INFECTION AND IMMUNITY, Sept. 1986, p. 498–504
0019-9567/86/090498-07$02.00/0

Vol. 53, No. 3

Hormonal Factors in Vaginal Candidiasis in Rats

OONAGH S. KINSMAN* AND ANGELA E. COLLARD

Chemotherapy Department, Glaxo Group Research, Greenford Road, Greenford, Middlesex UB6 OHE, United Kingdom

Received 6 February 1986/Accepted 14 May 1986

The hormonal status of rats affected vaginal infection with *Candida albicans*. Four hours after infection viable counts were higher and germ tubes were longer in those animals in estrous than in other animals. However, the infection was not maintained with the change in epithelial cell type which occurred as part of the estrous cycle. Estrogen dosing following ovariectomy predisposed toward infection, while progesterone dosing did not. In rats injected with progesterone, germ tube clumping was seen, leukocytes were present, and elimination occurred before hyphal growth was evident. In rats injected with estrogen, however, infection was maintained, with hyphal growth extending throughout the cornified epithelial layer. Vaginal washings from rats dosed with estrogen promoted elongation of germ tubes in vitro to a greater extent than washings from other rats. Preincubation of blastospores in progesterone and subsequent infection of rats in pseudoestrous promoted clumping of germ tubes in the vagina. Strains of *C. albicans* varied in their virulence, which correlated with their ability to produce germ tubes in vitro. Loss of virulence occurred on subculture of a clinical isolate.

Hormonal changes appear to predispose women to vaginal candidiasis, which is one of the most common gynecological complaints in Europe and North America. Thus, pregnancy seems an important predisposing factor (13); indeed, Hurley (6) recognized vaginal thrush as one of the most common infectious diseases of pregnancy. When pregnant and nonpregnant women were experimentally inoculated with *Candida albicans*, a greater incidence of infection occurred in the pregnant women (2). Also, vaginal carriage of candida is greater in pregnant women than in nonpregnant women (13). There have been conflicting reports regarding the role of oral contraceptives in vaginal candidiasis, possibly arising from the lack of attention to the particular type of pill used. Apisarnthanarax et al. (1) reviewed the contrasting views but also noted that the use of a combined estrogenic type of oral contraceptive was more commonly associated with candidiasis than the sequential type. With regard to recurrent *Candida* infections, little has been documented on the time of the recurrence in the menstrual cycle, but the infections tend to become clinically evident premenstrually (13).

The role that mammalian hormones may play in predisposing to *Candida* infections has not been elucidated. Moreover, there have been few investigations of the virulence of this organism with specific reference to vaginal infection. A rat model for vaginal candidiasis which was developed for chemotherapeutic purposes (12, 17, 18, 21) has recently been used to investigate the pathogenicity of candida in vaginal infections (20, 21). In the present study we used the rat model to further investigate the effect of host hormonal factors on infection and virulence differences between strains of *Candida*.

MATERIALS AND METHODS

Candida strains. *C. albicans* C316 was subcultured continuously for many years. *C. albicans* 2402E was a fresh isolate from a case of vaginal candidiasis that was subcultured only twice and maintained as a stock culture in liquid nitrogen [designated strain 2402E(N)]. Unless otherwise stated experiments were carried out with strain 2402E(N). Further subcultures of this strain once a week for 6 months were designated 2402E(S). Strain 2402E(V) was reisolated from the rat vagina 1 day after infection with strain 2402E(S).

Animals. Female CD rats (weight range, 150 to 200 g; Charles River Breeding Laboratories, Ltd., Margate, Kent, England) were used throughout the study. Ovariectomy was performed at least 1 week before infection. Hormone dosing was given subcutaneously at least 3 days before infection and weekly thereafter. Hormones included 1 mg of estradiol benzoate, 10 mg of progesterone, or both prepared in ethyl oleate (Paines and Byrne Ltd., Greenford, Middlesex, England). Ovariectomized animals dosed with estradiol were in pseudoestrous.

Experimental infection and subsequent collection of vaginal scrapings and washings. Blastospores, obtained by incubation on Sabouraud maltose agar at 37°C overnight were suspended in 5% sodium carboxymethyl cellulose. When blastospores were incubated in hormones prior to infection, 5×10^8 blastospores per ml were incubated at 37°C in distilled water, 0.1% methanol, 1 μM estrogen in 0.1% methanol, 1 μM progesterone in 0.1% methanol, or a combination of both hormones, and suspended in sodium carboxymethyl cellulose as described above. In further experiments (see below), distilled water was replaced with normal vaginal washings.

Each animal received 10^7 CFU of *C. albicans* intravaginally by inserting the end of a 1-ml syringe and discharging a 0.1-ml suspension.

Vaginal scrapings were taken with a 1-μl plastic loop and dispersed in a drop of distilled water on a slide. Samples were then air dried and stained by the Papanicolaou method (5). The stage of the estrous cycle was determined by the epithelial cell type and the presence or absence of leukoytes (20). Cornified epithelial cells present under estrous conditions stained orange, while noncornified cells present under diestrous stained green. Leukocytes were present only under diestrous conditions. The number of each type of cell in a scraping was scored (± to +++). For scanning electron microscopic examination, the scraping was placed in glutaraldehyde (12% [vol/vol] in phosphate buffer [pH 7.0]) for 1 h, and centrifuged at 1,500 × *g* for 5 min. A suspension of the pellet in one drop of buffer was placed on a cover slip, air

* Corresponding author.

TABLE 1. Effect of the rat estrous cycle on vaginal infection with *C. albicans* 2042E

Inoculum size (CFU) and rat no.	Phase of estrous cycle at infection	Log_{10} CFU/ml at 4 h	Mean germ tube length (μm)[a]	Predominance of leukocytes
10^6				
1	Estrous	4.85	33	
2	Estrous	4.41	34	
3	Late estrous	4.76	21	
4	Late estrous	4.20	36	
5	Early diestrous	3.76	16	+
6	Early diestrous	3.60	16	
7	Diestrous	2.56	15	
8	Diestrous	2.54	—[b]	+
9	Late diestrous	ND[c]	22	
10	Late diestrous	ND	—	+
10^7				
1	Early estrous	4.88	18	
2	Estrous	4.49	23	
3	Estrous	4.26	14	
4	Estrous	4.19	27	
5	Early diestrous	3.60	—	+
6	Early diestrous	2.18	—	+
7	Late diestrous	3.35	19	
8	Late diestrous	3.24	—	
9	Late diestrous	3.17	—	

[a] Mean of 25 germ tubes.
[b] —, not sufficiently numerous or absent.
[c] ND, Not detectable (<1.0).

dried, and subsequently coated for the scanning electron microscope.

Vaginal washings were obtained by introducing 1 ml of saline into the vagina with a syringe and collecting the expelled fluid. For viable count determinations, dilutions were plated on Sabouraud maltose agar. Results of previous work (7) have shown a direct correlation between counts obtained by vaginal lavage and counts obtained by macerating vaginal tissue, although the majority of organisms remained bound to vaginal epithelium when lavage took place. In this study results of experiments in which lavaged and nonlavaged rats were compared indicated that daily lavage did not reduce viable counts. To test the reproducibility of the sampling technique, 10 normal rats were injected with 6.2×10^7 CFU of *C. albicans* and immediately sampled. The mean log_{10} CFU/ml of washing was 6.60 ± 0.29 (standard deviation).

When vaginal washings were collected from uninfected animals for germ tube tests, the washings were taken on 5 consecutive days from groups of five rats, pooled within groups, centrifuged at 1,500 × *g* for 5 min to sediment epithelial cells, filtered with a Millipore filter, and stored at −20°C. The level of estriol in urine and vaginal washings was determined with a radioimmunoassay kit (Amerlex; Amersham International p.l.c., Amersham, Bucks, England).

Germ tube test. Mouse kidney extract was prepared by emulsifying 10 kidneys from adult mice in 5 ml of saline with a tissue grinder, followed by centrifugation at 5,000 × *g* for 5 min to remove cell debris. The homogenate was filter sterilized. Serum and kidney extracts were stored at −20°C prior to the test and were diluted in saline. Suspensions of blastospores were stored frozen in liquid nitrogen and diluted in the test medium or saline to give 2×10^6/ml. The percentage of germination at 37°C was determined with a hemocytometer, and germ tube length was measured with an eye-piece scale which was calibrated against a stage micrometer.

RESULTS

Effect of the rat estrous cycle on vaginal infection with *C. albicans*. Four hours after intravaginal infection with a blastospore suspension of *C. albicans* 2402E, higher counts were obtained in vaginal washings in those rats which were in estrous compared with those that were in diestrous (Table 1). The phase of the estrous cycle was indicated by the epithelial cell type in a Papanicolaou stain of a vaginal scraping. The rats with the highest counts showed orange-stained cornified epithelial cells and an absence of leukocytes, which is indicative of the estrous phase. Germinated blastospores were detectable in most scraping samples; however, the lengths of the germ tubes were longer in those rats in the estrous phase (Table 1). Clumping of the blastospores was evident in some rats that were in diestrous. Elimination of all infection occurred between 4 and 48 h after infection, as changes in the epithelium occurred with a concomitant influx of leukocytes (Table 2) as part of the estrous cycle. Although the cycle lasted for 4 to 5 days, cornified cells were present for less than half of the cycle.

Effect of ovariectomy followed by hormone treatment on infection. The hormone status of rats was artificially manipulated by ovariectomy followed by weekly injections of estrogen, progesterone, or both; and the infection was followed by viable counting of vaginal washings, microscopy of Papanicolaou smears, and histology of infected animals. In Fig. 1 are shown the viable counts detected in vaginal washings after an inoculum of 10^7 blastospores of *C. albicans* 2402E. Estrogen administration promoted maintenance of infection, while progesterone administration did not. The results of dosing with progesterone and estrogen were similar to estrogen dosing alone. Other experiments (data not shown) confirmed that infection was not maintained in those cases in which no hormone was given. Germ tubes developed in each rat group, but in the case of progesterone-dosed rats clumping of the germ tubes was seen (Fig. 2A), leukocytes were present (Fig. 2B), and elimination occurred before hyphal growth was evident. However, with estrogen-dosed rats infection was maintained, with hyphae extending throughout the epithelial layer (Fig. 3). When no hormone was given to ovariectomized rats, the cell type was variable. Epithelial scrapings examined by scanning electron microscopy revealed that in samples from rats given progesterone the coat of the blastospore appeared rough (Fig. 4); however, in samples from rats given estrogen, clumping did not occur and the blastospore appeared smooth.

Vaginal washings were pooled from uninfected rats receiving the same hormone treatments. Development of germ tubes in these washings was measured after 3 h of incubation in vitro with the same strain of *C. albicans* (2402E). In Table 3 are shown the values that were obtained. Although germ tubes developed in all groups, the washings from estrogen-dosed rats stimulated the elongation of the germ tubes to a greater extent than the other groups. Addition of 0.1 μM (27 ng/ml) estradiol to washings from normal rats did not increase the level of germination. By a radioimmunoassay no estriol was detectable (0- to 100-ng/ml range) in the vaginal washings of the estrogen-treated rats, although 3.7 ng/ml was detectable in the urine of these animals.

Effect of preincubation in hormones on the infectivity of *C. albicans* 2024E. Rats which were in pseudoestrous were infected with 10^7 blastospores which previously had been

TABLE 2. Effect of change in the estrous cycle on vaginal infection with *C. albicans* 2042E

Inoculum size and rat no.	Cell type prior to infection[a]	Changes at the following times (h) postinfection:					
		4		24		48	
		Cell type[a]	Log_{10} CFU/ml	Cell type[a]	Log_{10} CFU/ml	Cell type[a]	Log_{10} CFU/ml
10^6							
1	G+L+++	G+L++	2.54	G++O+L+	0	O+G+	0
2	O++G+	L++	4.76	O++G+	1.69	O+++	2.46
3	O+++G+	O++G+	4.41	O+G+	0	O+G+	0
4	O++G+	O+G+	3.60	G++O++L++	0	O+G+	0
5	G+L+++	G+L+++	3.76	G+L+	0	G+O+L+	0
6	O+++	O++G±	4.20	G+L+++	0	G++O++	0
7	G+++O±	G++O+	0	O+++G±	0	G+++O+L++	0
8	G++O++	O+++G±	4.85	G++L+++	0	O+G+L+	0
9	G+L+++	G+L+	0	G++O+L+	0	O++G+	0
10	G+++O±	G+	2.56	G±	0	O++G+	0
10^7							
1	O+++G+	O++	4.19	G+L+	0	G+O+	0
2	O++G+	O++G+	4.49	G++	0	G+	0
3	G++L++	G+L++	2.68	G+L+	0	O+G+	0
4	O++G+	O+	4.26	G+L+++	2.14	G+L+++	0
5	O++G+	O++	4.78	G+O+L+	0	G+L++	0
6	G+	G++O+	3.35	O++	2.25	G+L++	0
7	G++	G++	3.17	O++	2.91	G+L++	0
8	G+	G++	3.24	O++G+	2.60	G+L++	0
9	G+L++	G+L+++	3.60	G+L+	1.0	O++	0

[a] With the Papanicolaou stain, the presence of orange cells (O) predominant in estrous, green cells (G) predominant in diestrous, and leukocytes (L) present in diestrous was scored (± to +++).

incubated in distilled water or normal vaginal washings containing 1 μM estrogen, 1 μM progesterone, 1 μM oestrogen–1 μM progesterone, or the solvent control. Vaginal scrapings taken 4 h after infection revealed that preincubation in progesterone or progesterone–estrogen caused clumping of the blastospores in vivo. Such clumping was not evident in vitro. Scrapings from rats infected with blastospores which had been preincubated in estrogen showed germ tubes that were longer than those from other groups. There was an overall drop in viable counts between 4 and 24 h in each group, but in the comparison of preincubation in estrogen with preincubation in progesterone (Table 4) it was shown that greater elimination again occurred when progesterone was present.

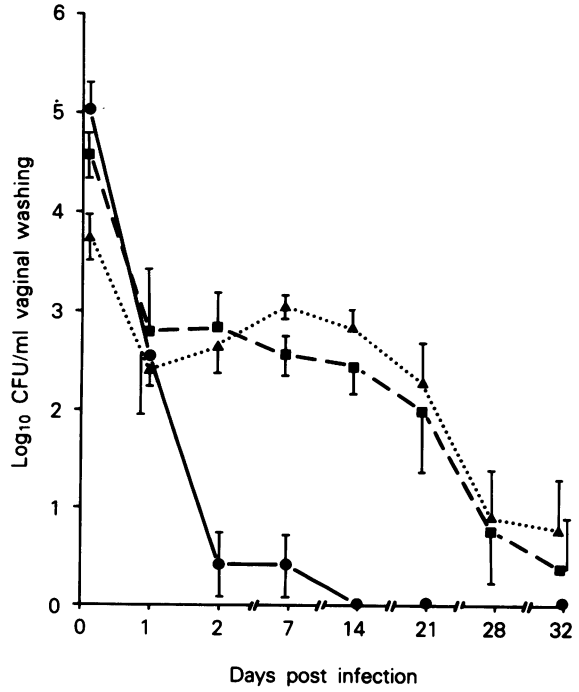

FIG. 1. Effect of hormone treatment on candida vaginitis in the rat. Symbols ▲, estrogen treatment; ●, progesterone treatment; ■, treatment with estrogen and progesterone. Counts represent the mean of five rats per group (± standard error).

Variation in virulence of *C. albicans* strains in rat vaginitis. A subcultured strain (C316) and a recent isolate (2402E) were compared for their ability to produce vaginitis following inoculation of 10^7 blastospores into rats in pseudoestrous. Viable count results are shown in Fig. 5. C316 was eliminated in all rats by 2 weeks after infection, while 2402E was not. When the two strains were compared for their ability to produce germ tubes in vitro, it was found that the more virulent strain (2402E) produced germ tubes more readily than the less virulent strain (C316) (Table 5). Vaginal washings taken from rats in pseudoestrous induced a low level of germination in both strains after 2 h of incubation, but again, 2402E gave a higher result (5.6%) than C316 (3.2%).

In Fig. 6, however, is shown the effect of subculturing on the virulence of *C. albicans* 2402E. Virulence was reduced in the continuously subcultured strain [2420E(S)], and the virulence of the original isolate was not regained by one passage of the subcultured strain [2402E(V)] in the rat vagina. Rats sacrificed for histology 11 days after infection showed that hyphae were most evident in rats infected with the minimally subcultured strain 2402E(N). Interestingly, when a small amount of infection remained with the other two strains, the hyphae often only remained within the epithelial layers in the folds of the vagina high up, near the vaginal fornix.

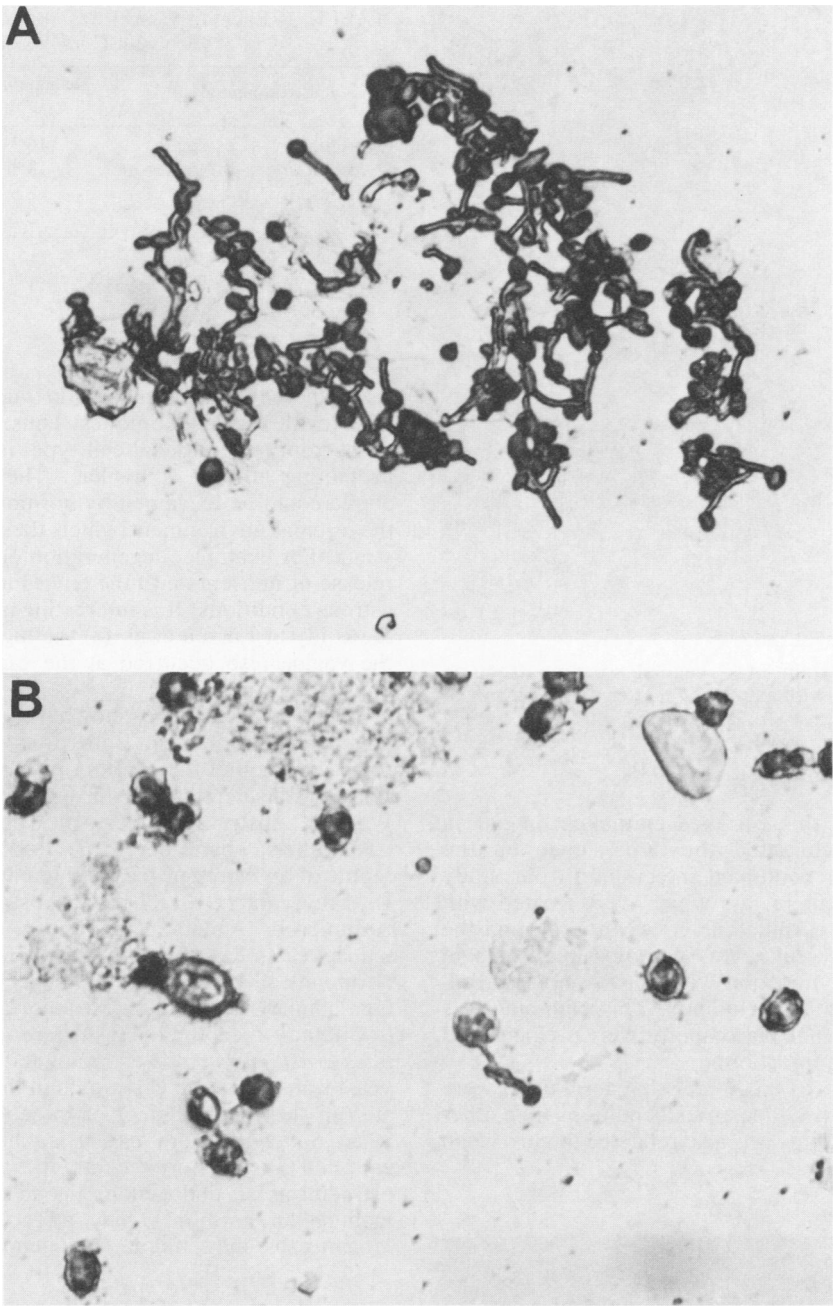

FIG. 2. Papanicolaou stain of a vaginal scraping from an ovariectomized rat treated with progesterone 4 h after infection with *C. albicans* showing the presence of clumped germ tubes (A) and the presence of leukocytes (B). Magnification, ×472.

All three strains were investigated for their ability to produce germ tubes in vitro. The percentage of germination was as follows: *C. albicans* 2402E(N), 90%; 2402E(S), 21%; 2402E(V), 17%. Again, the strain with the greatest ability to produce germ tubes in vitro was the most virulent in vivo.

DISCUSSION

Results of this study indicate that host factors in the rat which predispose toward vaginal infection with *C. albicans* include the presence of a cornified epithelium and the absence of leukocytes. These conditions are present at estrous or with artificial manipulation involving ovariectomy and the administration of estrogen. A predisposition to candida infection in animals given estrogen has been noted before in mice (9, 24) and in rats (7, 18), and has been explained by greater adherence of blastospores to vaginal cells from animals in estrous or pseudoestrous in vivo (9, 19) and in vitro (J. D. Sobel and G. Muller, Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 693, 1984). In the present study we have identified an additional factor, namely, that maintenance of infection is only possible when yeast hyphal elements penetrate the cornified layer and thus are not readily eliminated with cell turnover. Sobel et al. (23)

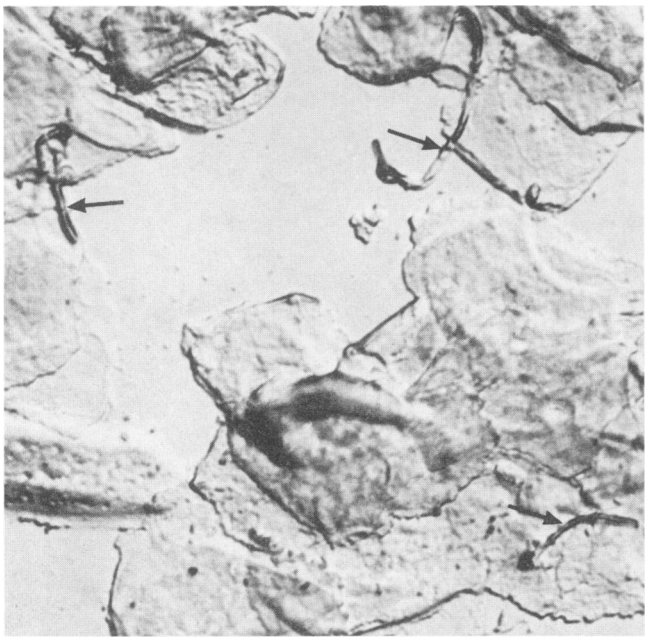

FIG. 3. Papanicolaou stain of a vaginal scraping from an ovariectomized rat treated with estrogen 4 h after infection with *C. albicans*. Magnification, ×472. The arrows point to hyphal elements.

also recently described this cornified epithelium in rats in pseudoestrous and highlighted the dependence on the pseudoestrous state for continued infection. In our study penetration did not occur in rats which were treated with progesterone when the cornified layer was absent, and the removal of candida by leukocytes was possible. Also in these animals, 4 h after injection with blastospores, germinated cells were present but clumped. This clumping was again observed in vivo when blastospores were preincubated in progesterone prior to infection.

Of particular interest was the finding that germ tubes were longer under estrous or pseudoestrous conditions than other hormonal states and that this was also reflected in vitro when

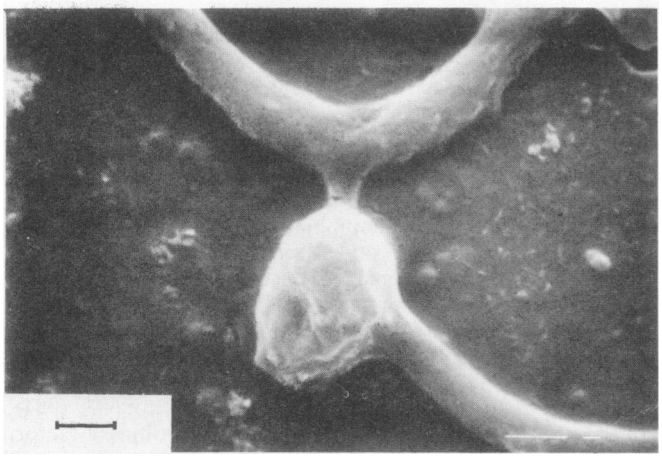

FIG. 4. Scanning electron micrographs of blastospores taken from the infected rat vagina in animals treated with progesterone. Bar, 1 μm.

TABLE 3. Effect of vaginal washings on germ tube formation in vitro with *C. albicans* 2402E

Rat treatment group	Mean germ tube length (μm) ± SE[a]	% Germination (range)[b]
Ovariectomy + estrogen	16.5 ± 0.6[c]	11.2 (7–17)
Ovariectomy + progesterone	13.1 ± 0.5[c]	14.0 (12–17)
Ovariectomy	8.9 ± 0.3	3.7 (3–5)
Normal	12.5 ± 0.5	11.5 (18–14)

[a] Mean of 100 germ tubes.
[b] Based on four estimations of 100 cells per estimation.
[c] $P < 0.05$ by Student's t test.

blastospores were germinated in vaginal washings from rats dosed with various hormones. Thus, the microenvironment, apart from the epithelial cell type, is an important factor in promoting growth of hyphae. The relationships reported above could be explained by hormonally related changes in the vaginal environment which then affect the *C. albicans* strain. For example, degeneration of cells with concomitant release of nutrients into the environment could occur under estrous conditions. It is interesting in this connection that in the study of Larsen et al. (8) the highest bacterial counts in the vagina also occurred at the same time as the estrous phase in the rat.

Recent studies have shown that *C. albicans* can bind mammalian hormones. Both progesterone and corticosterone were bound to a protein in the cytosol (10, 14), while Wagner considered progesterone to coat the yeast cell (G. E. Wagner, Abstr. 23rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1037, 1983). There has been one report of an estrogen receptor in candida (B. L. Powell, C. L. Frey, and D. J. Drutz, Abstr. 23rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 751, 1983). Other yeasts have also been shown to bind to mammalian hormones. *Coccidiodes immitis* has been reported to have a high-affinity receptor for estradiol and progesterone and a low-affinity receptor for testosterone (15, 16). *Paracoccidiodes brasiliensis* binds estradiol and inhibits the mycelial to yeast transformation (11) which in this organism is the initial step in the establishment of infection. The nonpathogenic yeast *Saccharomyces cerevisiae* has been found to bind estradiol (3), and more recently these authors have described estradiol as an endogenous ligand of this yeast (4). Thus, mammalian hormones may affect the metabolism of *C. albicans* or may mimic endogenous steroid compounds

TABLE 4. Effect of preincubation of blastospores in hormones prior to infection of rats in pseudoestrous

Blastospore treatment	Log_{10} CFU/ml of vaginal washing at [a]:	
	4 h	24 h
Preincubation in:		
1 μM estrogen in 0.1% methanol	3.95 ± 0.12	3.04 ± 0.13
1 μM progesterone in 0.1% methanol	3.83 ± 0.32	1.85 ± 0.46
Normal vaginal washings containing:		
1 μM estrogen in 0.1% methanol	4.73 ± 0.18	3.48 ± 0.05
1 μM progesterone in 0.1% methanol	4.15 ± 0.43	1.90 ± 0.95

[a] Mean ± standard error of five rats.

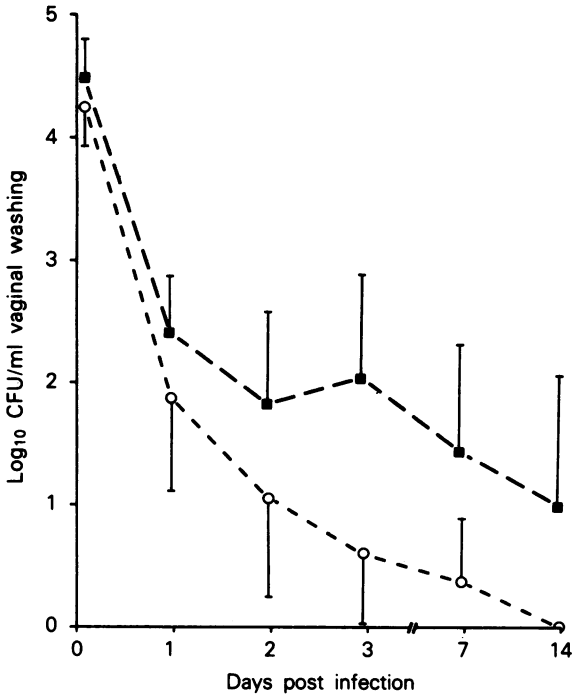

FIG. 5. Comparison of *C. albicans* C316 (○) and *C. albicans* 2402E (■) in inducing vaginitis in the rat. Counts represent the mean of seven rats per group (± standard error).

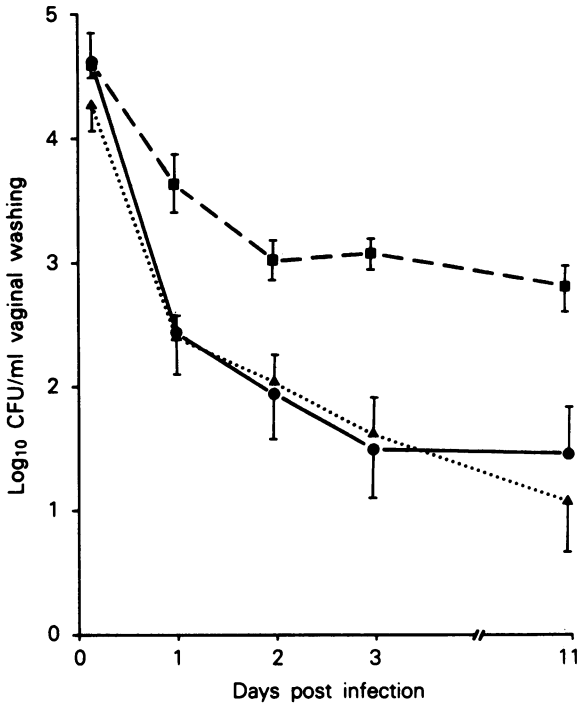

FIG. 6. Effect of subculture on the virulence of *C. albicans* 2402E in experimental rat vaginitis. Symbols: ■, *C. albicans* 2402E(N); ●, *C. albicans* 2402E(S); ▲, *C. albicans* 2042E(V). Counts represent the mean of 10 rats per group (± standard error).

involved in metabolic control, or the hormones may induce a host metabolite which then affects the *C. albicans* strain. We could not detect any direct effect of the hormones on the *C. albicans* strain, but it is possible that a steroid transformation occurred in vivo which was then active on the *C. albicans* strain. Thus, there appears to be a relationship between hormonal factors, the microenvironment, and the pathogenic process; and further investigations of this relationship are important.

The rat model of vaginal candidiasis described here detected differences in the virulence in strains of *Candida*. Subculturing in vitro resulted in the selection of less-virulent organisms, and this was correlated with a reduction in the ability to produce germ tubes in vitro. This supports the work of Sobel et al. (21, 22), who have described the capacity to produce hyphae as an important virulence factor in the pathogenesis of candidal vaginitis. Patients with symptomatic vaginitis almost always have germinated organisms in their secretions. Further basic research into pathogenic mechanisms may elucidate the likely factors involved in the transformation from the commensal to the pathogenic state in humans.

TABLE 5. Production of germ tubes in vitro by *C. albicans* C316 and *C. albicans* 2402E

Growth medium	% Germination in the following strains:			
	C316		2024E	
	1 h	2 h	1 h	2 h
20% Normal human serum	0.5	5.5	25	28
20% Normal mouse serum	0.5	2.5	25	54
20% Mouse kidney extract	0	1.5	23.5	67

ACKNOWLEDGMENTS

We thank P. Acred for constant interest and H. Smith for helpful discussions. Technical assistance was ably provided for by Mary-Anne Malone.

LITERATURE CITED

1. **Apisarnthanarax, I., S. J. Self, G. Ramer, and L. F. Montes.** 1974. Oral contraceptives and candidiasis. Cutis **14**:77–82.
2. **Bland, P. B., A. E. Rakof, and I. J. Pincus.** 1937. Experimental vaginal and cutaneous moniliasis. Clinical and laboratory study of certain monilias associated with vaginal and cutaneous thrush. Arch. Dermatol. Syphilol. **36**:760–780.
3. **Feldman, D., Y. Do, A. Burshell, P. Stathis, and D. S. Loose.** 1982. An estrogen binding protein and endogenous ligand in *Saccharomyces cerevisiae*. Possible hormone receptor system. Science **218**:297–298.
4. **Feldman, D., L. G. Tokes, P. A. Stathis, S. C. Miller, W. Kurz, and D. Harvey.** 1984. Identification of 17β-estradiol as the estrogenic substance in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **81**:4722–4726.
5. **Gurr, E.** 1962. Staining animal tissues: practical and theoretical, 340–343. Leonard Hill, London.
6. **Hurley, R.** 1975. Inveterate vaginal thrush. The Practitioner **215**:753–756.
7. **Larsen, B., and R. P. Galask.** 1984. Influence of estrogen and normal flora on vaginal candidiasis in the rat. J. Reprod. Med. **29**:863–868.
8. **Larsen, B., A. J. Makovetz, and R. P. Galask.** 1977. Relationship of vaginal cytology to alterations of the vaginal microflora of rats during the estrous cycle. Appl. Environ. Microbiol. **33**:556–562.
9. **Lehrer, N., E. Segal, and L. Barr Nea.** 1983. *In vitro* and *in vivo* adherence of *C. albicans* to mucosal surfaces. Ann. Microbiol. (Paris) **134B**:293–306.
10. **Loose, D. S., D. A. Stevens, D. J. Schurman, and D. Feldman.** 1983. Distribution of a corticosteroid binding protein in candida and other fungal genera. J. Gen. Microbiol. **129**:2379–2385.
11. **Loose, D. S., E. P. Stover, A. Restrepo, D. A. Stevens, and D.**

Feldman. 1983. Estradiol binds to a receptor like cytosol binding protein and initiates a biological response in *Paracoccidiodes brasiliensis*. Proc. Natl. Acad. Sci. USA **80**:7659–7663.
12. **McRipley, R. J., R. A. Schwind, P. J. Erhard, and R. R. Whitney.** 1979. Evaluation of vaginal antifungous formulations *in vivo*. Postgrad. Med. J. **55**:648–652.
13. **Odds, F. C.** 1979. Candida and candidosis. Leicester University Press.
14. **Powell, B. L., and D. J. Drutz.** 1983. Confirmation of corticosterone and progesterone binding activity in *C. albicans*. J. Infect. Dis. **147**:359.
15. **Powell, B. L., and D. J. Drutz.** 1984. Identification of a high affinity binder for estradiol and a low affinity binder for testosterone in *Coccidiodes immitis*. Infect. Immun. **45**:784–786.
16. **Powell, B. L., D. J. Drutz, M. Huppert, and S. H. Sun.** 1983. Relationship of progesterone and estradiol binding proteins in *Coccidiodes immitis* to coccidiodal dissemination in pregnancy. Infect. Immun. **40**:478–485.
17. **Ryley, J. F., R. G. Wilson, M. B. Gravestock, and J. P. Poyser.** 1981. Experimental approaches to antifungal chemotherapy. Adv. Pharm. Chemother. **18**:49–176.
18. **Scholer, H. J.** 1960. Experimentelle vaginal candidiasis der ratte. Path. Microbiol. **23**:62–68.
19. **Segal, E., A. Soroka, and N. Lehrer.** 1984. Attachment of candida to mammalian tissues. Clinical and experimental studies. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **257**:257–265.
20. **Short, D. J., and D. P. Woodnott.** 1969. The I.A.T. manual of laboratory animal practice and techniques. Crosby Lockwood, London.
21. **Sobel, J. D., and G. Muller.** 1984. Comparison of itraconazole and ketoconazole in the treatment of experimental candidal vaginitis. Antimicrob. Agents Chemother. **26**:266–267.
22. **Sobel, J. D., G. Muller, and H. R. Buckley.** 1984. Critical role of germ tube formation in the pathogenesis of candidal vaginitis. Infect. Immun. **44**:576–580.
23. **Sobel, J. D., G. Muller, and J. F. McCormick.** 1985. Experimental chronic vaginal candidosis in rats. Sabouraudia J. Med. Vet. Mycol. **23**:199–206.
24. **Taschdjian, C. L., F. Reiss, and P. J. Kozinn.** 1960. Experimental vaginal candidiasis in mice, its implications for superficial candidiasis in humans. J. Invest. Derm. **34**:89–93.