A GROWING CAMPAIGN AGAINST PARACOCCIDIOIDOMYCOSIS[1]

The imposing name "paracoccidioidomycosis" belongs to a dangerous mycotic infection endemic to South and Middle America. Caused by the fungus Paracoccidioides brasiliensis, *the disease is difficult to treat and apt to recur years later in patients who have been clinically cured. It also presents a number of puzzles for researchers that six decades of investigation have left unsolved. Recognizing the need for an active forum on this subject, the Pan American Health Organization and the University of Antioquia, Colombia, sponsored the First Symposium on Paracoccidioidomycosis, which was held in Medellín, Colombia, on 25-27 October 1971. The two articles appearing on the following pages are based on papers presented at that meeting. Other contributions to the Symposium are contained in the volume* Paracoccidioidomycosis *published by the Pan American Health Organization.*[2]

EFFECT OF STEROID HORMONES ON THE GROWTH OF *PARACOCCIDIOIDES BRASILIENSIS*[3, 4]

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Growth of P. brasiliensis, *the agent of paracoccidioidomycosis, is strongly inhibited by high concentrations of the steroid sex hormone estradiol. This finding does not imply that estrogen causes the relatively low incidence of paracoccidioidomycosis in women by inhibiting* P. brasiliensis *growth; but it does support evidence that estrogen inhibits other types of fungal growth and shows the need for further investigation of this process.*

Introduction

The great preponderance of male patients is a striking feature of the published reports on proven cases of paracoccidioidomycosis. Of 1,601 patients in four studies, 1,485 were males and 116 were females—a ratio of 13.2:1 (*1, 3, 10, 13*). This circumstance is usually attributed to the fact that men are more frequently exposed to the etiologic agent in the course of their work. However, the incidence of delayed hypersensitivity to intradermal paracoccidioidin is the same in the two sexes. Restrepo and her colleagues (*14*), testing nearly 4,000 subjects, found that 9.9 per cent of the males and 9.4 per cent of the females reacted to paracoccidioidin. This slight difference is not statistically significant at the 5 per cent confidence level.

Investigating the possible role of factors other than exposure to explain the greater frequency of the disease in males, we cultured seven isolates of *Paracoccidioides brasiliensis* in the presence of steroid sex hormones to determine whether or not these substances exert any effect on the growth of this fungus *in vitro*.

Materials and Methods

P. brasiliensis isolates were obtained from

[1] Also known as South American blastomycosis.

[2] *Scientific Publication PAHO 254* (1972). For further information see p. 41.

[3] Paper presented at the First Pan American Symposium on Paracoccidioidomycosis held at Medellín, Colombia, on 25-27 October 1971. Published in English in *Scientific Publication PAHO 254* (1972), pp. 300-304. Also appearing in Spanish in *Boletín de la Oficina Sanitaria Panamericana*, Vol. LXXV (1973).

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Dr. Libero Ajello and Dr. Angela Restrepo M. Multiple budding in the yeast phase was verified in all seven isolates used. The test procedures were carried out on brain-heart infusion agar (BHIA) by Difco, and cultures were maintained on this same medium.

Pure, non-esterified steroid hormones—estradiol, testosterone, and progesterone—were obtained from the Upjohn Co., Kalamazoo, Michigan, USA. Each of these was in naturally-occurring, physiologically active form. Cholesterol and diethylstilbestrol were also tested.

These substances were dissolved in methanol to a concentration of 4 mg/ml. Appropriate amounts of the resulting stock solutions were then added to the sterile BHIA, and slants were made. Final concentration of steroids varied from 0.05 to 10 μg/ml, but the methanol concentration was held constant at 0.25 ml per 100 ml of medium.

Inocula were prepared from each *P. brasiliensis* isolate by transferring several loopfuls of a vigorously growing yeast-phase culture to 5.0 ml of the BHIA in a screw-capped tube containing several glass beads. A uniform, turbid suspension of approximately 10^5 viable particles per ml was obtained by vigorous agitation with a Vortex® mixer, and one drop (0.05 ml) of the suspension was used to inoculate each agar slant. All test concentrations and controls were inoculated in triplicate, and each isolate was completely tested on five separate occasions.

Duplicate sets of each test series were incubated at 37° and 25°C. Tubes were inspected daily for 14 days, and growth was recorded as confluent (4), heavy (3), moderate (2), sparse (1), or none (0). Similar studies on isolates of *Histoplasma capsulatum* were also carried out, and these will be briefly mentioned below.

Results

The effect of the steroid hormones and other substances on the growth of *P. brasiliensis* at 25° and 37°C are summarized in Tables 1 and 2. Estradiol and diethylstilbestrol in concentrations of 10 μg/ml completely inhibited the growth of all seven isolates at both 25° and 37°C. Cholesterol, in the same concentration, showed no effect on the growth of any of the isolates. The effects of progesterone varied in the different isolates: at 25°C, isolate 4 was most inhibited, numbers 2, 3, 6, and 7 were partly inhibited, and numbers 1 and 5 were not inhibited at all; for all isolates, including numbers 1 and 5, the inhibitory effect on the yeast

TABLE 1—Growth of *Paracoccidioides brasiliensis* at 25°C on steroid-containing media.

Test substance	Concentration	Isolate number						
		1	2	3	4	5	6	7
None (control)	0	4	4	4	3	4	4	4
Methanol (control)	0.25%	4	4	4	3	4	4	4
Cholesterol	10 μg/ml	4	4	4	3	4	4	4
Testosterone	"	4	4	4	2	4	4	4
Estradiol	"	0	0	0	0	0	0	0
Stilbestrol	"	0	0	0	0	0	0	0
Progesterone	"	4	3	3	1	4	3	2

4 = confluent growth
3 = heavy "
2 = moderate "
1 = sparse "
0 = no "

TABLE 2–Growth of *Paracoccidioides brasiliensis* at 37°C on steroid-containing media.

Test substance	Concentration	Isolate number						
		1	2	3	4	5	6	7
None (control)	0	4	4	4	3	4	4	4
Methanol (control)	0.25%	4	4	4	3	4	4	4
Cholesterol	10 μg/ml	4	4	4	3	4	4	4
Testosterone	"	4	3	3	2	4	4	3
Estradiol	"	0	0	0	0	0	0	0
Stilbestrol	"	0	0	0	0	0	0	0
Progesterone	"	2	3	3	1	3	2	2

phase (at 37°C) was somewhat more pronounced than that on the mycelial phase (at 25°C). Testosterone showed very little effect: isolates 2, 3, 4, and 7 showed a slight reduction of growth at 37°C, while only isolate 4 showed any inhibition at all at 25°C. However, number 4 never exhibited as rapid or as vigorous growth as did the other isolates, either on the BHIA or on any of the several other media that were tested. Essentially similar results were obtained with *H. capsulatum* isolates: estradiol and stilbestrol inhibited all growth at 10 μg/ml, and neither testosterone or cholesterol at this concentration had any effect.

The effects of lower concentrations of estradiol, stilbestrol, and progesterone on the growth of two of the *P. brasiliensis* isolates are shown in Table 3. The minimum inhibitory concentration (MIC) of estradiol for both isolates lay between 5 and 10 μg/ml. Stilbestrol was slightly more inhibitory, showing an MIC between 1 and 5 μg/ml. Progesterone, again, was somewhat less inhibitory than either estradiol or stilbestrol. With all the substances the

TABLE 3–Growth of *Paracoccidioides brasiliensis* with various concentrations of steroid substances.

Test substance	Concentration	Isolate 6		Isolate 7	
		25° C	37° C	25° C	37° C
None (control)	0	4	4	4	4
Methanol	0.25%	4	4	4	4
	1.0 "	4	3	3	3
	2.5 "	3	2	2	1
	5.0 "	0	0	0	0
Estradiol	1 μg/ml	4	4	4	3
	5 "	0	2	2	1
	10 "	0	0	0	0
Stilbestrol	1 μg/ml	2	4	2	1
	5 "	0	0	0	0
	10 "	0	0	0	0
Progesterone	1 μg/ml	4	3	4	4
	5 "	2	3	2	1
	10 "	0	2	0	0

— =minimal inhibitory concentration (MIC).

inhibitory effect was slightly greater on mycelial-phase growth (25°C) than on yeast-phase growth (37°C).

Discussion

The foregoing results indicate that the natural female hormones estradiol and progesterone have a certain inhibitory effect on the *in vitro* growth of *P. brasiliensis* and *H. capsulatum*, and that their inhibitory activity is greater than that of testosterone or cholesterol under the same conditions. Stilbestrol, a synthetic compound that has estrogenic activity, demonstrated greater inhibitory effects than any of the other substances tested.

The concentrations required to produce the *in vitro* inhibitory effects reported here are much higher than those that occur in the human body. We do not suggest or imply that the simple inhibitory effects of natural estrogen are responsible for the low incidence of clinical paracoccidioidomycosis reported in females.

Whereas a breakdown by sex of 484 histoplasmosis patients (*7, 9, 12*) plus the 1,601 paracoccidioidomycosis patients mentioned earlier (*1, 3, 10, 13*) shows that males far outnumber females, tabulations of large numbers of results from paracoccidioidin (*14*) and histoplasmin (*2, 11, 14*) skin-test surveys indicate that the proportion of male to female reactors is not significantly different at the 5 per cent confidence level. These figures are summarized in Table 4.

If the demonstration of delayed hypersensitivity to these two sensitins is considered an indicator of infection in the human test subject, then male-to-female infection ratios for these fungi may be calculated and compared to the clinical disease ratio for the two sexes. Table 5 shows these two ratios for paracoccidioidomycosis and histoplasmosis. It can be seen that the infection ratio of males to females is very similar for both the fungal agents, suggesting that contact or exposure is also quite similar, or the same, for the two sexes. On the other hand, the preponderance of males among patients with clinically recognizable disease leads us to believe that the famele possesses considerably greater resistance to *P. brasiliensis* and *H. capsulatum* than does the male.

TABLE 5—Fungal infection and disease ratios in males and females.

Fungus	Infection ratio[a] male:female	Disease ratio male:female
P. brasiliensis	1.05:1	13:1
H. capsulatum	1.13:1	11:1

[a] Based on positive skin test.

The reasons for this apparently greater resistance in females to the fungal diseases in question are undoubtedly based on many complex and interrelated factors. The investigations of Nicol and co-workers (*8*) demonstrated differences in efficacy in phagocytes derived from male and female animals, which led the

TABLE 4—Delayed hypersensitivity to fungal sensitins in males and females.

Sensitin	Test subjects		
	Total No. tested	No. positive	% positive
Paracoccidioidin[a]			
Male	1775	177	9.9
Female	2163	204	9.6
Histoplasmin[b]			
Male	9125	3414	37.4
Female	7739	2554	33.0

[a] Colombia (difference not significant).

[b] Colombia and USA (difference not significant).

authors to propose that these phagocytic differences, and hence resistance to experimental bacterial disease, are related to estrogen: androgen ratios in the animals. Landay *et al.* (*4*) demonstrated in hamsters that females are significantly more resistant than males to experimentally induced disseminated blastomycosis, and they speculated that sex may therefore play a role in the morbidity picture of this disease in humans.

We have previously reported (*5, 6*) that the growth of *Allescheria boydii* and several *Aspergillus* species are inhibited by estradiol and stilbestrol, and in concentrations lower than those reported here for *P. brasiliensis* and *H. capsulatum.* These findings, together with the results of the present studies, would suggest that the inhibition of fungal growth by estrogen may be a widespread phenomenon.

We do not offer our demonstration of small inhibitory effects of estradiol and progesterone on *P. brasiliensis* and *H. capsulatum* as an explanation for the remarkable differences in sex incidence of paracoccidioidomycosis or histoplasmosis, but we do suggest that further study of all aspects of this question is justified.

SUMMARY

Despite evidence that roughly equal numbers of men and women are exposed to paracoccidioidomycosis, the bulk of those developing the disease seem to be males. To investigate possible reasons for this difference, the authors cultured seven isolates of *Paracoccidioides brasiliensis* in media containing the steroid sex hormones testosterone, estradiol, and progesterone, as well as diethylstilbestrol and cholesterol. Similar experiments were also conducted with *Histoplasma capsulatum,* the agent of histoplasmosis.

The results showed that estradiol and diethylstilbestrol strongly inhibited growth of both organisms at concentrations well above those found in the human body. This should not be taken as evidence that natural estrogen is responsible for the low incidence of paracoccidioidomycosis in women. However, estradiol and diethylstilbestrol also seem to inhibit growth of other fungi; the data thus suggests that estrogen inhibition of fungal growth may be a fairly widespread phenomenon.

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PROLONGED THERAPY FOR PARACOCCIDIOIDOMYCOSIS: APPROACHES, COMPLICATIONS, AND RISKS[6]

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Paracoccidioidomycosis has a long incubation period and is hard to cure. This article discusses various types of available drug therapy and indicates general guidelines for treating the disease.

The successful management and treatment of the paracoccidioidomycosis patient depends, more than anything else, on the physician's knowledge of and personal experience with the disease.

The experience acquired to date in the endemic areas of this mycosis tends to support the theory that *Paracoccidioides brasiliensis* exists freely in nature, probably in soil, and that its portal of entry is the respiratory tract, with infection taking place in the lungs. As with coccidioidomycosis and other systemic fungus diseases with similar patterns, the primoinfection is usually asymptomatic and can only be diagnosed by skin testing.

For background on the development of the foregoing postulates, as well as on the classification of the clinical forms of the disease, we refer to our 1947 and 1964 publications on these subjects. In the first of these (*43*), we called attention to the long period of incubation observed on occasion with this mycosis—as much as 40 years or more—according to information provided in the patients' anamneses. Time-spans of this length between an asymptomatic period of residence in an endemic area, when the primoinfection was very probably acquired, and the appearance of the first clinical symptoms in a nonendemic area, have been recorded more than once. Further

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