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Experimental Paracoccidioidomycosis in Mice

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Virulence and infectivity of nine strains of *Paracoccidioides brasiliensis* were investigated in groups of mice which were inoculated intranasally or intravenously, and some of each were treated with corticosteroids. Fatal infections were not often seen among untreated mice, but mortality usually occurred when corticosteroids were given, regardless of the route of fungus inoculation. Prior treatment did not uniformly increase the incidence of infection, however; only in the case of intranasally inoculated mice was this effect seen. Most strains appeared to be more virulent when administered intravenously, with the exception of a single strain which, under the influence of corticosteroids, repeatedly displayed greatest virulence when given intranasally. All animals that died early in the course of the disease, irrespective of route of inoculation, always had acute pulmonary lesions and usually no other organ was involved. Animals which died later or were sacrificed always had chronic lung lesions. Whether or not chronically diseased animals had additional organ involvement correlated with how the organisms were administered; intravenously inoculated animals usually had extrapulmonary as well as pulmonary lesions, but lesions of those inoculated intranasally were almost exclusively pulmonary. Corticosteroids did not alter the histologic characteristics of either the acute or the chronic type of lesion, but the lesions of treated animals were usually more extensive. Most of the survivors appeared healthy even when infection was extensive.

Several investigators have demonstrated that experimental animals such as mice, guinea pigs, rabbits, rats, and hamsters are susceptible to infection with *Paracoccidioides brasiliensis*, the etiologic agent of South American blastomycosis (3-5, 7, 9, 14, 16, 21, 23). In most animals, however, the disease is not apparent until long after inoculation and, in certain cases, only under special conditions such as housing at low temperature. Furthermore, there are few studies designed to elucidate the pathogenesis of this disease. In the present report, the virulence of nine strains of *P. brasiliensis* was investigated by intravenous and intranasal inoculation of mice with and without prior corticosteroid treatment. The histology of the lesions obtained and their organ distribution are presented.

MATERIALS AND METHODS

Culture materials. *P. brasiliensis*, strain 3, was supplied by J. D. Schneidau, Tulane University School of Medicine, New Orleans, and was identified by him as C81; strain 17 was 15989 from the collection of L. Pollak, Departamento de Bacteriologia, Centro Regional de Referencia de la O.M.S. para Bacteriologia de la Tuberculosis, Caracas, Venezuela; strains 1, 7, 9, 10, 11, 12, and 19 were obtained from A. Restrepo, Universidad de Antioquia, Medellin, Colombia, identified by her as Ardila, Alvarez, Rendon, Jimenez, Suarez, Ospina, and Castaneda, respectively.

Cultures were maintained in the mycelial phase on Sabouraud's glucose agar at about 25 C. For the preparation of mouse inocula, the cultures were converted to the yeast phase by incubation in 1% yeast extract-2% glucose broth, at 34 C with constant agitation, 180 oscillations per min (gyrotory shaker, New Brunswick Scientific Co., New Brunswick, N.J.). They were then twice transferred and incubated for 3 days, each time with constant agitation as described. The number of yeast particles was estimated by direct hemocytometer and confirmed by spread plate counts on a Brain Heart Infusion agar. The number of viable units in the actual mouse inocula was determined in triplicate immediately before and after each series of inoculations. Maximal counts were obtained after 5 days of incubation at 37 C.

Determination of pathogenicity. Six-week-old, male, Swiss albino mice, strain CD1 from Charles River Farms (Wilmington, Mass.), were used in all experiments; the animals were housed at 25 C. Each fungus strain was inoculated intravenously and intranasally into mice in groups of 10 (5 pretreated with steroid and 5 nontreated). For each series of inoculations, five animals were given steroid but not fungus. As a single exception to this standard protocol, group

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sizes were doubled when an attempt was made to confirm results obtained with strain 7. The inocula were suspended in a final volume of 0.05 ml for the intranasal inoculations and 0.2 ml for the intravenous. The pretreated animals were given either 5 mg of cortisone or 2.5 mg of prednisolone intramuscularly 2 days prior to fungus inoculation and were protected with antibiotics as described by Sidransky and Friedman (22). Animals that died were autopsied with particular attention to macroscopic lesions of mouth, skin, anus, and internal viscera including the luminal surface of the intestines. Lung, liver, and spleen were cultured at 25 C on Sabouraud's glucose agar containing 0.03% chloramphenicol and 0.05% cycloheximide. Tissue from lung, liver, spleen, kidney, and intestines, irrespective of the presence of macroscopic lesions, was fixed in 10% Formalin for histological study. Other tissues were fixed only if macroscopic lesions were seen. Sections were stained with hematoxylin and eosin. Surviving animals were sacrificed between 50 to 60 days after inoculation and studied by the same methods used for those that died spontaneously.

RESULTS

Untreated mice were susceptible to paracoccidioidomycosis, but frequently the process was not fatal. Of the combined total of 110 animals inoculated either intranasally or intravenously with one or another of the nine fungal strains, only eight died. Prior administration of a corticosteroid, however, considerably increased mortality; 33 of 110 animals so pretreated succumbed (Table 1). Most of the deaths occurred in the first 20 days after inoculation. After that period, only a few deaths were observed, and these were at variable times (Table 2).

By combining the numbers of animals that died (Table 1), irrespective of pretreatment or route of inoculation, evidence emerged of variation in virulence according to strain, since an equivalent or smaller number of yeasts of some strains produced higher mortality than did others. For example, strain 1, for which the inoculum was 5,000 viable particles, caused death of 6 of 20 animals; and strain 7, at a dose of 15,000 to 20,000, killed 13 of 60. In contrast, only 2 of 20 animals that were given larger doses of strains 11 and 17 died. Even less virulent were strains 9, 10, and 19, which did not fatally infect any of a combined total of 60 mice.

There appeared also to be variations in virulence according to the route by which the organism was administered. There was more often a fatal paracoccidioidomycosis following intravenous than intranasal inoculation, with the striking exception of strain 7 in which a higher mortality occurred when the organism was given by the respiratory route.

By pooling data from all of the animals inoculated, irrespective of strain and route of inoculation, there was a suggestion of variation in capacity of a strain to produce infection, albeit not fatal. Of all the animals inoculated with strains 1, 7, and 11 (disregarding variability of treatment), most were found to have gross lesions at autopsy, following spontaneous death or sacrifice, and were confirmed as paracoccidioidal. In contrast, animals inoculated with strains 9, 10, or 19 had lesions at autopsy less often. Morbidity results with strains 3, 12, and 17 were not compared because the number of viable particles in the inoculum was notably larger, and thus the effect observed could have been dose-dependent.

Further compilation of data obtained with the 9 fungus strains revealed a difference in morbidity

TABLE 1. *Cumulative mortality and morbidity among mice inoculated with various strains of Paracoccidioides brasiliensis and observed for 50 to 60 days*

Strain no.	No. of viable yeast particles ($\times 10^3$)	Pretreatment				
		Corticosteroids[a]	Corticosteroids[b]	None[b]	None	Corticosteroids[d]
1	5	4, *5*[e]	2, *5*	0, *5*	0, *0*	0, *0*
19	10	0, *4*	0, *2*	0, *2*	0, *0*	0, *0*
9	15	0, *2*	0, *0*	0, *2*	0, *1*	0, *0*
7	15	1, *5*	5, *5*	0, *5*	0, *2*	0, *0*
7	20	0, *10*	6, *7*	0, *10*	1, *6*	0, *0*
11	15	1, *5*	1, *4*	0, *5*	0, *2*	0, *0*
10	16	0, *5*	0, *1*	0, *2*	0, *0*	0, *0*
17	35	2, *5*	0, *2*	0, *5*	0, *2*	0, *0*
3	60	5, *5*	0, *4*	5, *5*	0, *5*	0, *0*
12	100	2, *4*	4, *4*	1, *5*	1, *1*	0, *0*

[a] Two days prior to fungal inoculation, groups 3, 7, 11, 12, and 17 were given 2.5 mg of prednisolone acetate and 30,000 units of long-lasting penicillin (Bicillin, Wyeth Laboratories, Philadelphia, Pa.), intramuscularly. For groups 1, 9, 10, and 19, 5 mg of cortisone acetate was used instead of prednisolone. Throughout the period of observation, tetracycline hydrochloride (50 mg, liter) was added to the drinking water of all pretreated animals. Fungus was administered intravenously.

[b] Fungus was administered intranasally.

[c] Fungus was administered intravenously.

[d] No fungus was administered.

[e] The first number represents the number that died; the second number (italicized) is the total number found to have gross and microscopic lesions at autopsy, confirmed as paracoccidioidal by culture, and includes both the animals that died and those that were sacrificed at the end of the observation period. There were five mice per group except for the second trial with strain 7 when the group size was 10.

TABLE 2. *Time until death of mice during 50 days after intravenous or intranasal inoculation with Paracoccidioides brasiliensis*[a]

Days after inoculation	Route of fungus inoculation			
	Intravenous[b]	Intranasal[b]	Intravenous[c]	Intranasal[c]
0–10	7	15	5	1
11–20	5	1	0	1
21–30	0	0	0	0
31–40	1	2	1	0
41–50	2	0	0	0
Total dead	15	18	6	2

[a] Composite data from all strains (Table 1), 55 mice per each of the above four composite groups.
[b] Pretreated with corticosteroids.
[c] Not pretreated.

TABLE 3. *Cumulative mortality and morbidity of mice 50 to 60 days after inoculation intravenously or intranasally with yeast cells of Paracoccidioides brasiliensis*[a]

Experimental group	Route of inoculation	No. of animals	No. dead	No. infected[c]
Pretreated with corticosteroids and antibiotics[b]	Intravenous	55	15 (27)	50 (90)
	Intranasal	55	18 (32)	34 (61)
No previous treatment	Intravenous	55	6 (11)	46 (83)
	Intranasal	55	2 (3)	19 (34)
Treated with corticosteroids and antibiotics	(No fungus)	50	0	0

[a] Composite data from all strains (Table 1). Numbers in parentheses are percentages.
[b] Two days prior to fungal inoculation mice were given 2.5 mg of prednisolone acetate or 5 mg of cortisone acetate, and 30,000 units of long-lasting penicillin (Bicillin, Wyeth Laboratories, Philadelphia, Pa.), intramuscularly. Throughout the period of observation, tetracycline hydrochloride (50 mg per liter) was added to the drinking water.
[c] Includes animals that died of paracoccidioidomycosis as well as survivors found to have lesions when sacrificed.

among intranasally inoculated animals, dependent upon whether or not corticosteroids and antibiotics were administered. In Table 3, it is shown that 34 of 55 (61%) pretreated and intranasally inoculated animals had gross lesions at autopsy after either spontaneous death or sacrifice. In comparison, only 19 (34%) of another pool of 55 had evidence of infection, and they also had been intranasally inoculated but not pretreated. There was no apparent difference between pretreated and untreated animals when the inoculum was given intravenously.

Examining data from animals inoculated with all of the fungus strains, without regard to pretreatment, it was observed that there was little tendency to dissemination among intranasally inoculated animals as compared with those given the fungus intravenously. As shown in Table 4, the incidence of extrapulmonary lesions among intranasally inoculated animals did not in any instance exceed 4% of the total number of animals with evidence of infection after inoculation by that route. In the case of intravenously inoculated animals, however, lesions in liver, spleen, kidney, heart, skin, voluntary muscle, and intestine or pancreas, or both, were seen in 4 to 79% of 96 such animals found infected.

Most of the animals that survived through the period of observation appeared remarkably healthy and active but were found to have extensive lesions in the lungs, other organs, or both, as extensive as seen in the mice that succumbed.

Animals that died in the first 15 days after infection had acute pneumonia in a phase of red hepatization with predominance of congestion and hyperemia. The pulmonary parenchyma in certain areas was virtually replaced by multiple focal lesions of different sizes having numerous yeast cells, some of them budding. Yeasts were observed also in the alveolar spaces. There was intense cellular infiltration around the fungal cells, predominantly of neutrophils, lymphocytes, and plasmocytes; no eosinophils were observed. The lesions were localized in the proximity of the bronchi and bronchioli in the animals inoculated intranasally and around the blood vessels in those inoculated intravenously. The acute histological reaction, typical of either intranasally or intravenously inoculated animals, is shown in Fig. 1.

Animals that died after approximately 2 weeks had multiple granulomatous lesions, always of lungs, with other organs less frequently involved (Fig. 2). The lesions were of different sizes, but all were well circumscribed, and the parenchyma often was unrecognizable. They were composed largely of apparently healthy fungal cells, some of them budding, and in the immediate vicinity there were few-to-moderate numbers of lymphocytes, neutrophils, and fibroblasts but rarely eosinophils. Numerous giant cells and macrophages could be seen, some with phagocytized yeasts. Foamy and epitheloid cells surrounded these granulomas. Around the blood vessels the reaction was inflammatory with lymphocytes, plasmocytes, and Russel's corpuscles predominating. In certain areas, destruction of the walls

TABLE 4. *Distribution of lesions of 149 mice[a] and 36 humans[b] with paracoccidioidomycosis*

Localization of lesions	No. and percentage[c] of mice		Data from A and B pooled[c]	No. and percentage[c] of human cases
	A. Intravenous route	B. Intranasal route		
Oral mucosa	0 (0)	0 (0)	0 (0)	8 (22)
Lung	96 (100)	53 (100)	149 (100)	27 (75)
Liver	76 (79)	2 (4)	78 (52)	10 (28)
Spleen	72 (75)	2 (4)	74 (50)	1 (3)
Kidney	55 (57)	1 (2)	56 (38)	3 (8)
Heart	49 (51)	1 (2)	50 (34)	1 (3)
Striated muscle	35 (36)	1 (2)	36 (24)	1 (3)
Intestine	23 (24)	2 (4)	25 (17)	1 (3)
Anorectal	4 (4)	0 (0)	4 (3)	
Pancreas	12 (12)	0 (0)	12 (8)	
Skin	4 (4)	0 (0)	4 (3)	1 (3)

[a] Ninety-six were inoculated intravenously; 53 were inoculated intranasally. These data are not differentiated according to strain of fungus or as to whether or not corticosteroids were administered. They represent a composite of the animals described in Tables 1 and 2.

[b] Human autopsy cases reported by Brass in 1969 from Venezuela.

[c] Numbers in parentheses are percentages.

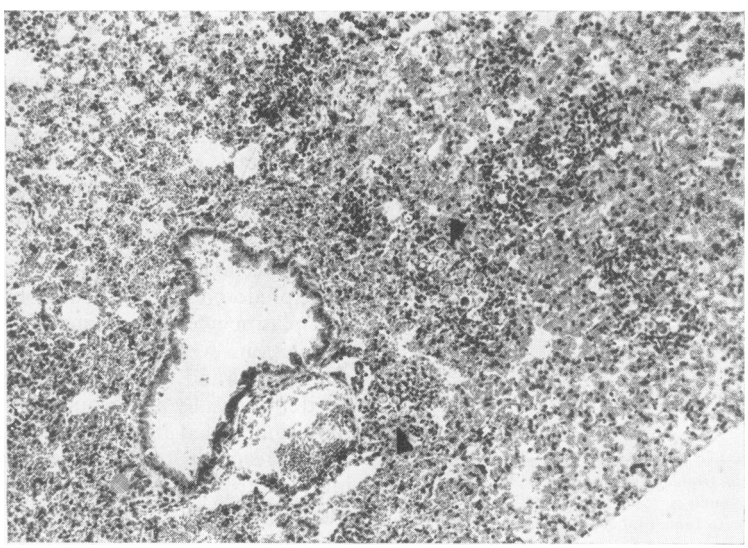

FIG. 1. *Acute fungal pneumonia in an intravenously inoculated mouse. Note intense inflammatory reaction, numerous yeasts in clusters (arrows), edema, and congestion. Hematoxylin and eosin stain.* × *300.*

of the vessels was observed and yeasts were seen in the lumen.

No differences were observed in the characteristics of the lesions between those animals receiving corticosteroids and antibiotics and those that did not, except for size; lesions of animals pretreated with steroids were more extensive.

When intestinal lesions were seen (in sacrificed animals and more frequently after intravenous inoculation, irrespective of pretreatment), there was involvement of mucosa, submucosa, and lymphatic follicles. The organisms were numerous but there was a scarce infiltrate of lymphocytes and neutrophils, although many giant cells with phagocytized fungal cells could be seen. The integrity of the surface of the mucosa was conserved, and no organisms were observed in the lumen of the intestine or in the muscular layers. Lesions in the anorectal region involved striated muscle, submucosa, and mucosa, usually without ulceration. These lesions also had numerous microorganisms surrounded with few neutrophils and lymphocytes, but, in contrast, few giant cells; some fibrosis was observed.

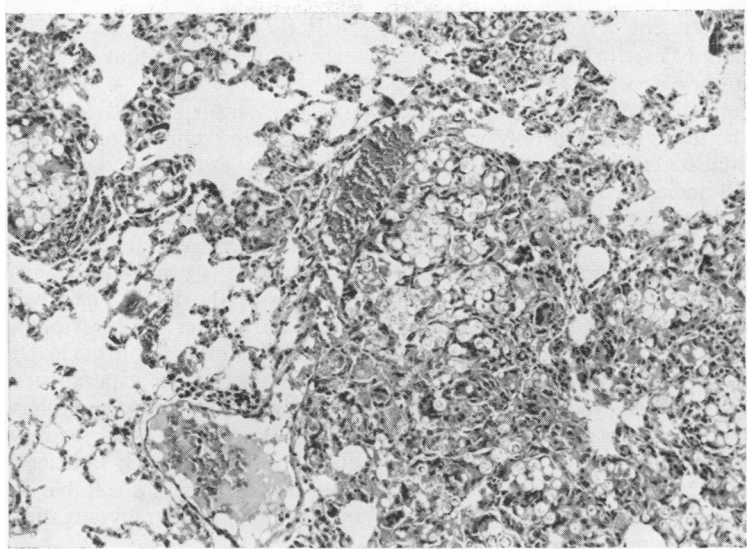

FIG. 2. *Granulomatous reaction in lung of a chronically diseased mouse that died 48 days after intravenous inoculation of Paraoccidioides brasiliensis. Note focal and chronic inflammation around blood vessels, many yeasts (some phagocytized) with fibrosis, giant cells, and moderate leukocytic infiltration. Hematoxylin and eosin stain.* × *400.*

No lesions were found in the oral cavity.

DISCUSSION

Limitations of group size in the present study preclude extensive interpretation, but certain conclusions seem justified. Whereas murine paracoccidioidomycosis does not precisely mimic the disease as seen in man, there are many comparable features; and the mouse, particularly when pretreated with corticosteroids, was found to be a suitable experimental model for the study of this disease. This is especially true when a fatal process of short evolution is desired, for most of the deaths occurred within 20 days. Beyond this period, death usually did not occur. But even without an immunosuppressant, normal mice were susceptible to infection, although most of the infections were chronic and, for the most part, did not terminate fatally, at least not within 50 to 60 days. The intensity of the infections seemed to be determined largely by the strain and dosage used.

Our experience of relative resistance of normal mice to the disease is in agreement with that of other investigators (3, 9; G. del Negro and T. de Brito, Proc. XIIth Congr. Trop. Med., Rio de Janeiro, **3:** 118, 1963), although Mackinnon (14) found that with longer periods of observation infected mice often succumb, even when an immunosuppressant has not been used to modify susceptibility. Had our animals been permitted to live longer, some of them also may have died eventually of paracoccidioidomycosis. It is noteworthy, nevertheless, that while many of these animals subsequently proved to be extensively infected, at no time did they appear in ill health. In fact, this trait of general good health in spite of widespread disease has been noted also in human infections (6, 11; A. Restrepo, *personal communication*) and is an indication of how well the disease is often tolerated. In the case at least of human disease, this unusually harmonious relationship between host and parasite may persist through many years (1).

The chronic lesions observed in the present study, among those animals that lived relatively long, were similar to chronic lesions reported by other investigators (4, 5, 7, 9, 14, 21, 23), namely, that of a granulomatous reaction. Only Mackinnon (12) has reported the acute type of lesions that we observed in animals dying during the first 2 weeks after inoculation, but he saw these only in mice sacrificed (on the 4th and 7th day) rather than dying spontaneously.

Another similarity between our studies and those of previously mentioned investigators, including those who used other animal species, was the observation that pulmonary involvement was common even when the organism was administered intravenously. This predilection for pulmonary tissue in experimental infections is consistent with recorded data from autopsy of naturally acquired human infections (1, 2). Similarly, Restrepo and her associates (19) re-

ported the most frequent clinical manifestation of paracoccidioidomycosis in their series of patients was respiratory. It should be noted, however, that in our experimental study exclusive localization in the lungs occurred only when the fungus was administered intranasally. Furthermore, intranasally inoculated animals seemed to have a remarkable capacity to confine the organisms within the lung, for the incidence of dissemination, despite frequent extensive pulmonary involvement, was low, and even then was seen only among those that were sacrificed. When animals were inoculated intravenously, other organs frequently were involved although not so uniformly as were the lungs. Furthermore, the almost exclusive propensity for lung in our intranasally induced murine infections corresponds more closely to the organ distribution in the above mentioned series of human cases (Table 4) than does the distribution of lesions in mice following intravenous inoculation. This similarity in organ distribution, observed also in many human case reports of human disease (10, 18, 20) is consistent with the hypothesis proposed by other colleagues (8, 15, 17) that, under natural conditions, inhalation of the organism is the most frequent mode of infection in humans, the primary lesion being localized to the respiratory tract and evolving either to healing or, after a relatively long period, to dissemination.

Perhaps even more important in the present study was the paucity of mucosal lesions, which were uncommon except when the inoculum was administered intravenously, and then were primarily intestinal, although in a few instances rectal lesions were seen. Even after intravenous inoculation, intestinal involvement occurred far less frequently than pulmonary. Mackinnon (13) also found some rectal and intestinal lesions but only after intravenous inoculation. This scarcity of mucosal lesions in parenterally and intranasally infected animals suggests the possibility that many mucosal lesions in naturally acquired human infections may result from direct local inoculation.

Beyond these generalizations it is difficult to make comparisons with the work of other investigators because of variable factors such as source of inoculum and route of administration. It does seem, however, that among the animal species tried none is completely resistant to *P. brasiliensis*, although in most reports no mention is made of strain variability in infection or virulence. Conant and Howell (3) stated that some of their isolates were more virulent for mice than others; a limitation of this study was that usually only one animal was used per strain. In our own trials, despite a similar restriction, there was some suggestion of variation in the aforementioned strain characteristics. A notable observation was that one strain seemed to differ in that it repeatedly caused higher mortality when inoculated intranasally as compared with intravenous inoculation.

Another acknowledged limitation of our work was the use of yeast inocula rather than mycelial forms, for it is probable that man is usually infected with the latter phase which presumably occurs in nature. To contribute to a better understanding of the still unclear epidemiology of this disease, further experiments should be conducted with the saprophytic phase of the fungus. At the time the present study was undertaken, difficulties in obtaining sufficiently homogeneous mycelial particles of a suitable size precluded use of this phase for the investigation.

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LITERATURE CITED

1. Benaim Pinto, H. 1961. La paracoccidioidomicosis brasiliensis como enfermedad sistemica. Comentarios a la casuistica Venezolana. Mycopathol. Mycol. Appl. 15:90–114.
2. Brass, K. 1969. Observaciones sobre la anatomia pathologica, patogenesis y evolucion de la paracoccidioidomicosis. Mycopathol. Mycol. Appl. 37:119.
3. Conant, N. F., and A. Howell, Jr. 1942. The similarity of the fungi causing South American blastomycosis (Paracoccidioidal granuloma) and North American blastomycosis (Gilchrist's disease). J. Invest. Dermatol. 5:353–370.
4. Conti-Diaz, I. A., L. A. Yarzabal, and J. E. Mackinnon. 1959. Lesiones cutaneas, orofaringeas, rectales y musculares por inoculacion intracardiaca de *Paracoccidioides brasiliensis* al cobayo y al conejo. An. Fac. Med. Univ. Repub. Montevideo 44:601–607.
5. de Brito, T., and C. Fava Netto. 1963. Disseminated experimental South American blastomycosis of the guinea pig; a pathologic and immunologic study. Pathol. Microbiol. 26:29–43.
6. Goncalves, A. P., and C. Bardy. 1946. Aspectos clinicos a radiologicos da blastomicose brasileira pulmonar. Hospital (Rio de Janeiro) 30:1021–1041.
7. Guimaraes, F. N. 1951. Infeccao do hamster (*Cricetus auratus* Waterhouse) pelo agente da micose de Lutz (blastomicose sul-americana). Hospital (Rio de Janeiro) 40:515–520.
8. Lacaz, C. da S. 1955–56. South American blastomycosis. An. Fac. Med. Univ. Sao Paulo 29:9–120.
9. Lacaz, C. da S., S. T. Iaria, M. Ferreira, A. A. Martins, and V. S. Vega. 1949. Blastomicose experimental (nota preliminar). Hospital (Rio de Janeiro) 36:341–349.
10. Londero, A. T. 1968. Aspectos de la blastomicosis sudamericana en Rio Grande do Sul, Brasil. Torax 17:56–59.
11. Machado Filho, J., and J. L. Miranda. 1960. Consideracoes relativas a blastomicose sul-americana. Localizacoes, sintomas iniciais, vias de penetracao disseminacao em 313 casos consecutivos. Hospital (Rio de Janeiro) 58:99–137.
12. Mackinnon, J. E. 1959. Blastomicosis sudamericana experimental evolutiva por via pulmonar. An. Fac. Med. Univ. Repub. Montevideo 44:355–358.

13. Mackinnon, J. E. 1959. Miositis en la blastomicosis sudamericana experimental. An. Fac. Med. Univ. Repub. Montevideo **44**:149–155.
14. Mackinnon, J. E. 1959. Pathogenesis of South American blastomycosis. Trans. Roy. Soc. Trop. Med. Hyg. **53**:487–494.
15. Mackinnon, J. E. 1968. Actualizacion sobre patogenia de la blastomicosis sudamericana. Torax **17**:40–45.
16. Mackinnon, J. E., I. Conti-Diaz, L. A. Yarzabal, and N. Tavella. 1960. Temperatura ambiental y blastomicosis sudamericana. An. Fac. Med. Univ. Repub. Montevideo **45**:310–318.
17. Negroni, P. 1966. Las blastomicosis y coccidioidomicosis, p. 142. *In* Micosis profundas, vol. III. Comision de Investigacion Cientifica de Buenos Aires.
18. Passos Filho, M. D. da R. 1966. Blastomicose sulamericana. Comentarios em torno de 83 casos de localizacao pulmonar. Classificacao radiologica. Hospital (Rio de Janeiro) **70**:109–134.
19. Restrepo, A., M. Robledo, F. Gutierrez, M. Sanclemente E. Castaneda, and G. Calle. 1970. Paracoccidioidomycosis (South American blastomycosis). A study of 39 cases observed in Medellin, Colombia. Amer. J. Trop. Med. Hyg. **19**:68–76.
20. Rodriguez, C., N. L. Rincon, and G. Troconis-Garcia. 1961. Contribucion al estudio de la paracoccidioidomicosis brasiliensis en Venezuela. Consideraciones sobre 62 casos estudiados con especial referencia a las localizaciones respiratorias. Mycopathol. Mycol. Appl. **15**:115–138.
21. Segretain, G., and E. Drouhet. 1955. Blastomycoses experimentales du hamster dore. Ann. Inst. Pasteur (Paris) **89**: 593–595.
22. Sidransky, H., and L. Friedman. 1959. The effect of cortisone and antibiotic agents on experimental pulmonary aspergillosis. Amer. J. Pathol. **35**:169–183.
23. Teixeira, G. de A., J. Machado Filho, and J. L. Miranda. 1965. Blastomicose sul-americana experimental. Estudo experimental em ratos com consideracoes relativas a patogenia das lesoes. Hospital (Rio de Janeiro) **68**:1081–1096.