides immitis* in vitro. 17β-Estradiol, progesterone, and testosterone were highly stimulatory for the parasitic phase of *C. immitis* growth, whereas cholesterol, ergosterol, and 17α-estradiol (a physiologically inactive stereoisomer of 17β-estradiol) lacked such effects. Rates of spherule maturation and endospore release were accelerated, in a dose-dependent fashion, by concentrations of 17β-estradiol occurring in normal women, with the most striking effects seen at levels encountered in advanced pregnancy. A stimulatory effect of 17β-estradiol on the saprobic phase of fungal growth was also detected. The nonsteroidal "antiestrogens" tamoxifen and nafoxidine had either stimulatory or inhibitory effects, depending on fungal strain and experimental conditions. Diverse strains of *Cryptococcus neoformans*, *Candida* sp, and *Petriellidium boydii* were unaffected by hormones that had distinct effects on *C. immitis*. These studies suggest that direct stimulation of *C. immitis* by human sex hormones may help to account for sex- and pregnancy-related predisposition to dissemination of coccidioidomycosis.

Coccidioidomycosis is usually a self-limited pulmonary infection. However, progressive hematogenous dissemination occurs in a variable proportion of patients, particularly those predisposed by virtue of race, immunosuppression, or pregnancy. Coccidioidal dissemination during pregnancy is especially noteworthy because women are otherwise substantially less susceptible than men to extrapulmonary spread of this infection (12). Previous studies have shown that exogenous estradiol and testosterone increase the susceptibility of mice to experimental infection with *Coccidioides immitis*, puportedly by altering host defense mechanisms (32).

Fungi are eucaryotic organisms and possess mating types, the interaction of which results in the sexual or "perfect" state (1). Many fungi use hormonal control mechanisms in the process of their mating, growth, and development (8, 19, 20, 28, 40, 51, 55). The "mating hormones" antheridiol and oogoniol from the water mold *Achlya* are derivatives of fucosterol and bear a structural similarity to mammalian sex hormones derived from cholesterol. Female strains of *Achlya* secrete antheridiol, which induces in the male strain both the production of sex organ initials and the secretion of oogoniol. The oogoniol acts upon the female partner by inducing the formation of female sex organs (36). Mammalian cell estrogen receptors are also capable of binding estrogenic "mycotoxins" such as zearalenol and zearalenone from *Fusarium* species; these fungal sex hormones then initiate certain biochemical events normally associated with the binding of estradiol to its receptor (35). A physiological role for sex hormones in the reproductive cycle of fungi pathogenic for humans has not been demonstrated.

Because susceptibility to coccidioidal dissemination is related to gender and to the presence or absence of pregnancy (23, 47, 53, 54), we conducted studies to determine the effect of selected sex hormones on *C. immitis* in vitro.

MATERIALS AND METHODS

Fungi. Fungi used in these studies included three strains of *C. immitis* (C-60, cerebrospinal fluid isolate; C566, sputum; C599, transtracheal aspirate), four strains of *Cryptococcus neoformans*, and one strain each of *Candida albicans*, *C. tropicalis*, *C. parapsilosis*, and *Petriellidium boydii*.

C. immitis was maintained in the mycelial phase on GYE agar plates containing 1% glucose, 0.5% yeast extract, and 1.5% agar at room temperature, as described (49). The other fungi were maintained on Sabouraud dextrose agar. All manipulations with *C. immitis* were performed under strict biohazard precau-

tions in a P-3 facility. Studies with other fungi were conducted in laminar flow hoods.

Hormones and other sterols. 17α-Estradiol (molecular weight 272), 17β-estradiol (molecular weight 272), testosterone (molecular weight 288), progesterone (molecular weight 314), cholesterol (molecular weight 389), and ergosterol (molecular weight 397) were obtained from Sigma Chemical Co., St. Louis, Mo. Nafoxidine (molecular weight 462) was obtained from the Upjohn Co., Kalamazoo, Mich.; tamoxifen (molecular weight 564) was obtained from Stuart Pharmaceutical Division of ICI, Wilmington, Del. All compounds were dissolved in 95% ethanol and studied over a range of 10^{-6} to 10^{-22} M, as indicated.

Fungus-hormone interaction (i) *C. immitis*. The influence of the above compounds on the growth and maturation of *C. immitis* at various stages of its life cycle (Fig. 1) was determined as follows. Solutions of study compounds were prepared in 95% ethanol at concentrations of 10^{-4} to 10^{-20} M and then diluted 1:100 in modified Converse agar medium (31). Controls contained either ethanol (0.95%) or distilled water, the latter to assure that normal fungal growth was commencing during the experiment. Five-milliliter portions of the melted agar were dispensed immediately into sterile box-type petri dishes (50 by 12 mm; Falcon Plastics, Oxnard, Calif.) and allowed to gel. Plugs of agar (1-cm square) were transferred to slide cultures (49), using two plugs for each slide.

For studies involving initiation of the parasitic growth phase from arthroconidia (Fig. 1; A → S), quadruplicate slide cultures were inoculated with 2×10^6 to 4.5×10^7 arthroconidia from 1- to 2.5-month-old mycelial-phase cultures. All suspensions were standardized for viable count and verified by plating to colony-forming units. Slide cultures were incubated under moist conditions in 20% CO_2–80% air at 40°C for 5 to 6 days, depending on the strain of *C. immitis* (49). One slide culture was removed for reading at each study period (generally 24, 48, 72, and 120 or 144 h). Because of the light sensitivity of tamoxifen, all plates in all studies were wrapped in foil.

For studies involving initiation of the parasitic growth phase from endospores (Fig. 1; H → S), arthroconidia were harvested by the stirring-bar technique as described (27) and then inoculated to the surfaces of Nuclepore membranes (15 mm, 0.4 μM; Nuclepore Corp., Pleasanton, Calif.) which had been distributed, four per plate, on Converse medium in petri dishes (50 by 12 mm). Plates were incubated under 20% CO_2 at 40°C for 2 days until the liquid medium had been absorbed into the agar and were then inverted and incubated for an additional 2 to 3 days. This technique resulted in the growth of the parasitic phase on the Nuclepore membrane, with copious endospores available after the 5- to 6-day incubation period. Endospores were harvested in saline and transferred to quadruplicate slide cultures containing various amounts of hormones under the conditions described above.

The effect of drugs on fungus cells was determined by two parameters: (i) rate of growth and (ii) proportion of cells reaching full maturity. Rate of growth was obtained by daily measurement and calculation of the average cell volume (usually 100 cells) on each agar plug for the first 3 days (Fig. 1, A or F to P). For

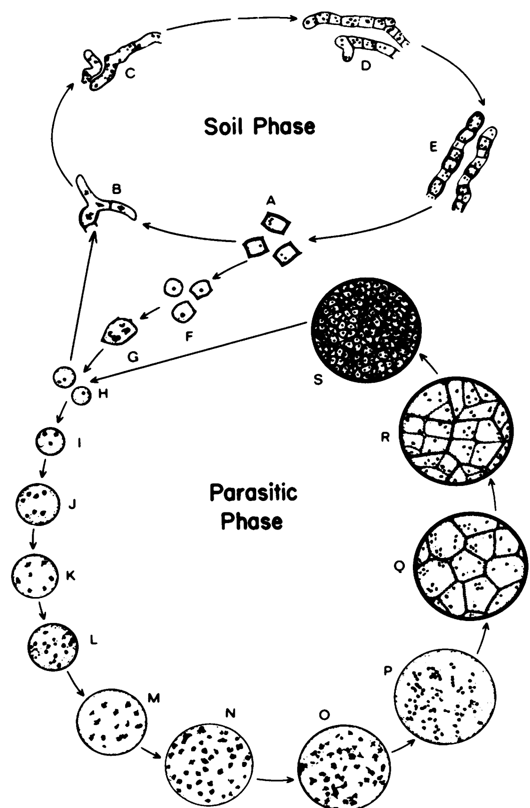

FIG. 1. *Growth cycle of C. immitis, including both soil (arthroconidia plus mycelia) and parasitic (spherule-endospore) phases. (Adapted from reference 49 by permission.) A, Arthroconidia; H, endospores or converting arthroconidia; R, near-mature spherule with cytoplasmic segmentation indicating formation of endospores; S, fully mature spherule with endospores, immediately before endospore release.*

arthroconidia and the earliest developing forms of the parasitic growth phase (Fig. 1, A → F), the volume of their generally cylindrical shape was calculated from the formula $V = \pi r^2 L$, with r (radius) and L (length) determined by direct measurement. For cells with round contours, the volume of their generally spherical shape (Fig. 1, H → P) was calculated from the formula $V = 4/3\ \pi r^3$. Calculated volumes in cubic micrometers were converted to $\log_{10}$ to simplify the plotting of data, which would have been widely divergent on linear graph paper. Geometric means were recorded because of proportionate data, i.e., r^2 and r^3 (48). The volumes of arthroconidia at t_0 (before hormone exposure) ranged from 1.47 to 1.76 $\log_{10}\ \mu m^3$; those of endospores averaged about 1.87 $\log_{10}\ \mu m^3$.

The proportion of cells reaching full maturity was determined on day 5 or 6 (strain variation) by counting 100 cells and noting the percentage of these which had matured to at least stage S in Fig. 1 or had actually released their endospores.

Studies of the effect of 17β-estradiol, tamoxifen, and nafoxidine on the mycelial phase of *C. immitis* (Fig. 1,

A → E) were conducted in Erlenmeyer flasks containing 50 ml of Converse liquid medium, 2.1×10^6 to 4.5×10^7 colony forming units of arthroconidia, and ethanol with or without the appropriate hormonal agent. Flasks were incubated at 30°C in a rotary shaker for approximately 5 days, depending on the growth characteristics of the particular strain. When a suitable amount of growth was obtained in the control flasks, 1% Formalin was added to each flask. After 3 days, the killed fungal growth was collected on a membrane filter (0.45 μm; Millipore Corp., Bedford, Mass.) and allowed to dry. The dried filtrate was peeled from the filter under a dissecting microscope and transferred to preweighed vials. After desiccation at 60°C for 3 days, the dry weight of the mycelium was obtained.

(ii) *Candida* spp. and *C. neoformans*. Preliminary experiments established that *Candida* spp. and *C. neoformans* would not grow in Converse medium. However, yeast nitrogen base agar, a defined constituents medium (Difco Laboratories, Detroit, Mich.), when supplemented with glucose and asparagine, was found to support the parasitic growth phase of *C. immitis* and to allow demonstration of the stimulatory effects of 17β-estradiol. Therefore, yeast nitrogen base medium was used to study the effects of hormones on *Candida* spp. and *C. neoformans*.

Fungi from stock cultures were inoculated into Sabouraud dextrose broth on a shaking water bath incubator at 37°C. After 24 to 36 h, 10^5 fungi were inoculated to a series of borosilicate tubes containing 5 ml of yeast nitrogen base or Sabouraud dextrose broth to which had been added appropriate concentrations of hormones dissolved in ethanol as above. Tubes were wrapped in foil and incubated for 48 h on a shaking incubator at 37°C. Parameters monitored at the beginning of the experiment (t_0) and at 24-h intervals included: hemacytometer chamber count (and degree of clumping); colony count; viability by methylene blue dye exclusion; cell diameter, with and without inclusion of the capsule (*C. neoformans*); and percentage of budding.

(iii) *P. boydii*. Fungi were grown for 14 days at 37°C on Sabouraud dextrose agar. From these plates, 9-mm circles of confluent fungal growth were removed with a cork borer and pressed in the centers of nine petri dishes, each containing 24 ml of congealing Sabouraud dextrose agar. Two-millimeter wells were cut in each of four quadrants on each plate. For each of the three hormones, three plates were used, with wells located 13 mm from the center on one plate, 18 mm from the center on the second, and 23 mm from the center on the third. On each plate, well 1 was filled with 0.2% ethanol; wells 2 through 4 were filled with 5×10^{-4}, 5×10^{-6}, and 5×10^{-8} M hormone, respectively. In some experiments, wells were recharged every 48 h to ensure that hormone effect had not been lost in the incubation process. Plates were wrapped in foil and incubated at 37°C for 7 days; plates were checked daily for evidence of growth inhibition or stimulation by the material in the wells.

Statistical methods. As noted above, geometric means were calculated as the measure of central tendency for covenience of presenting the results and because of a general familarity with interpretation of this presentation. Nevertheless, no assumptions were made about normal distributions of data, especially because of its proportional rather than arithmetic nature and because of the morphogenetic change in *C. immitis* from cylindrical arthroconidia to spherical endospores and spherules. Therefore, significance of the differences observed among the various experi-

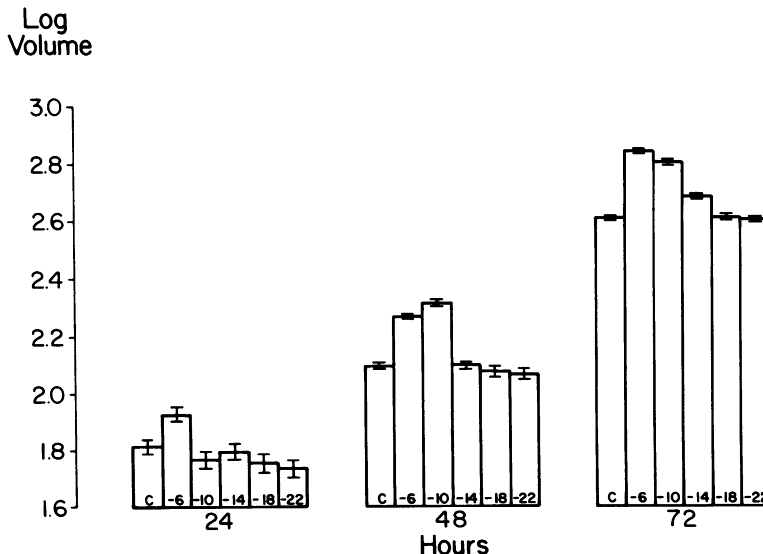

FIG. 2. *Effect of 17β-estradiol (10^{-6} to 10^{-22} M) on the growth of C. immitis strain C60 arthroconidia as a function of time. The ordinate in this and subsequent figures measures volume in micrometers cubed, expressed as the $\log_{10}$; $n = 100$ for all determinations. C, Control; -6 to -22, estradiol molarity to the base 10. Values shown are geometric means ± 2.58 × standard deviation (99% conficence limits). P values (versus control for each time period): 24 h (10^{-6} M), $P < 0.005$; 48 h (10^{-6} and 10^{-10} M), $P < 0.001$; 72 h (10^{-6} and 10^{-10} M), $P < 0.001$, (10^{-14} M), $P < 0.005$.*

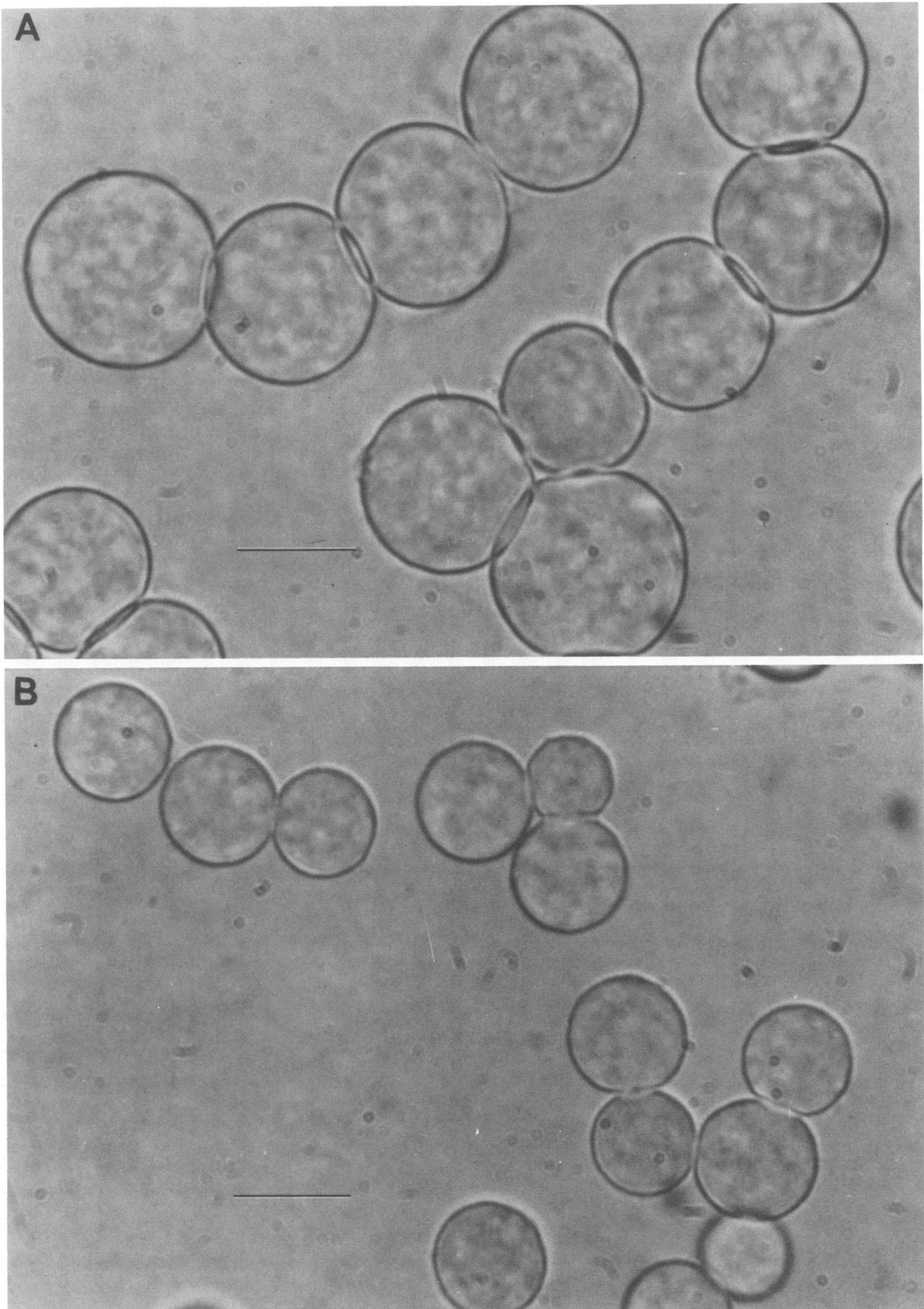

FIG. 3. *Comparative size of spherules developing from C. immitis strain C60 arthroconidia after 48 h of exposure to 17β-estradiol (10^{-6} M) (A) or the ethanol control (B). Photographs enlarged from an original magnification of 900×; bar, 10 μm.*

mental groups was tested by the nonparametric Mann-Whitney *U* test (46) rather than the *t* test.

For the data concerning maturation and release, results were analyzed by chi square for number of cells among 100 counted which had matured, or not matured, to endosporulating spherules.

RESULTS

C. immitis. Figure 2 shows the growth rate of strain C60 *C. immitis* in response to concentrations of 17β-estradiol ranging from 10^{-6} to 10^{-22} M. Growth enhancement was detectable at a minimum concentration of 10^{-6} M at 24 h, 10^{-10} M at 48 h, and 10^{-14} M at 72 h of observation. The size differential between control fungi and those exposed to 17β-estradiol was readily identifiable by microscope examination (Fig. 3). The size of hormone-stimulated fungi did not exceed that ultimately reached by control fungi, but hormone-stimulated *C. immitis* differed from control fungi by being persistently 24 to 48 h ahead in all stages of the fungal growth cycle.

The enhanced growth response of *C. immitis* appeared to be specific to those sterols with definite sex hormone activity. Preliminary experiments established that neither ergosterol nor cholesterol stimulated the growth of *C. immitis*. 17α-Estradiol (Fig. 4), a physiologically inactive stereoisomer of 17β-estradiol differing only in the orientation of a hydroxyl group (30), also had no effect on *C. immitis*. In contrast, both progesterone and testosterone were highly stimulatory for fungal growth.

Figure 5 examines the effect of 17β-estradiol and the nonsteroidal antiestrogens tamoxifen and nafoxidine (25, 29) on the rate of growth of arthroconidia and endospores of three strains of *C. immitis* as a function of time. 17β-Estradiol was highly stimulatory for the growth of endospores of all three strains and for the arthroconidia of C60 and C599. The effects of tamoxifen and nafoxidine were stimulatory, indifferent, or inhibitory, depending on the fungus, the duration of incubation, and whether arthroconidia or endospores contributed the starting inoculum. In the case of strain C599, nafoxidine at a concentration of 10^{-6} M was uniformly stimulatory for the growth of both arthroconidia and endospores ($P < 0.0075$). A dose-response curve involving C599 arthroconidia (Fig. 6) revealed a stimulatory effect for nafoxidine that was equivalent to that of 17β-estradiol over a range of 10^{-6} to 10^{-10} M. In contrast, tamoxifen was stimula-

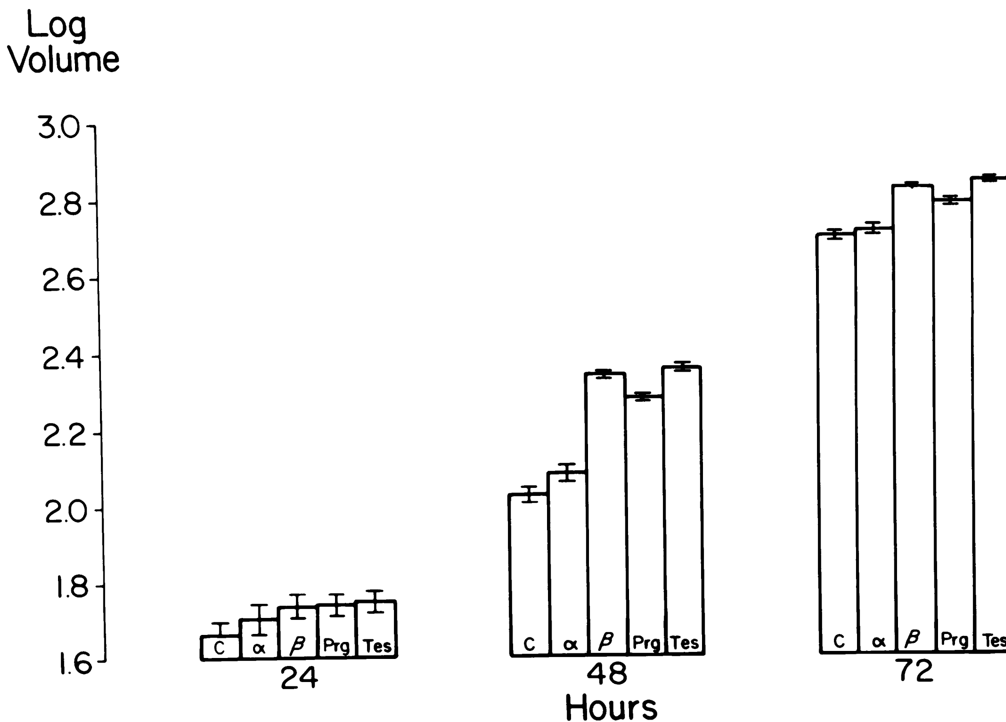

FIG. 4. *Growth response ($\log_{10}$ micrometers cubed) of C. immitis strain C60 arthroconidia to 17α-estradiol (α), 17β-estradiol (β), progesterone (Prg), and testosterone (Tes) (all at 10^{-6} M) as a function of time; $n = 100$ for all determinations. Values shown are geometric means ± 99% confidence limits. P values (versus control for each time period): 24 h (β ↑, $P = 0.05$; Prg ↑, $P = 0.04$; Tes ↑, $P = 0.005$); 48 and 72 h (β ↑, Prg ↑, Tes ↑, $P < 0.001$).*

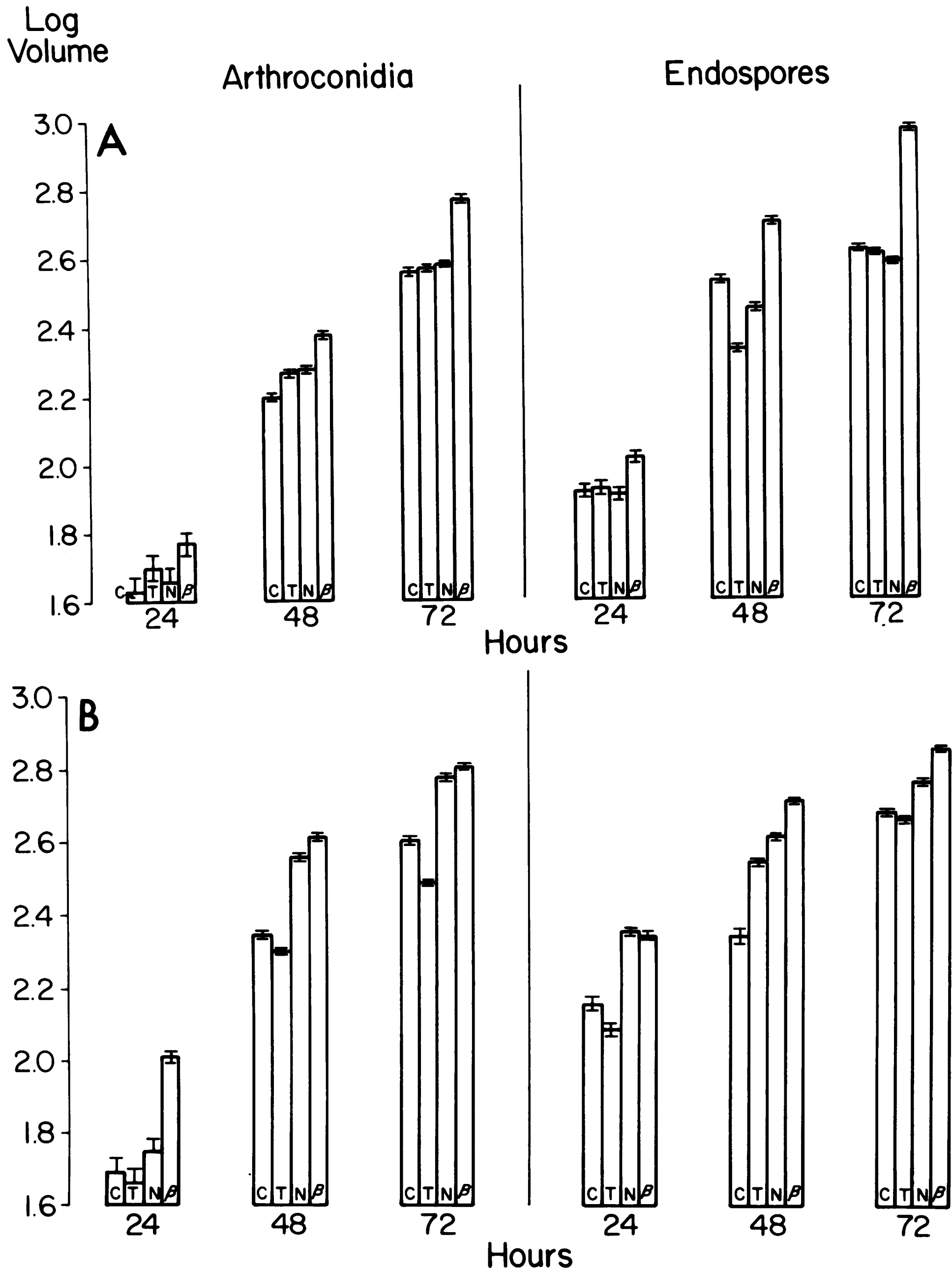

FIG. 5.

tory at 10^{-8} and 10^{-10} M, but inhibitory at 10^{-6} M.

Figure 7 shows the effect of estrogens and antiestrogens on spherule maturation (fungi reaching stage S in Fig. 1). Estradiol at 10^{-6} M stimulated spherule maturation from arthroconidia of C60 and from endospores of C566 and C599. Tamoxifen at 10^{-6} M inhibited fungal maturation from C60 and C566 arthroconidia, whereas nafoxidine stimulated maturation from C599 endospores.

To determine whether the estradiol effect was unique to the parasitic phase of fungal growth, studies were carried out with mycelial-phase *C.*

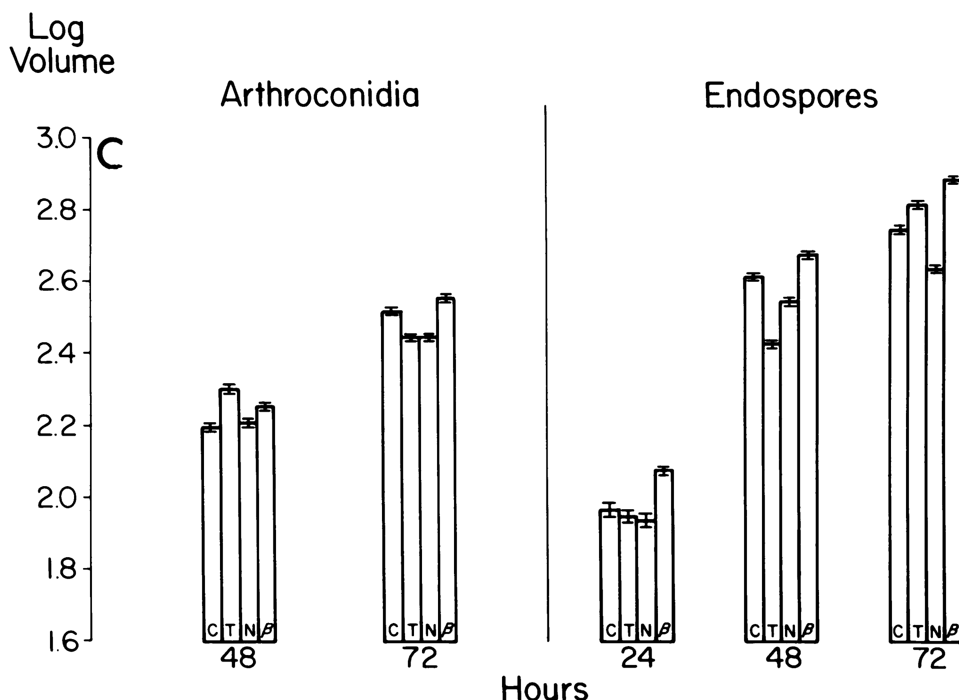

FIG. 5. *Growth response (log_{10} micrometers cubed) of C. immitis arthroconidia and endospores to tamoxifen (T), nafoxidine (N), and 17β-estradiol (β) (all at 10^{-6} M) as a function of time. Values shown are geometric means ± 99% confidence limits. (A) strain C60. P values (versus control for each time period): Arthroconidia (n = 100): 24 h (T ↑, P = 0.004; β ↑, P < 0.001); 48 h (T ↑, P = 0.086; N ↑, P = 0.014; β ↑, P < 0.001); 72 h (β ↑, P < 0.001). Endospores (n = 100): 24 h (β ↑, P < 0.001); 48 h (T ↓, β ↑, P < 0.001; N ↓, P = 0.028); 72 h (N ↓, P = 0.018; β ↑, P < 0.001). (B) Strain 599. P values (versus control for each experiment) were as follows. Arthroconidia (n = 20 to 100): 24 h (β ↑, P < 0.001); 48 h (N ↑, P = 0.004; β ↑, P < 0.001); 72 h (T ↓, P < 0.001; N ↑, β ↑, P < 0.001). Endospores (n = 100): 24 h (N ↑, β ↑, P < 0.001); 48 h (T ↑, P = 0.04; N ↑, β ↑, P < 0.001); 72 h (N ↑, P = 0.018; β ↑, P < 0.001). (C) Strain 566. P values (versus control for each period) were as follows. Arthroconidia (n = 50): 48 h (T ↑, P = 0.019). Endospores (n = 100): 24 h (β ↑, P = 0.003); 48 h (T ↓, P < 0.001); 72 h (T ↑, P = 0.003; N ↓, P < 0.001; β ↑, P < 0.001).*

immitis, using dry weight of the fungal mat as the indicator of cell growth. Estradiol stimulated the growth of C60 more than that of C566 (Fig. 8). Both fungi showed dose-dependent estradiol effects, but mycelial growth appeared to be less stimulated by higher concentrations of estradiol. Tamoxifen and nafoxidine were variably inhibitory for C60 and C566.

Other fungi. Tamoxifen, nafoxidine, and 17β-estradiol had no effect on the growth or development of any other fungi tested.

DISCUSSION

Under ordinary circumstances, men experience coccidioidal dissemination with a frequency four to six times that of women (14). This situation is drastically altered by pregnancy (54). The later in pregnancy that coccidioidomycosis is acquired, the more likely is dissemination to occur (23). The factors that predispose men and pregnant women to coccidioidal dissemination are not understood. Although animal studies have shown that treatment with estradiol or testosterone accelerates the rapidity with which death occurs from coccidioidomycosis, the authors' conclusion that the hormones acted by impairing host defense mechanisms was not directly substantiated (32).

An explanation commonly offered for the effect of pregnancy on coccidioidomycosis is depression of cell-mediated immunity. Immunological concomitants of pregnancy include depression of delayed-type skin test reactivity (15), impaired skin graft rejection (4), altered lymphocyte blastogenic responses (15, 26, 42, 56), and defective chemotaxis of phagocytic cells (34). These defects are considered to contribute to the process that prevents rejection of the antigenically foreign fetus.

Pregnant women are reputed to have increased susceptibility to paralytic poliomyelitis (5), herpes simplex type 2 genital infections (3),

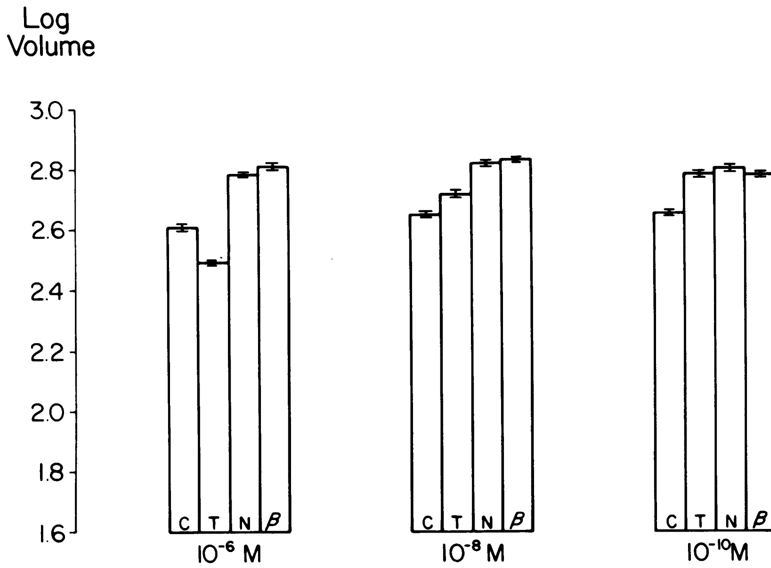

FIG. 6. *Effect of tamoxifen (T), nafoxidine (N), and 17β-estradiol (β) on the 72-h growth (log_{10} micrometers cubed) of C. immitis strain C599 arthroconidia as a function of molarity (10^{-6} to 10^{-10} M); $n = 100\text{-}200$ for all determinations. Values shown are geometric means ± 99% confidence limits. P values (versus controls for each molarity): 10^{-6} M (T ↓, $P < 0.001$; N ↑, β ↑, $P < 0.001$); 10^{-8} M (T ↑, $P = 0.025$; N ↑, β ↑, $P < 0.001$); 10^{-10} M (T ↑, N ↑, β ↑, $P < 0.001$).*

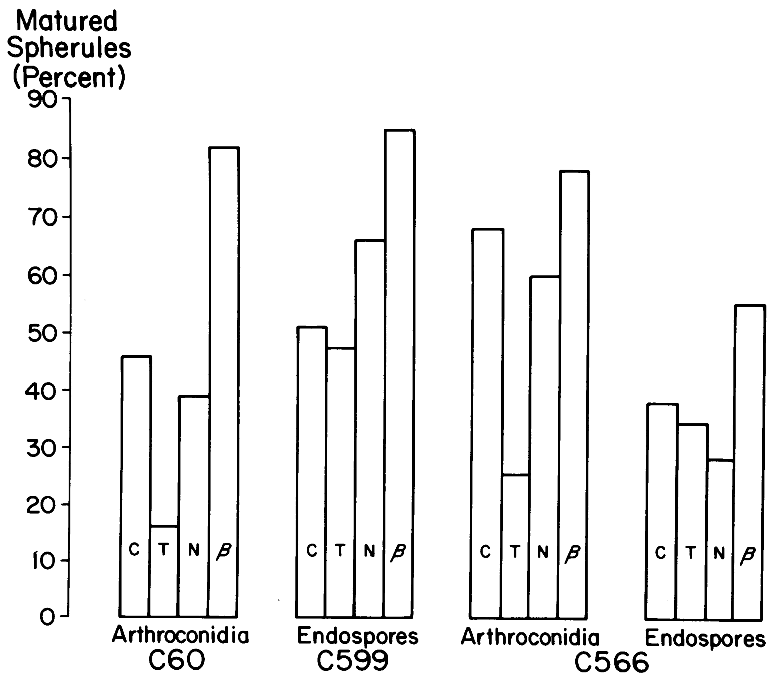

FIG. 7. *Percentage of spherules showing full maturation (stage S or stage S → H, Fig. 2) after 5 to 6 days of exposure to 10^{-6} M tamoxifen (T), nafoxidine (N), or 17β-estradiol (β); $n = 100$ for all determinations. P values (versus control for each time period): C60 arthroconidia (T ↓, $P < 0.001$; β ↑, $P < 0.001$); C599 endospores (N ↑, $P < 0.005$; β ↑, $P < 0.001$); C566 arthroconidia (T ↓, $P < 0.001$); C566 endospores (β ↑, $P < 0.001$).*

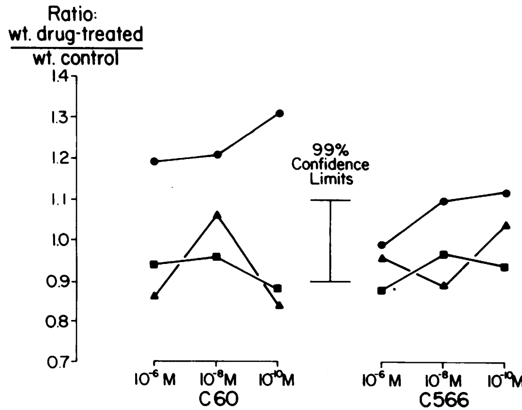

FIG. 8. *Effect of 17β-estradiol (●), tamoxifen (■), and nafoxidine (▲) on the growth of mycelial-phase C. immitis strains C60 and C566 as a function of molarity (10^{-6} to 10^{-10} M). Values are plotted as a ratio of dry weight of drug-exposed mycelial mat/dry weight control mycelial mat and are compared with the control value (= 1.0).*

influenza pneumonia (16), coxsackievirus B infection (41), hepatitis (41), chickenpox (41), smallpox (43), tuberculosis (22), vaginal candidiasis (6), group A beta-hemolytic streptococcal infection (50), and disseminated gonococcal infection (24). Only those associations pertaining to disseminated gonococcal infection, poliomyelitis, smallpox, vaginal candidiasis, and herpesvirus infections appear well established (22, 41). To whatever extent clinically significant suppression of immunity is encountered during pregnancy, there does not seem to be the sort of global susceptibility to opportunistic pathogens that characterizes more profound immune deficiency states, or that would be predictive of a markedly heightened predisposition to dissemination of coccidioidomycosis.

Because fungi are eucaryotic organisms with the capability of sexual reproduction, and because sex hormones, both steroidal and nonsteroidal, have been shown to play a role in the mating of saprophytic fungi (8, 19, 20, 28, 40, 51, 52, 55), we have explored the possibility that *C. immitis* might be stimulated directly by human sex hormones, perhaps through confusion with its own putative mating hormones. Estradiol, testosterone, and other mammalian sex hormones have been shown to influence the growth and development of plants, apparently successfully substituting for plant sex steroids (18).

Because the perfect form of *C. immitis* has never been identified, we had no way of knowing whether the strains we studied were positive or negative in terms of their sexuality (if this species should prove to be heterothallic). The three strains we used were selected at random, differing only in the sites from which they were isolated. Our studies indicate distinct stimulation of the rate of spherule growth and maturation, endosporulation, and endospore release by 17β-estradiol for all three strains of *C. immitis*. Stimulation was demonstrable with a starting inoculum of either arthroconidia (the form of the fungus that is inhaled and that initiates pulmonary infection) or endospores (the form that is released from mature spherules and that perpetuates or spreads infection within the host). More detailed studies with strain C60 arthroconidia revealed a threshold of stimulation at a concentration of 10^{-14} M with a dose-related response that extended through the physiological range of the normal nonpregnant female (7.35×10^{-13} to 2.43×10^{-10} M [2]) into the range of unconjugated estradiol encountered late in pregnancy (i.e., 3.68×10^{-7} to 8.82×10^{-7} M [45]).

The lack of stimulation of C60 arthroconidia by ergosterol, cholesterol, and 17α-estradiol indicated that there was great specificity to the hormonal action of 17β-estradiol and that the sterols were not being utilized merely as carbon or nitrogen sources in Converse medium (7). (Preliminary experiments with asparagine plus glucose supplementation of Converse medium had also failed to show a growth-stimulatory effect.) 17α-Estradiol is a naturally occurring hormone which is present in increased concentration during pregnancy (30). Although it has been considered to have relatively little physiological activity, recent studies indicate that it does possess estrogenic activity in vitro in the MCF-7 human breast cancer cell line (13). In the present studies, 17α-estradiol had no effect on *C. immitis* growth, suggesting that the fungus was capable of recognizing subtle differences in these otherwise identical stereoisomers of estradiol.

Studies with the nonsteroidal antiestrogenic compounds tamoxifen and nafoxidine over a range of 10^{-6} to 10^{-10} M failed to show a uniform pattern of response by *C. immitis*. However, there were two exceptions: (i) the growth and maturation of strain C599 arthroconidia and endospores were strongly stimulated by nafoxidine at all concentrations tested; and (ii) growth and maturation of all three strains of *C. immitis* were suppressed by tamoxifen at a concentration of 10^{-6} M. Antiestrogens are well known to manifest either antiestrogenic or estrogenic effects as a function of the system within which they are tested (25, 29). The stimulatory effect of nafoxidine for C599 indicates that the growth of at least some strains of *C. immitis* may be stimulated by compounds with estrogenic activity that are nonsteroidal in character.

Studies with testosterone and progesterone demonstrated a stimulatory effect on C60 arthroconidia equivalent to that of 17β-estradiol. The significance of these observations in terms of specificity of hormone action is unclear because fungi, like mammalian cells, are capable of carrying out biochemical transformations of steroid hormones (9, 57). Mammalian cells convert testosterone to estradiol (17), and it is possible that fungal cells could do the same. Because no data exist relative to the issue of steroid conversion by *C. immitis*, it is not possible on the basis of present data to be certain which hormone is actually responsible for stimulating the growth of the fungus in these studies.

The failure to demonstrate an effect of estradiol, tamoxifen, or nafoxidine on *C. neoformans*, *Candida* spp., or *P. boydii* indicates that our observations relative to *C. immitis* possess specificity. *P. boydii* was studied because a clinical case report had suggested some relationship between infection with this microorganism, the stage of the menstrual cycle, and the occurrence of pregnancy (38). *Candida* spp. were studied because of the strong association between pregnancy and vaginal candidiasis (6). *C. neoformans* was studied because it has been the subject of previous investigations relative to the antifungal activity of estrogenic compounds. In those studies, estradiol at a concentration of 0.05 μg/ml (1.84×10^{-7} M) had no effect on *C. neoformans*. However, at 1 μg/ml (3.68×10^{-6} M), estradiol was strongly inhibitory for the growth of seven strains of *C. neoformans* (39). Many steroids are inhibitory for the growth of diverse fungi at concentrations above 10^{-6} M (10, 11, 21, 44). In our studies, a concentration of 10^{-6} M 17β-estradiol was not exceeded, and no effect on *C. neoformans* growth was demonstrated.

In summary, these studies demonstrate that the rate of growth and maturation of *C. immitis* is intensely stimulated by selected human sex sterols. They do not indicate that 17β-estradiol, testosterone, and progesterone are the only hormones to which *C. immitis* is capable of response; the levels of many other sterol and protein hormones are elevated during pregnancy (17). Estradiol was selected for study because it is present at extremely high effective levels during pregnancy (33).

Our studies are compatible with the possibility that the propensity of pregnant women to develop coccidioidal dissemination may be related both to the suppression of cell-mediated immunity that characterizes late stages of pregnancy and to the stimulation of *C. immitis* by markedly elevated levels of estrogenic hormones. This stimulation is seen as an increased rate at which cells of *C. immitis* reach the stage of morphological differentiation to endosporulating spherules and increased number of fully matured endosporulating spherules. The sum effect of this accelerated spherule-endospore life cycle would be exposure of the pregnant patient to massive numbers of endospores released from the increased number of matured spherules. The extremely low concentration of estrogens effecting stimulation of *C. immitis* suggests the presence of receptors in this fungus. Additional experiments are being conducted to test this hypothesis.

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