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Paracoccidioidomycosis

Anatomic Study with Complete Autopsies

KARLHANNS SALFELDER, GOETZ DOEHNERT and HANS-RUDOLF DOEHNERT

Departamentos de Anatomía Patologica de las Universidades de Mérida
(Director: Prof. Dr. K. SALFELDER),
y Barquisimeto (Director: Prof. Dr. H. R. DOEHNERT), Venezuela

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Parakokzidioidomykose

Eine anatomische Studie mit vollständigen Autopsien

Zusammenfassung. 11 Sektionen und 20 durch Biopsie diagnostizierte Fälle von Parakokzidioidomykose wurden untersucht; bei den letzteren lag eine röntgenologische Thoraxuntersuchung vor.

Makroskopische Befunde, Gewebsreaktionen und die Morphologie der Pilze im Gewebe wurden beschrieben.

Zusätzlich zu den positiven histologischen Befunden wurde auch angegeben, welche Organe mikroskopisch untersucht worden waren. Ohne diese Angaben sollten in Zukunft Untersuchungen zu ähnlichen Themen nicht publiziert werden.

Die Sektionsbefunde ergaben einige Unterschiede im Vergleich zu anderen Mitteilungen über diese Erkrankung. Das bezieht sich vor allem auf den Befall von Lungen, Lymphknoten, Darmtrakt und Nebennieren. Die Gewebsreaktion ist in jedem Fall gemischt; infolgedessen erscheint eine Klassifikation nach Typen nicht angezeigt.

Pilze fanden sich auch im Pleuraexsudat. Leber- und Milzvergrößerung wurde nicht festgestellt. Cor pulmonale kam in mehr als zwei Dritteln der Fälle vor. Endlich waren zusätzliche Erkrankungen in diesen Fällen häufig. Sie erschwerten die klinische und makroskopische Diagnose. In einem Fall von generalisierter Histoplasmose zusätzlich zu einer Parakokzidioidomykose mit Streuung, lagen besondere Befunde vor.

Die Ergebnisse der Untersuchungen weisen darauf hin, daß hauptsächlich die Lungen als Eintrittspforte der Pilze in Frage kommen.

Summary. Eleven autopsy and 20 biopsy cases of paracoccidioidomycosis were studied; in the latter chest X-rays were required. The gross anatomic features, the tissue reaction, and the morphology of the organisms in the tissue are described.

In addition to the positive histologic findings, it is indicated which organs were subjected to microscopic study, a sine qua non recommended for all similar studies.

Necropsy revealed some differences from other reports on this disease as regards lung, lymph node, intestinal, and adrenal gland involvement. Tissue reaction was mixed and did not allow type classification.

The fungus could be found in pleural effusions. No hepatic or splenic enlargement was observed. Cor pulmonale occurred in more than two-thirds of the cases, and associated diseases were frequent, the latter making clinical and gross diagnosis difficult. A case of disseminated histoplasmosis coexistent with generalized paracoccidioidomycosis showed distinctive findings.

This study suggests that the lungs are the portal of entry of this fungus.

The deep mycosis known variously as South American blastomycosis, Lutz-Splendore-Almeida disease; Brasilian blastomycosis, and paracoccidiodal granuloma, and found exclusively in South America, is caused by the biphasic or dimorphic fungus *Paracoccidioides brasiliensis* (*P. bras.*). The highest incidence is

found in the rural population of areas with tropical climates. In São Paulo, Brazil, over 1,500 cases had been reported by 1960 (Lacaz, 1960) and in Venezuela, more than 300 cases up to 1959 (Angulo and Carbonell, 1961). The habitat of *P. bras.* has yet to be determined; despite one positive claim (Batista *et al.* 1962), spontaneous blastomycosis does not occur in animals, and opinions about human pathogenesis are divided, especially concerning the portal of entry.

Reports of casuistic series based on complete autopsies are available in histoplasmosis (Schulz, 1954; Binford, 1955), blastomycosis (Schwarz and Baum, 1951), cryptococcosis (Zimmerman and Rappaport, 1954; Baker and Haugen, 1955; Littman and Zimmerman, 1956), and coccidioidomycosis (Huntington *et al.*, 1967). The important papers on paracoccidioidomycosis (Buengeler, 1942; Angulo, 1948 and 1959; Lacaz, 1960; Fialho, 1960, and Azulay, 1963), on the contrary deal with larger series summarily or with generalities about tissue reactions and without detailed autopsy findings. An exception is the work of Brass (1966), which, however, appeared in a local publication without wide circulation. Therefore it was felt that our material should be reviewed with emphasis on anatomic findings and pathogenesis.

Material and Methods

Thirty-one cases of paracoccidioidomycosis (P.) were studied (11 autopsies and 20 cases with surgical specimens). They are from the Departments of Pathology of Barquisimeto (B) — 7 autopsies and 14 biopsies — and Mérida (M) — 4 autopsies and 6 biopsies — from the years 1952 to 1968.

Barquisimeto, capital of the State of Lara, is situated at 500 m of altitude in the central occidental part of Venezuela. It is an agricultural region with a tropical climate. Mérida, capital of one of the three Andean states in the southwestern part of the country, is situated at an altitude of 1,650 m in the central valley. In addition to people from the mountains, hospital patients in Mérida come from the tropical regions on both sides of the Andes, toward the Lake of Maracaibo and towards the Llanos.

The protocols of autopsies and biopsies and the clinical histories were reviewed. Tissue sections were reexamined, all paraffin blocks and wet material recut, and partial serial sections restudied. Gross photographs made at the time of examination were used. Also lung X-rays were restudied.

Two autopsy and more than 30 biopsy cases with the diagnosis of P. from Barquisimeto were not included, since only protocols and slides of the autopsies and of the biopsies without lung X-rays were available.

Sections and smears were stained with hematoxylin and eosin, and by the Grocott, Weigerts gram, Ziehl-Neelsen, and Goldner staining technics. Occasional PAS, Gridley, and other staining procedures were used.

Cultures have been made only exceptionally. Some positive cultures have been obtained, generally from biopsies or from sputum of cases which later were autopsied.

Observations

Clinical Data. As shown in Table 1, 8 of the 11 autopsy cases were observed in tuberculosis sanatoria and only 3 in general hospitals. With the exception of one case in 1955, all others occurred in the 1960s. All were male "criollos", i.e. of Venezuelan extraction (mixed race). Ages ranged from 32 to 59 years. Six patients were agricultural workers and 9 of the 11 had lived in rural zones. Only one patient had lived in a town (in one case, residence was unknown). Duration of symptoms was not known in 5 cases; in the remainder it varied from 3 months to several years. One patient died as the result of an automobile accident, 2 had stayed only 4 and 10 days, respectively, in the hospital, 5 had been hospitalized for about 1 month, and 3 others, for 2, 3, and 4 months, respectively.

Table 1. *Clinical features of autopsy cases*

Case No.	Year	Age	Sex	Prof.	Residence	Duration Sympt.	Hospitaliz. days	Clinic. Diagn.	Treatment
1. AS 17 M	1955	50	M	Agric. work	St. Barb. Zulia	Years	31	Pulm. Tbc.	Anti Tbc.
2. AS 105 M	1964	59	M	Agric. work	Palmarito/Truj.	?	31	No tub. pulm dis.	—
3. AS 109 M	1964	55	M	Agric. work	Jaji/Mérida	?	118	Pulm. Tbc.	Anti Tbc.
4. AS 150 M	1967	48	M	Agric. work	Jaji/Mérida	8 mths	24	Parac. ?	—
5. AS 144 B	1960	32	M	Worker	Sanare/Lara	18 mths	36	Pulm. Tbc.	Anti Tbc.
6. A 4473 B	1960	46	M	Agric. work	Araure/Port.	3 mths	4	Parac.	—
7. A 5407 B	1961	40	M	?	Barquisimeto	?	1	Traumatism.	—
8. AS 161 B	1962	38	M	Mason	San Felipe	?	95	?	—
9. AS 173 B	1965	54	M	Agric. work	Lara State	?	20	No tub. pulm. dis.	—
10. A 17611 B	1968	56	M	?	Biscucuy/Truj.	8 mths	10	Tuberculosis ?	Anti Tbc.
11. AS 183 B	1968	58	M	?	?	8 mths	64	Pulm. Tbc.	Anti Tbc.

M = cases from Mérida. B = cases from Barquisimeto.
AS = cases from Tuberculosis Sanatoria.

Table 2. *Gross pathology*

Case No.	Lungs	Pleura	Other organs	Weight		Cor pulm.	Gr. Diagn.
				Liv.	*Spl.*		
1	Bilat. fibr. Nod. Induration Superf. Retract Emphysema	Bilat. Adhes.	Ulc. larynx; epi glot. Bilat. Adrenal caseificat.	950	200	+	Tuberc.
2	Fibr. cas. Nod. Superf. Retract. Emphysema	Bilat. Adhes.	Bilat. Adrenal Caseification	1,410	250	—	Tuberc.
3	Fibr. cas. Nod. Superf. Retract. Emphysema Bilat. cavit.	Unilat. dif. Adhes.	Bilat. Adrenal Caseificat.	1,165	112	+	Tuberc.
4	Fibrosis Superf. Retract. Emphysema Diff. Pneumon.	Bilat. Adhes. Sero-fi br. Pleur	Hil. ly. nodes absces. Mil. Gran. Spl. Nod. skin, Bowel	1,460	141	+	Parac.[a]
5	Fibr. Nod. Emphysema Bilat. cavit.	Bilat. Adhes.	Hil. ly nodes cas. foci Ulc. Duod. L. bow M. Gr. Spl. Liv.	2,220	210	+	Tuberc.[a] Parac.
6	Emphysema Lob. Pneumonia	—	Hil. ly nodes cas. foc. Mediast. Tumor. Ulc. Phar. Ulc. colit. Nod. sm. bow. Mes. ly nodes	1,772	335	—	Parac.[a] Kal. Azar
7	Fibr. Nod. diff. sizes. Emphysema	Bilat. Adhes.	Mult. Fract. Int. Hemorrh.	1,500	86	—	Parac.[a]
8	Fibr. Nodul. Emphysema Bilat. Cavit.	Bilat. Adhes.	Hil. ly nod, Cas. purul. foci. Ulc. Tons. Lar. Mil. Gran. Spl. Liv. Kid.	937	60	+	Tuberc.[a] Parac.
9	Fibrosis. Cas. foc. diff. siz. Emphysema	Unilat. Adhes. Diff. Oblit.	Hil. ly nodes purul. foci. Ulc. Lar.	1,450	80	+	Coccidioid[a]
10	Fibr. Nod. Superf. retract. Emphysema Mil. granulomas	—	Med. ly nod. cas. foc. Ulc. Lar. Ulc. Ileon colon Mes. ly nod. cas. foc.	1,265	62	+	Parac.[a]
11	Fibrosis Fibr. Nod.	—	Bilat. necr. foc. adrenals	1,150	123	+	Parac.[a]

[a] = Smears cut surface lung.

Only in one case paracoccidioidomycosis had been the clinical diagnosis; in another, P. was suspected. Tuberculosis was diagnosed 5 times; nontuberculous lung disease twice. In one case no clinical diagnosis had been made and in one, acute traumatisms were found without suspicion of lung disease. None of the patients had received sulfonamide, the specific therapy; 5 had been treated with tuberculostatic drugs.

Gross Pathology. At autopsy bilateral *pulmonary* lesions resembling chronic tuberculosis were seen in all cases (Table 2). Fibrosis and caseous and fibrocaseous nodules were frequent, as was scar tissue, often with retraction of lung

Table 3. *Tissues of autopsies examined histol./mycotic lesions*

Organs	Cases											Total
	1	2	3	4	5	6	7	8	9	10	11	
Skin				×	×				×			
Tongue	×			×							×	
Tonsils	×+			×+	×+			×+	×+		×	5
Epiglot	×+			×								1
Larynx				×		×+			×+		×	2
Trachea				×		×			×+		×	1
Lungs	×+	×+	×+	×+	×+	×+	×+	×+	×+	×+	×+	11
Pleur. eff.				×+				×+				2
Ly nod med.	×	×+	×+	×+	×	×+	×	×+	×+	×+	×+	8
Oesophag.				×								
Stomach				×		×		×		×		
Sm. bowel				×	×+	×				×+		2
Larg. bow.				×	×+	×				×+		2
Ly nod abd.				×	×+					×+		2
Liver	×+	×+	×	×+	×+	×	×	×	×	×	×+	5
Spleen	×+	×		×+	×+	×	×	×+	×+	×	×+	6
Pancreas	×	×	×	×			×			×	×	
Myocard	×	×	×	×+	×	×	×	×	×	×	×	1
Aorta				×								
Kidneys	×	×	×	×+	×	×		×	×	×	×	1
Bladder				×	×							
Prostate				×+	×	×			×	×	×	1
Sem. vesicl.				×								
Testes				×	×	×			×		×	
Epididym.				×								
Brain men.				×	×	×	×	×	×	×	×	
Chor. plex.				×								
Masto. sin.				×								
Sphen. sin.				×								
Pit. gland				×	×		×		×			
Pin. gland				×								
Thyr. gland		×		×	×	×		×	×+	×		1
Adren. gland	×+	×+	×+	×	×	×	×	×	×	×	×+	4
Spin. col.				×+		×						1
B. marr. fem.				×+		×						1
Skelet. mus.				×								
Sal. gland		×	×									

× = Examined histologically. + = Funguscontaining lesions.

surface, which was observed in almost all cases. Emphysema was present in 9 cases. In four, multiple cavities and in three, more or less extensive pneumonic areas were found. Miliary granulomas were seen only once; exclusive lung involvement also only once.

Pleural adhesions, generally bilateral, were seen in 8 cases; in one, fluid was present in the pleural cavities. The hilar and mediastinal *lymph nodes* were involved in 6 cases, showing enlargement and fibrocaseous foci of different sizes, often with purulent masses. In one case a substernal tumor mass of 3:7 cm was seen. In two cases the mesenteric lymph nodes were enlarged with necrotic foci. In the *skin*, multiple nodules were observed in one case. The mucosa of the *upper respiratory* and *digestive tract* was involved in 5 cases, in some cases showing ulcerative lesions at several sites. In the *intestinal tract*, ulcerative lesions were found in 3 and nodules in 2 cases. Large caseous areas with enlargement of the

adrenals (Fig. 1) were observed in 4 cases. Miliary granulomas were observed in 3 cases in the *spleen;* in two, in the *liver;* and in one, in the *kidney.* Neither the spleen nor the liver were enlarged. Right ventricle hypertrophy (*cor pulmonale*) was diagnosed in 8 cases, based on the thickness of the wall, the thick papillary muscles, and the apex forming right ventricle. Gross diagnosis was tuberculosis in 3 cases. Based on the additional examination of smears during autopsy, tuberculosis and P. were diagnosed in 2 cases, P. alone in 4, P. and kala-azar in one, and coccidioidomycosis in one.

Histology. Fungus-containing *tissue lesions* were found more frequently than grossly suspected (Table 3).

In all cases the *lungs* were involved, in one exclusively (case No. 7). Yeast cells were seen also in *pleural effusions.* In one case (No. 4), in the sediment of the liquid; in the other (case No. 8), fungus cells were attached to the pleural surface in sections.

In addition to the six cases in which gross lymph-node lesions were histologically confirmed, two additional cases showed mycotic lesions only on microscopic examination. The grossly described lesions in *mesenteric lymph nodes* (case No. 6) were due to *histoplasmosis;* but in cases Nos. 5 and 11, mycotic lesions were found histologically which had not been seen on gross examination. No paracoccidioidal skin lesions were detected.

In the upper respiratory and digestive tracts, paracoccidioidal lesions were seen in two more cases than at gross examination (tonsils, larynx, and trachea), to a total of 6 cases of involvement of this area. In one case (No. 10), gross larynx involvement was not confirmed microscopically.

The digestive tract showed lesions only in 2 cases. In the other three grossly suspected cases, no mycotic alterations were found (see associated diseases).

In spleen and liver, fungal lesions were seen histologically in three additional cases each, to a total of 6 cases of spleen and 5 of liver involvement. The miliary granulomas found grossly in the liver of case No. 8 were of tubercular origin, giving a total of 5 cases in which mycotic lesions were observed only microscopically. The gross lesions of the 4 cases with involvement of the adrenal glands were confirmed; no small lesions were seen microscopically in these organs in the rest of the cases.

In the thyroid gland, myocardium, kidneys, prostate, and bone marrow, in which involvement was found once in each on microscopy, gross lesions had not been observed.

Tissue reaction was found to be particular and mixed; in German, these would be called "specific" and "non-specific", and in English, granulomatous reactions with pyogenic inflammation. In addition to a pure leukocytic reaction with micro- or macroabscesses, pure tuberculoid granulomas (Fig. 2) were observed. Often giant cells occurred in a leukocytic exudate, or a considerable number of leukocytes were found in regions with caseous necrosis. Apparently cavities form from pure abscesses, from caseous necrotic foci, or are the result of tissue breakdown of mixed lesions. Caseous foci or abscesses are often seen in scar tissue with extensive fibrohyalinosis and a variable number of lymphocytes, macrophages and plasma cells. The scar or granulation tissue without formation

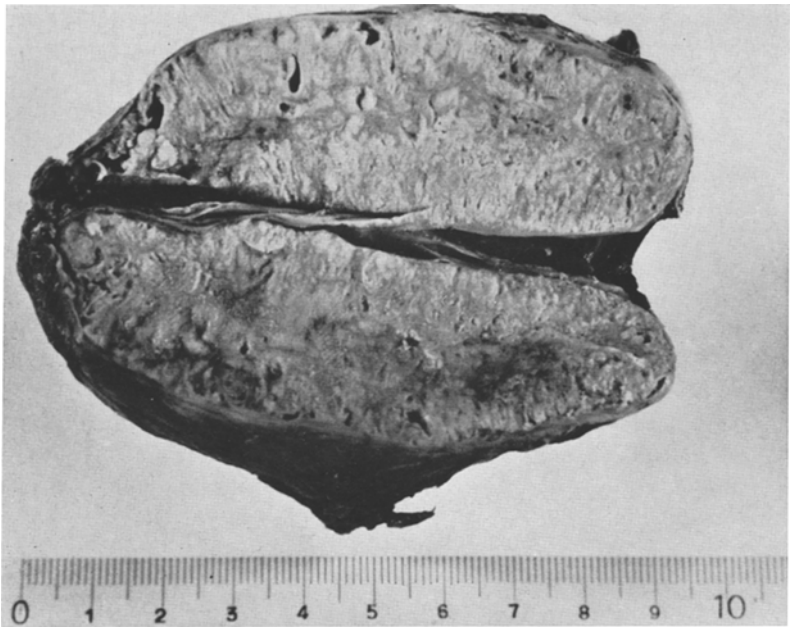

Fig. 1. Enlargement and diffuse casification of adrenal gland. Case No. 3

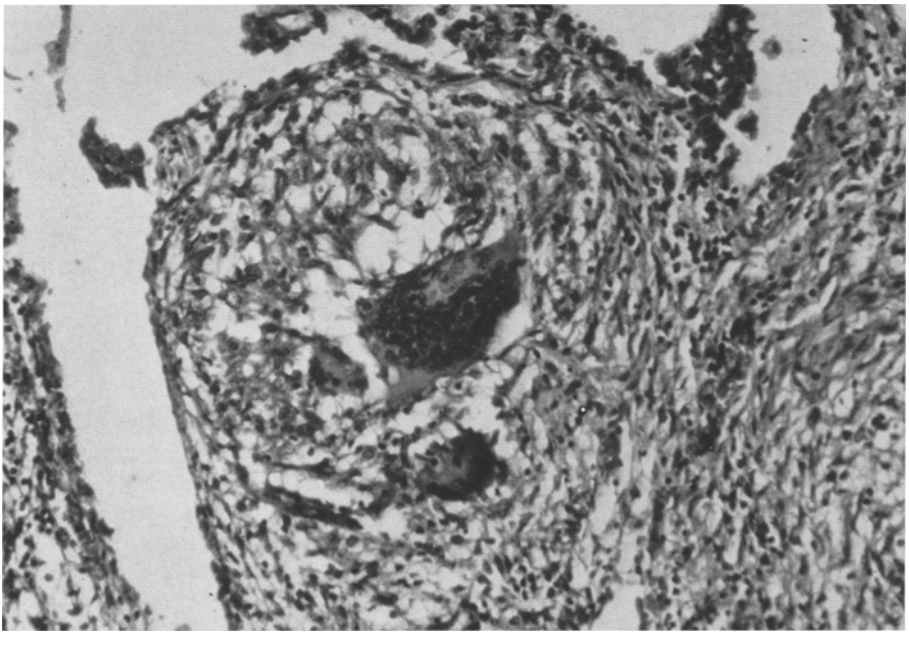

Fig. 2. Parac. miliary granuloma lung. Case No. 1. H & E; × 140

of granulomatous nodules may or may not show signs of a "specific" reaction, i.e. epithelioid and giant cells. *The complete lack of calcifications in our cases was characteristic.*

All the above-mentioned reactions occur also in the lungs. Granulomas result from the confluence of previous alveolar lesions, with small intraalveolar granulomas, or form in the interstitial space (Fig. 3). They may contain fibrous and hyalin material. Abscesses of different sizes form by breakdown of lung parenchyma. In addition to these features, in a few cases areas with intraalveolar pneumonia with leukocytic exudate, some giant cells, and scattered intraalveolar fungus cells were observed, which could be classified as *paracoccidioidal pneumonia* (Fig. 4). In one case, hyaline membranes were found in a pneumonic area (Fig. 5). Extensive fibrosis may develop interstitially or intraalveolarly (carnification) (Fig. 6).

In one case, multiple foci of scar tissue of different sizes and of irregular shape were observed, with extensive deposits of hyaline material and dust pigment. Small arteries often showed a marked thickness of walls, with sclerosis and narrowing of the lumen (Fig. 7).

No granulomas in the visceral pleura or perforations of subpleural mycotic lesions were seen. In one case, microabscesses containing yeast cells had formed in the thickened pleura.

In the lymph nodes, caseous foci of various sizes were generally found, occasionally with a small number of leukocytes, and often showing in addition fibrohyaline tissue. The foci were surrounded by a fibrotic granulation tissue with giant cells.

In the mucosa of the upper respiratory and digestive tracts, "specific" granulomatous tissue with giant cells and always with leukocytic exudate, was seen, often with a necrotic or ulcerated surface. Granulomas without ulceration of superficial layers were often observed, especially in the tonsils.

The mycotic lesions in the adrenals always consisted of large caseous necrosis with "specific" granulomatous tissue (Fig. 8). Leukocytic reactions were also seen; no pure abscesses were observed.

Of the two cases with intestinal involvement, case No. 5 showed a duodenal ulcer with only three small fungi-containing submiliary granulomas in the submucosa near the border of the ulceration (Fig. 9).

In the other case there was exclusively "non-specific" granulation tissue without yeast cells. In the three blocks from the large bowel ulcers, a "specific" reaction with fungus cells within giant cells (Fig. 10) could be found only in one, in a limited area. In the rest of the tissue of this ulceration and in the remaining two blocks a wide ulceration of mucosa with "non-specific" granulation tissue and without fungus cells was observed. In case No. 10, only granulomatous fungus-containing tissue reaction, with necrosis and ulceration of mucosa, was seen.

Exclusively miliary or submiliary granulomas containing yeast cells were found in spleen, liver (Fig. 11), bone marrow, myocardium, thyroid gland, kidney, and prostate.

Organisms in tissues were generally abundant. However, to detect the diagnostic multiple budding, many fields or slides often had to be reviewed. This

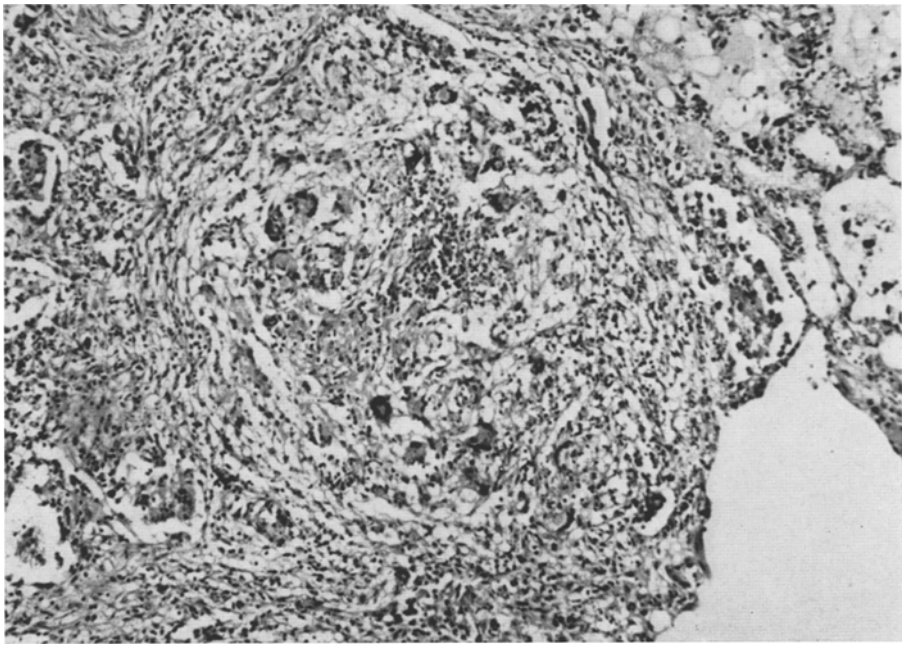

Fig. 3. Confluent parac. granulomas in lung P. Case No. 8. H & E; × 100

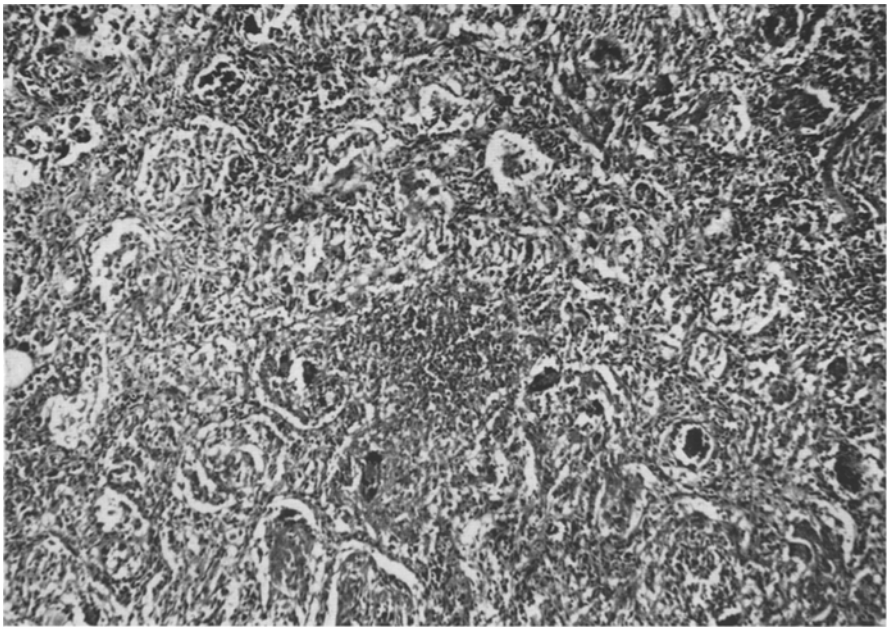

Fig. 4. Parac. pneumonia with multiple intraalveolar granulomas; in the center, formation of small abscess. Case No. 8. H & E; × 80

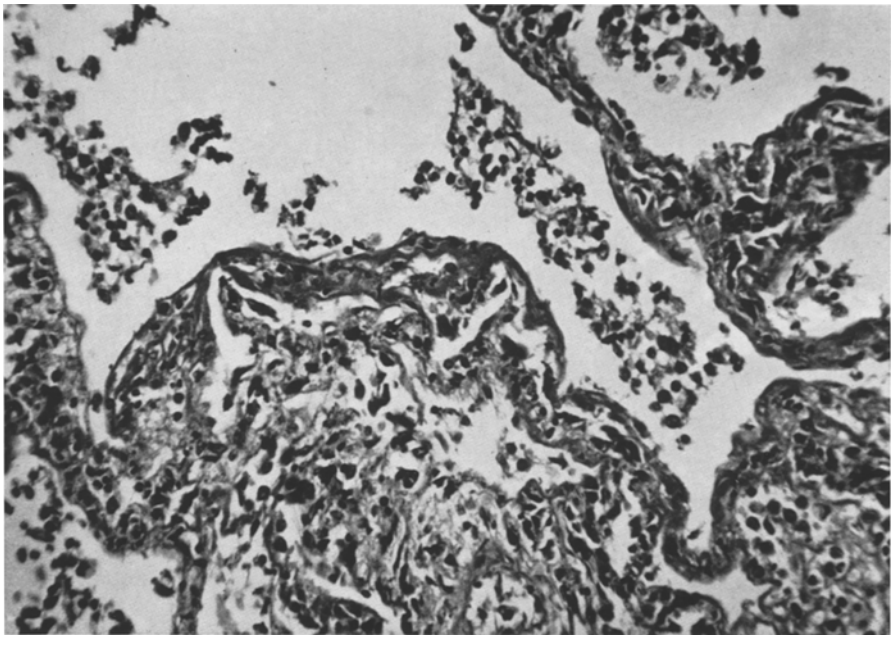

Fig. 5. Hyaline membranes in parac. pneumonia. Case No. 4. H & E; × 190

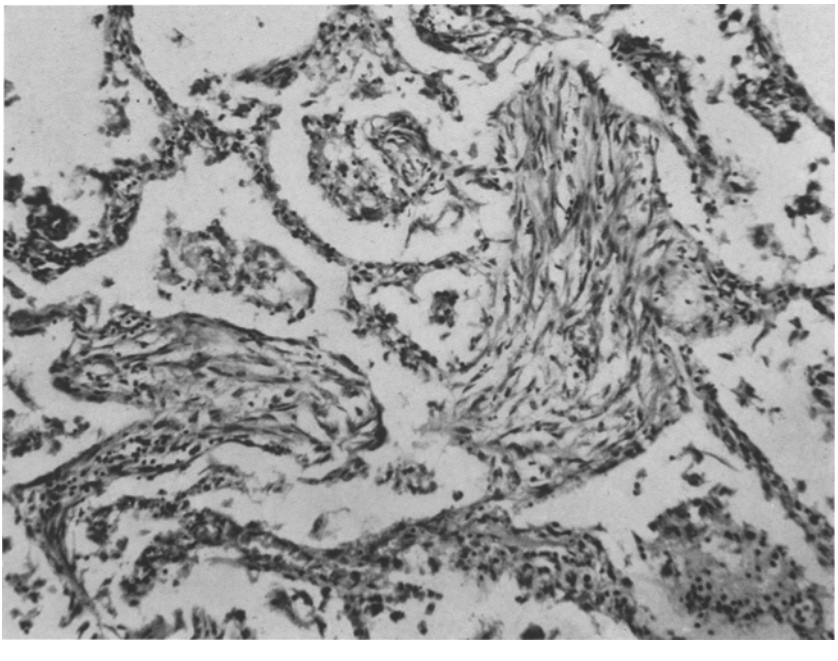

Fig. 6. Chronic parac. pneumonia with carnification (intraalveolar fibrosis). Case No. 4. H & E; × 80

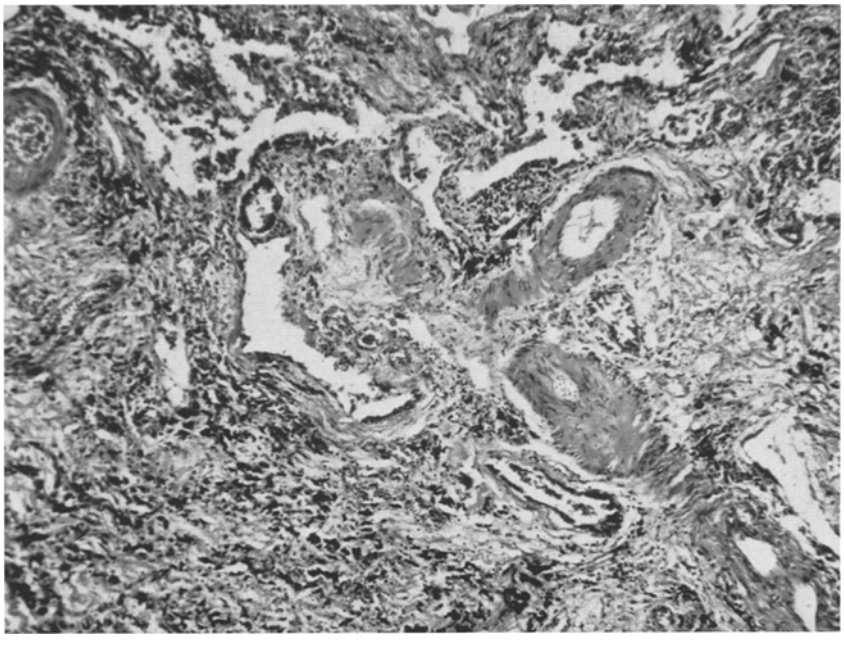

Fig. 7. Thick-walled small arteries in pulmonary parac. sclerosis. Case No. 1. H & E; × 50

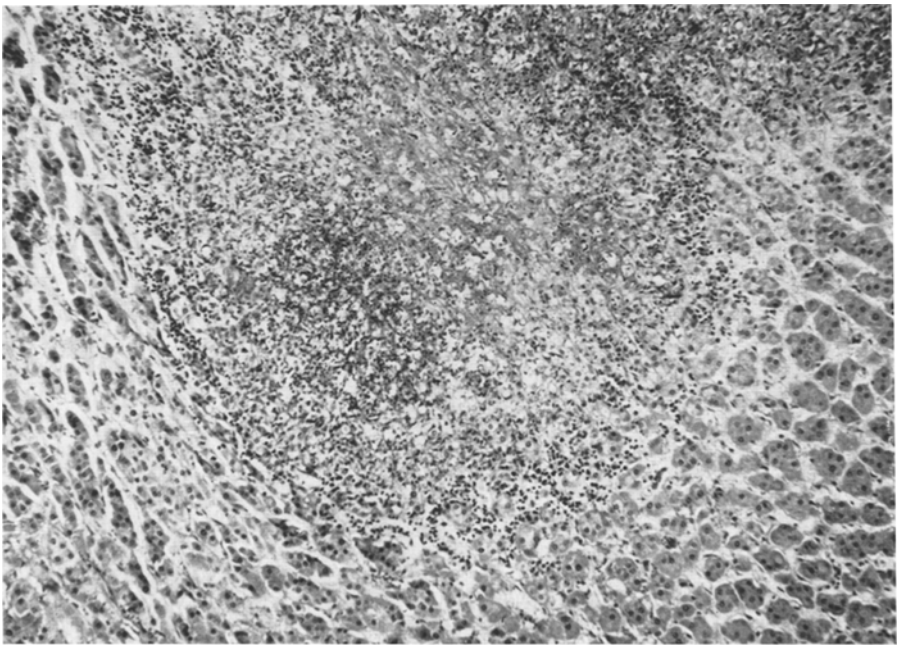

Fig. 8. Parac. focus with central necrosis in adrenal gland. Case No. 11. H & E; × 26

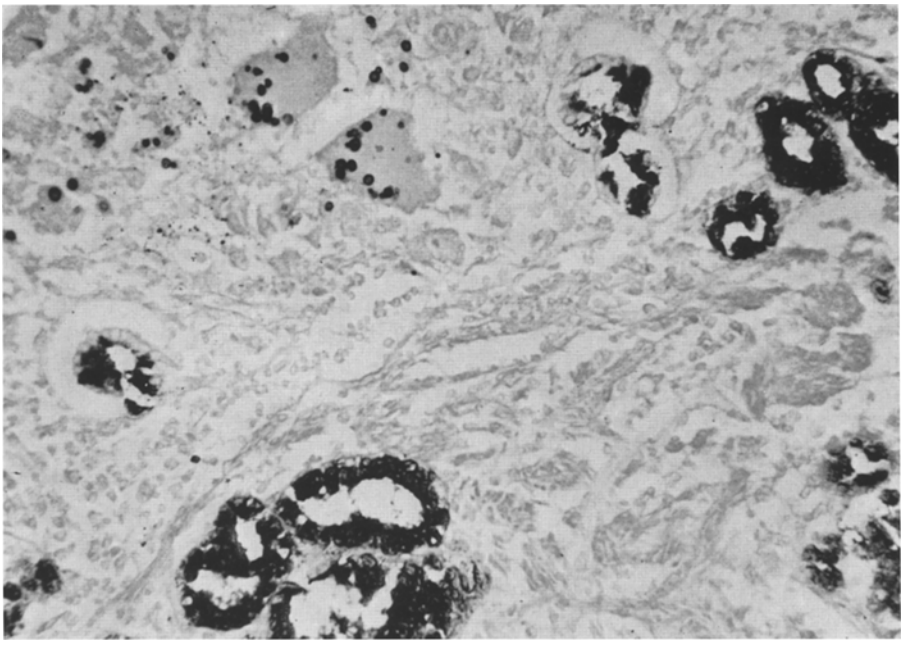

Fig. 9. Small parac. granulomas in duodenal submucosa, near border of peptic ulcer. Organisms in giant cells. Muc. glands also black-stained. Case No. 5. Grocott method; × 200

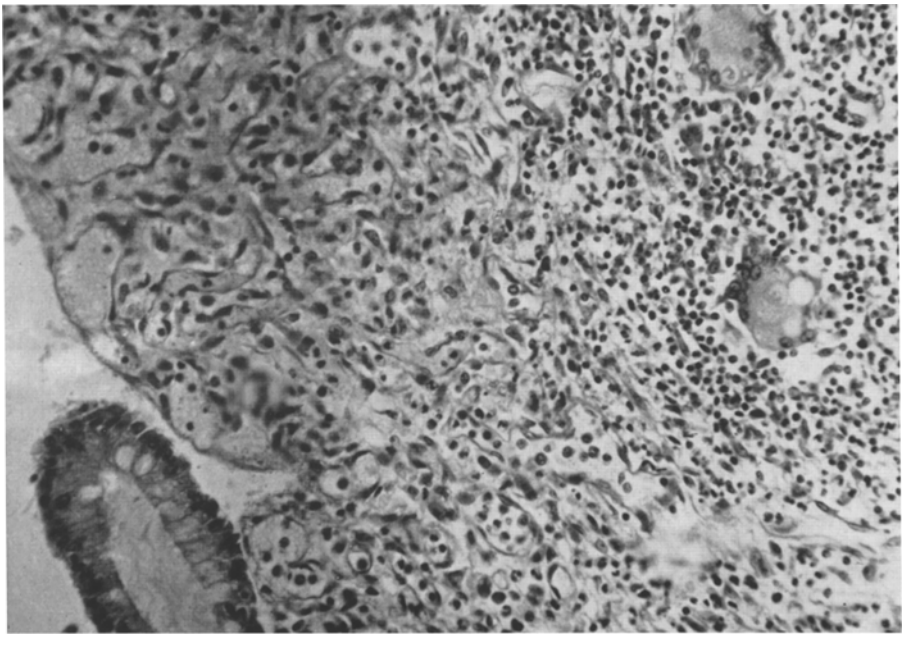

Fig. 10. Ulcerative colitis. Parac. granuloma with giant cells in sub-mucosa. Case No. 5. H & E; × 200

characteristic multiple budding of *P. bras.* is best and most clearly seen with the *Grocott staining method.* The buds are distributed uniformly about the whole surface of the ball-shaped fungus cell. The lateral or peripheral ones appear to protrude, giving a "steering wheel" structure (Fig. 12). The buds situated centrally, protruding into another plane at right angles to the lateral ones, can mimic endospores, the cell then having the appearance of a spherule of *C. immitis* (Fig. 13).

In sections stained with H & E, many fungus cells may be overlooked; the yeast cells can be confused with large tissue cells. Sometimes tumor tissue, especially cells of mucoid carcinomas, show structures resembling yeast cells of *P. bras.* The nucleus of the fungus cells is often seen with a halo between nucleus and cell wall. When the nucleus is missing, the yeast cells appear like vacuoles. Frequently vacuolic structures in giant cells are fungus cells (Fig. 14).

In Grocott-stained preparations many more fungus cells can be seen and single organisms can be detected. The nucleus stains only exceptionally with the Grocott method. The spherical or oval yeast cells of *P. bras.* more often show single than multiple buds or have no buds at all. Formation of hyphae or pseudohyphae is exceptional (Fig. 15). Asteroid body formation was seen only once (Fig. 16).

The size of the yeast cells varies from 3—5 to 30—40 μ and reaches exceptionally 60 μ in diameter. The walls seem not to be rigid, since bowl-, hat-, and sickle-shaped cells, which seem to be pressed in, can be found, and which may be mistaken for *Pneumocystis carinii* (Fig. 17). Ruptured and collapsed yeast cells are also seen. Frequently, dustlike structures were seen in Grocott-stained sections, generally in accumulations of numerous fungus cells with different forms of regressive alterations (Fig. 18). These represent Grocott-positive fungus-cell detritus. In lung and lymph nodes anthracotic pigment appears with similar structures. Since, however, the fungus-cell detritus has been found also in organs where anthracotic pigment does not occur, as, for instance, in adrenal glands, the nature of these dustlike structures as deriving from fungus cells cannot be doubted.

The organisms are often situated inside macrophages or giant cells, but can be seen also free in tissues. Accumulations of them form in the periphery of necrotic foci, giving an annular or garland-like appearance (Fig. 19).

The observation of sections of paraffin-embedded material with polarized light gave birefringency and showed Malta crosses in a certain number of organisms.

Associated Diseases. In six autopsy cases (Table 4), lesions of other diseases were observed which, on gross examination, were attributed to paracoccidioidal disease and which directly or indirectly played a role in causing death.

In the three cases with additional tuberculosis, acid-fast bacilli had been found in the sputum, leading in two cases to the clinical diagnosis of the lung disease and to specific treatment. In all three cases, both lungs were involved and acid-fast bacilli were found also in sections or smears. On morphologic grounds, the tuberculous lesions were similar to the paracoccidioidal ones, also showing leukocytic reactions, but without fungi in these areas or on slides. It was not possible to determine which infection preceded the other.

A residual calcified histoplasmic focus was seen in case No. 4 in a hilar lymph node containing numerous yeast cells compatible with *H. capsulatum* in the Grocott-stained sections of the decalcified material. In the same lymph node, extensive fibrocaseous paracoccidioidal

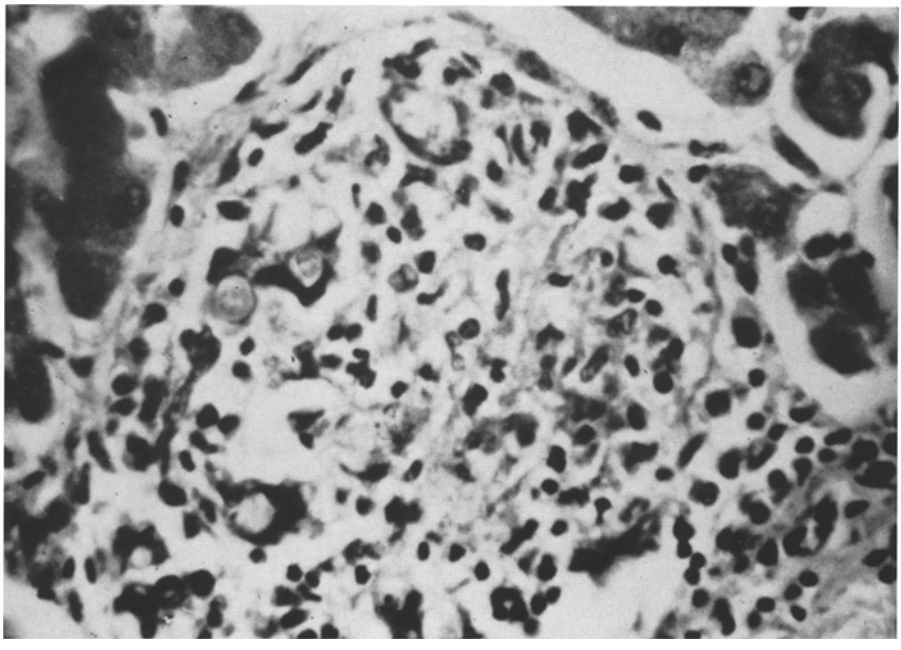

Fig. 11. Submiliary granuloma in the liver. Some intracellular yeast cells of *P. bras.* visible. Case No. 5. H & E; × 450

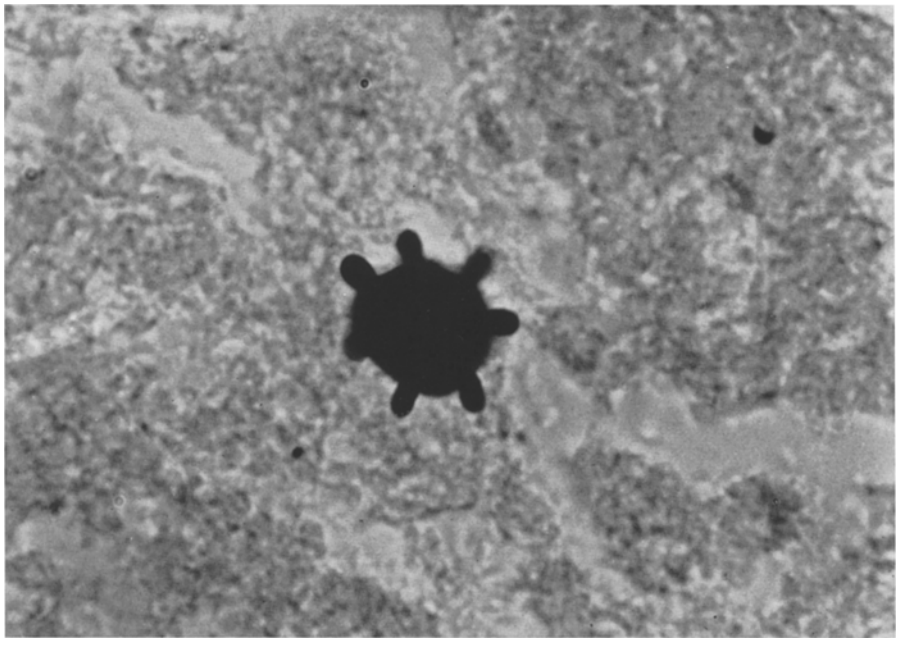

Fig. 12. Yeast cell of *P. bras.* with multiple budding (steering wheel). Lung tissue. Case No. 2. Grocott method; × 1,500

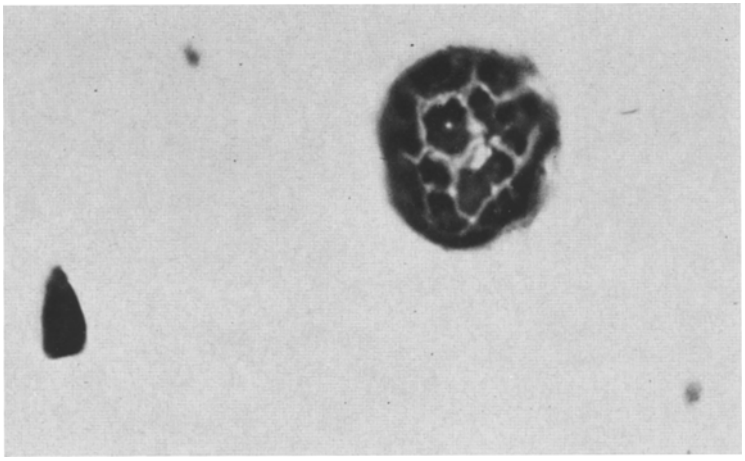

Fig. 13. Yeast cell of *P. bras.* with multiple budding. Buds can be confused with endospores. Lung tissue. Case No. 3. Grocott method; × 1,500

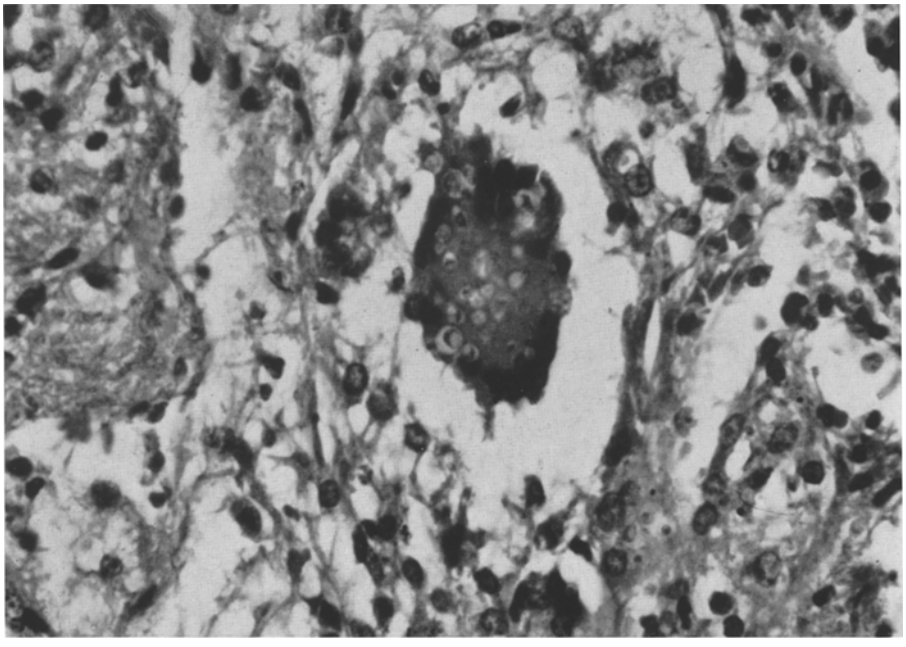

Fig. 14. Yeast cells of *P. bras.* in giant cell. Lung tissue. Case No. 8. H & E; × 500

lesions were present. In case No. 6 (Table 5), in addition to a partly generalized paracoccidioidomycosis, a widely disseminated histoplasmosis was found. Paracoccidioidal and histoplasmic lesions in some tissues were present simultaneously and mixed. The previous lesion — it is assumed that the paracoccidioidomycosis occurred first — were invaded by the other histoplasmic fungus. In the mediastinal lymph nodes, yeast cells of *P. bras.* and *H. caps.* occurred not only in the same area and microscopic field (Fig. 20) but also in the same cells (Fig. 21). Details of this case will be dealt with in another paper. Three types of benign tumors were

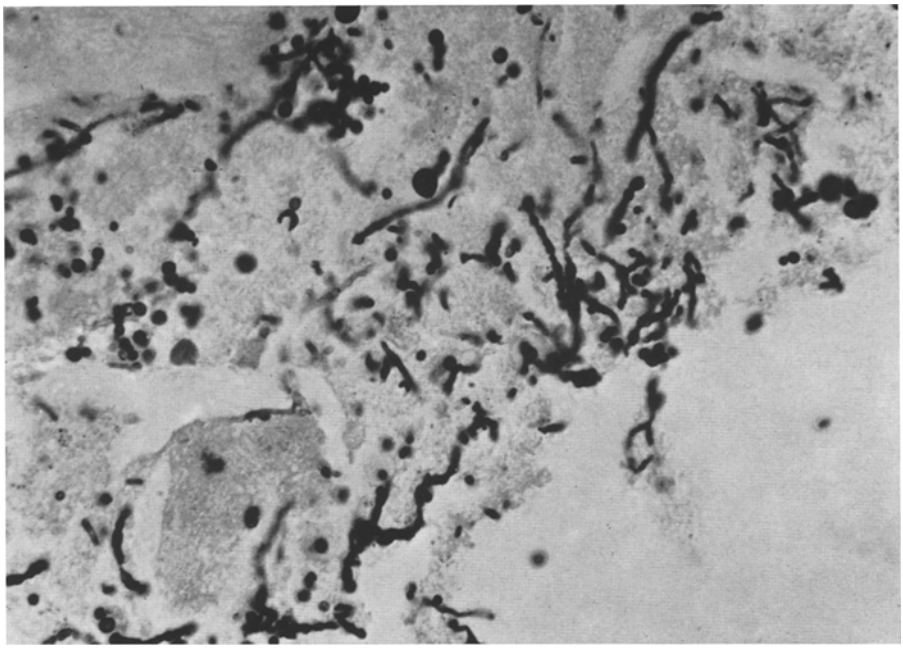

Fig. 15. Hyphae and pseudohyphae of *P. bras.* in caseous necrotic pulmonary lesion. Case No. 2. Grocott method; × 580

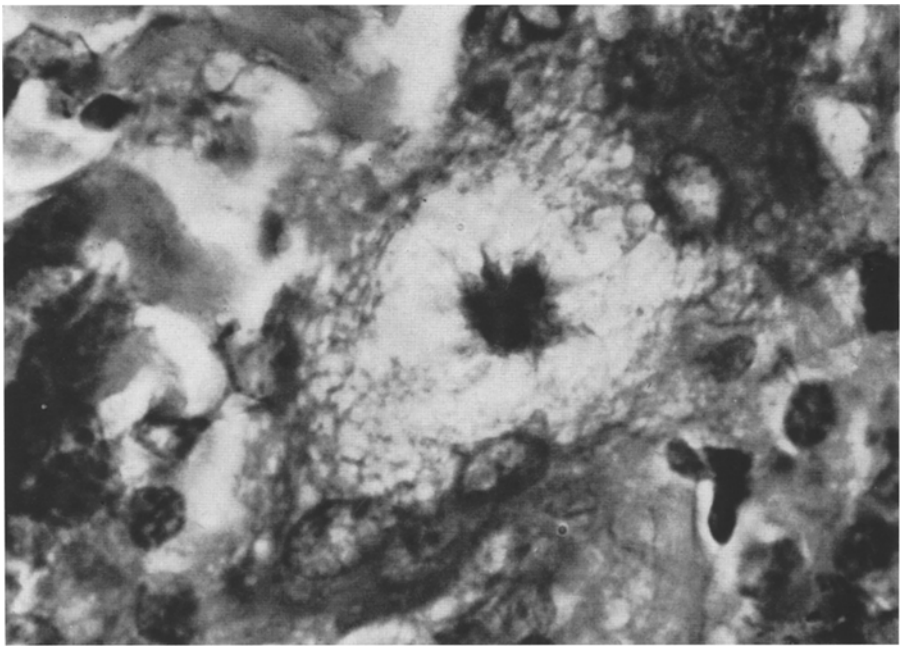

Fig. 16. Asteroid body with central yeast cell of *P. bras.* (not clearly seen in H & E). Lung lesion. Case No. 7. H & E; × 1,150

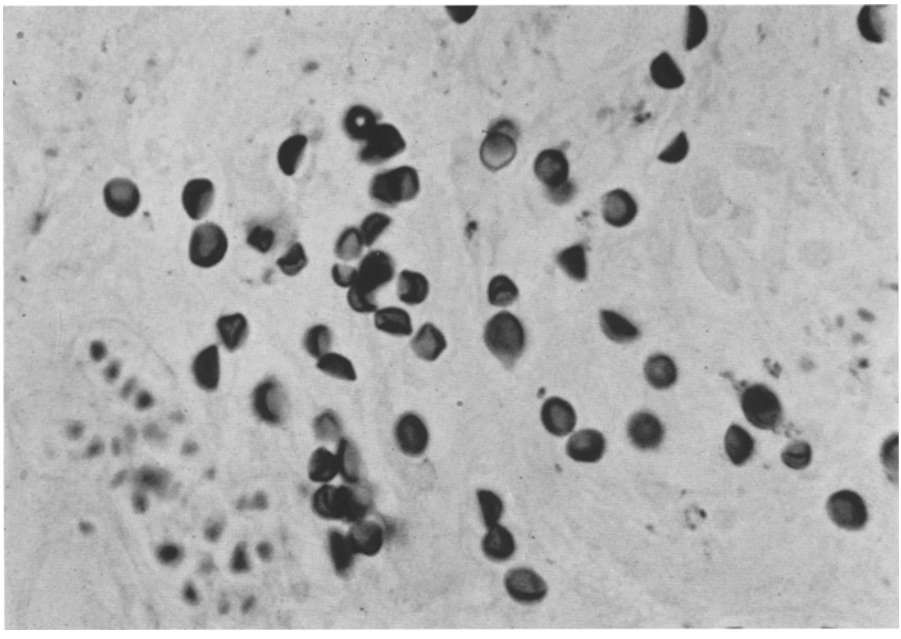

Fig. 17. Bowl, hat, and sickle-shaped yeast cells of *P. bras.* resembling Pneumocystis carinii. Lung lesion. Case No. 4. Grocott method; × 1,200

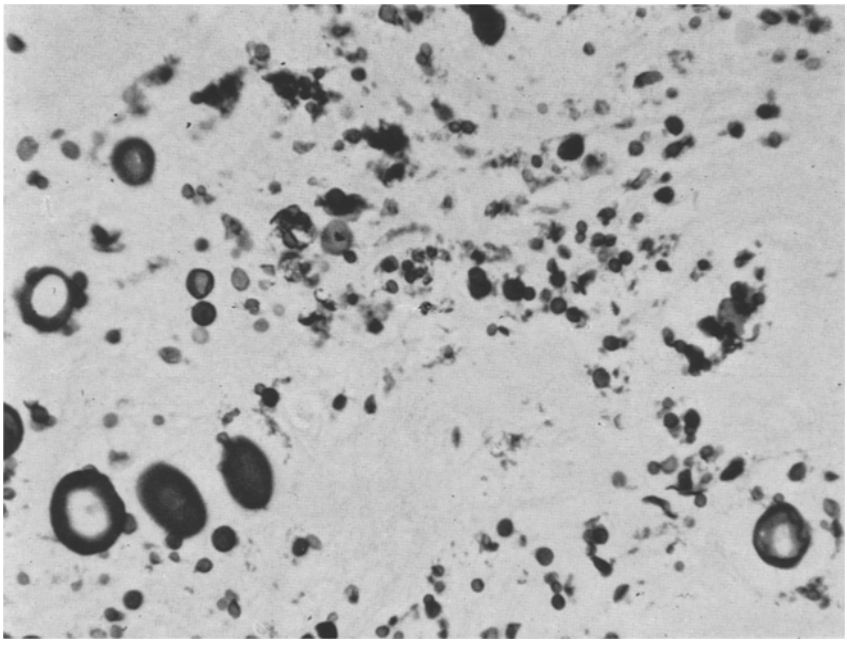

Fig. 18. Yeast cells of *P. bras.* with regressive alterations and dustlike fungus-cell detritus. Caseous adrenal lesion. Case No. 2. Grocott method; × 720

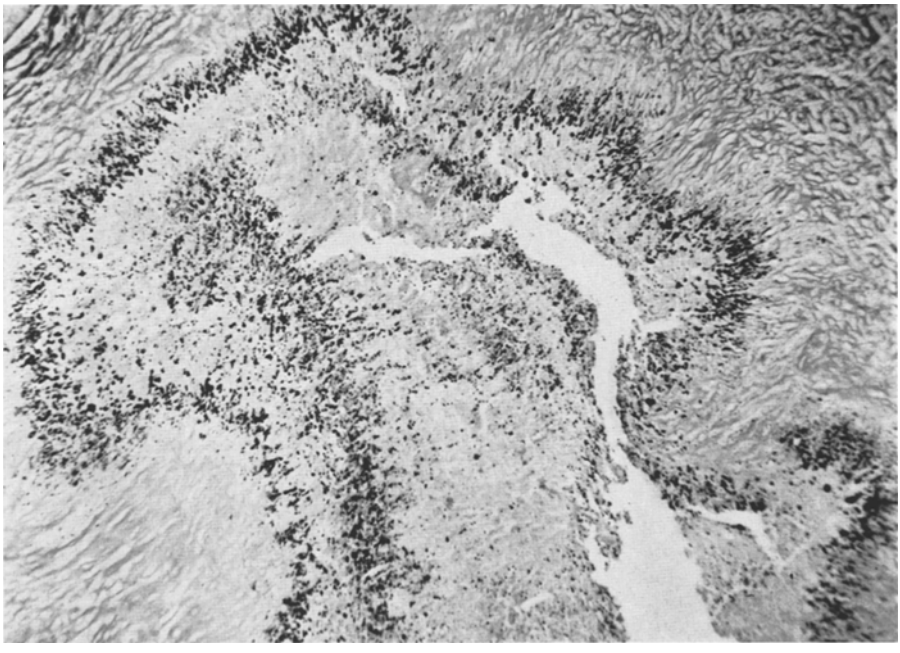

Fig. 19. Yeast cells of *P. bras.* accumulated in the periphery of caseous parac. lung lesion with breakdown of tissue in a garland-like fashion. Case No. 4. Grocott method; × 80

Table 4. *Associated diseases in autopsies*

Case No.	Tuberc.	Histopl.	Neurofibr.	Thymoma	Lipoma	Dys. Colitis	Myocardit.	Trauma
3	Lungs							
4		Hil. ly node	Skin; sm. bowel					
5	Lungs					+	+	
6		Genera-lized		+	+	+		
7								+
8	Lungs ly nod							

found in cases Nos. 4 and 6, in which skin, mediastinal, and small-bowel tumors could grossly be mistaken for mycotic lesions.

The ulcerative colitis in cases Nos. 5 and 6 was of a "non-specific" dysenteric type, most probably of bacterial etiology, as seen frequently in our environment. The lesions of case No. 5 were interpreted as duodenal ulcer and dysenteric ulcerative colitis with secondary paracoccidioidal infection, most probably due to hematogenous spread.

The myocarditis in case No. 5 was chronic, of unknown etiology with cardiac hypertrophy. This type of myocarditis is considered in our environment by many, mainly clinical, investigators as Chagas' disease; however, no parasites were found in the heart muscle fibers.

The fatal lesions in case No. 7 were of traumatic origin.

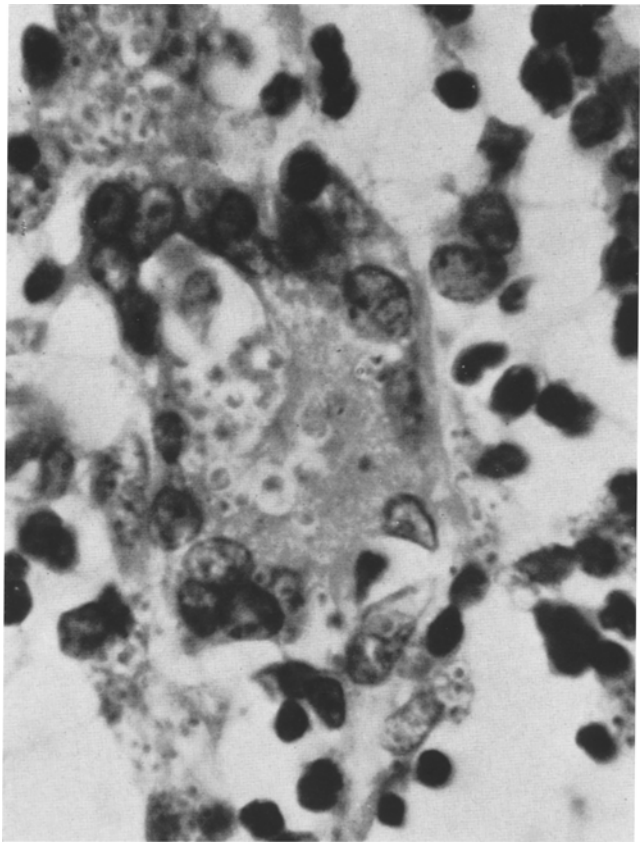

Fig. 20. Yeast cells of *P. bras.* and *H. caps.* in the same field. Hilar lymph node. Case No. 6. H & E; × 1,000

Table 5. *Case No. 6 generalized paracoccidioidomycosis and histoplasmosis*

Organs	Parac.	Histo-plasm.
Lungs	+	+
Mediast. ly nodes	+	+
Larynx	+	+
Pharynx	+	—
Thymoma, large bowel, liver, spleen Mes. ly nodes, adrenals, bone marrow	—	+

Surgical Material. Of the 20 cases of P. diagnosed in biopsies or by examination of smears (Table 6), 7 were from tuberculosis sanatoria. All were males and only one patient (10 years of age) was less than 32 years old; the oldest was age 70.

Localization of extrapulmonary lesions, with the exception of two cases (skin of the leg and invasion of the thyroid gland by contiguity), was restricted to the skin of face or neck and the mucosa of the oral cavity.

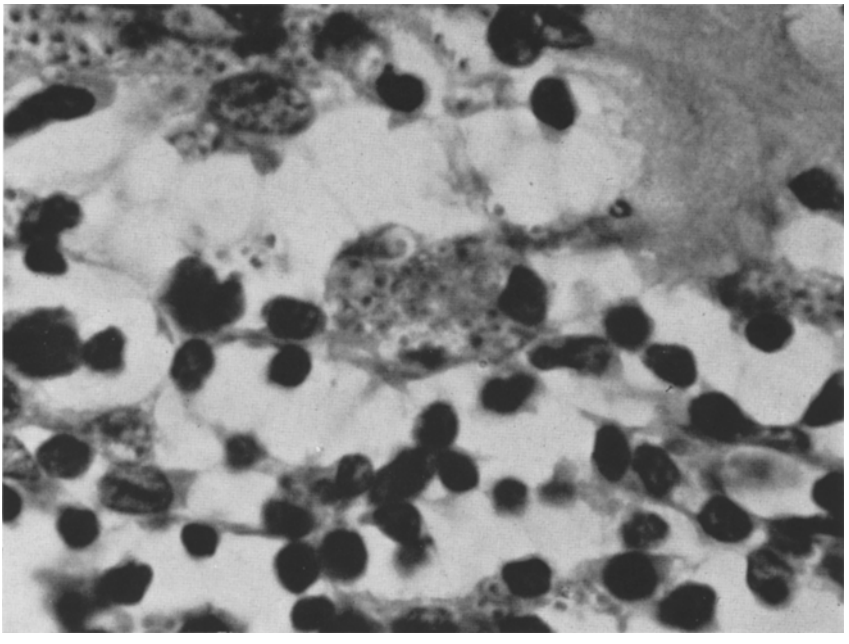

Fig. 21. Yeast cells of *P. bras.* and *H. caps.* in one macrophage. Three fungus cells of *P. bras.* are visible in the cell. Hilar lymph node. Case No. 6. H & E; × 1,200

Table 6. *Surgical material*

Case No.	Age	Sex	Site	X-ray lungs
1S 2003 B	41	Male	Skin; oral cavit.	++
2S 1190 B	35	Male	Skin; ly node neck	+
3 7916 B	10	Male	Skin; ly node neck	(+)
4 9695 B	41	Male	Larynx	+
5S 1136 B	39	Male	Tonsils	+
6 11795 B	32	Male	Oral cavit.	+
7 16221 B	40	Male	Lip; skin leg	+
8 16547 B	41	Male	Skin neck	−
9 19863 B	65	Male	Gum	+
10 32154 B	32	Male	Skin; ly node neck	++
11 37175 B	37	Male	Oral cavit. Tongue	+
12 37218 B	38	Male	Lip	+
13 43106 B	40	Male	Tongue	+
14 44092 B	56	Male	Larynx	+
15S 3753 M	60	Male	Skin neck	++
16 11221 M	55	Male	Skin; thyroid	+
17S 14251 M	36	Male	Bronchial wash.	++
18S 19062 M	70	Male	Sputum	+
19 21127 M	40	Male	Skin face	++
20S 28401 M	44	Male	Sputum	+

B = Barquisimeto. M = Mérida. S = Tuberculosis Sanatoria. (+) = Hilar lesion.
+ = Lesions in parenchyma. ++ = Cavitary lesions.

All patients, except one, showed more or less extensive pulmonary lesions on X-ray. In five cases, cavities were proved in tomograms. Only in one case was hilar or perihilar involvement found.

Comments

The age, sex, occupation, and residence of our autopsy and partly biopsy cases are more or less comparable with those in the literature. LACAZ (1960) mentions that out of more than 1,550 P. cases, only 28 were under 10 years of age; POTENZA *et al.* (1953) described a case of a 9-year-old boy.

As noted previously, all our cases were males. LACAZ (1960) found a male:female sex ratio of 14:1 and BRASS (1966) of 10:1. FIALHO (1960) saw only 4 females in 125 cases.

The great majority of our patients were agricultural workers from rural areas; the same was true in the material of FIALHO (1960), LACAZ (1960), AZULAY (1963), and BRASS (1966).

Paracoccidioidomycosis in our 11 autopsy cases was diagnosed clinically only once and suspected also only once, despite the fact that most of the patients had been hospitalized at least one month. In view of the similarity of the radiologic and anatomic gross lesions, it is possible that clinical diagnosis was often tuberculosis. Since tuberculosis was also present in three of our autopsy cases, clinical diagnosis efforts should not cease when one of these conditions is proved by the finding of the causative agent.

No specific (sulfonamide) treatment had been applied in our autopsy cases; hence the extensive scar formation and fibrosis was from natural evolution of the lesions.

Gross diagnosis at autopsy could be made only when *smears* of the caseous or purulent foci were examined. In these preparations, Leishmanias were once confused with *H. caps.*, and *C. immitis* with *P. bras.* *Without doing smears*, the lesions were frequently *misdiagnosed as tuberculous.* The signs which lead to suspicion of P. are the often multiple retractions of the lung surface, the suppuration in addition to or within caseous necrotic foci, and the fact that pulmonary vertices are often free of lesions. As noted also by BRASS (1966), hepato- and splenomegaly were not found in our autopsy cases. When present, they are often due to other (associated) diseases. Surprising was that in 8 of our 11 cases, cor pulmonale was present. FIALHO (1960) and BRASS (1966) mentioned this feature only once in 25 and 33 autopsies, respectively.

As seen in Table 3, in quite a few cases in which gross lesions were found during a complete autopsy, apparently normal organs were not examined histologically. Only in case No. 4 were all tissues reviewed. For investigations, this method is recommended. Not only positive histologic findings should be recorded, but also which tissues were examined. Lack of basic technical facilities, such as containers and fixative liquids, was one of the causes for not doing complete histopathologic studies of our cases.

Comparison of Tables 3 and 2 shows that tissues of the upper respiratory and digestive tract, spleen, liver, and lymph nodes, revealed more paracoccidioidal lesions than grossly suspected. Also fungal lesions in myocardium, kidneys, bone marrow, and thyroid gland, and fungi in pleural effusions, were seen only microscopically. On the other hand, tumorous lesions, eventually suspected as fungal, in skin, mediastinum, and bowel, were recognized histologically as tumors, and granulomas in viscera as being of tuberculous and not mycotic origin. In two

cases dysenteric ulcerative colitis was diagnosed as such, in one, with additional apparently hematogenous foci of P.

The *lungs* were involved in *all* our autopsy cases; in one case, they were the only organs involved. BRASS (1966) found 12 cases with exclusive lung involvement. Pulmonary involvement was considered rare by BUENGELER (1942) and AZULAY (1963) and secondary by EMMONS *et al.* (1963), apparently based on clinical or biopsy examinations. In autopsies, these organs showed fungal lesions in a high percentage: 24 of 33 (BRASS, 1966); 21 of 25 (FIALHO, 1960); and 9 of 11 (ANGULO, 1948).

Lesions in the pleura were described as frequent by AZULAY (1963); FIALHO (1960) mentions a case of perforation into the pleura of a paracoccidioidal cavity, the picture not showing these features clearly. Pleural adhesions were frequent in our cases. The finding of fungi in pleural effusions (twice in our small series) suggests the possibility of clinical diagnosis of P. by cytologic examination of fluids. Gross pleura perforation has not been seen; in one the presence of microabscesses in the pleura suggests that the organisms can reach the pleural cavity by perforation of small lesions.

The frequency of paracoccidioidal lesions of the *mucosa of the oral cavity, pharynx, and larynx* was as high as in other autopsy series. It must be admitted that small lesions could have been overlooked and that no systematic histologic examinations were done of these sites. On the other hand, not all lesions of these sites were of paracoccidioidal origin and the mucosa was not always ulcerated, suggesting the possibility of contamination by hematogenous spread.

Intestinal lesions were seen only twice, apparently secondary and due to hematogenous dissemination. BRASS (1966) observed intestinal lesions only once in more numerous autopsy material. ANGULO (1948) reports four cases of this kind out of 11. BUENGELER (1942), EMMONS *et al.* (1963), and FIALHO (1963) considered intestinal involvement as common. When ulcerative lesions in the intestinal mucosa are present, in our environment the possibility of associated dysenteric lesions must be considered, as was the case in two of our observations. P. can only be accepted when fungus-containing lesions are exclusively present histologically at these sites.

Lymph-node involvement was not the rule, as was noted also by BRASS (1966), who describes paracoccidioidal lesions only in one third of his autopsies. In our series, mediastinal lymph-node involvement was found in 8 of the 11 cases, the lymph nodes always being examined histologically without showing gross lesions. In the abdominal cavity, mesenteric lymph-node involvement was observed twice in the two cases of intestinal paracoccidioidomycosis, whereas BRASS (1966) generally saw involvement of paraaortic subdiaphragmatic lymph nodes, in contrast to majority reports in the literature. BUENGELER (1942) reports involvement of lymphatic tissue as very characteristic, FIALHO (1960) found it almost always involved, and AZULAY (1963) states that it always becomes involved in early or later stages of evolution. Both BRASS (1966) and BUENGELER (1942) commented on the aspect of lesions resembling those of HODGKIN'S disease.

Adrenal fungus disease was found only in four of our cases. More or less the same proportion was observed by FIALHO (1960) — 8 out of 25. Hence, the high frequency of lesions of these organs reported by BRASS (1966) — 27 out of

33 cases — seems amazing. It is not likely that lesions in our series were overlooked, since both adrenals were examined microscopically in all cases.

Liver and *spleen* involvement in the form of small granulomas was proved in about 50% of our cases. No large nodules in the spleen (FIALHO, 1960) were observed. Neither paracoccidioidal lesions in bone with osteomyelitis (FIALHO, 1960), of the aorta with thrombosis (ANGULO, 1948; BRASS, 1966), of the central nervous system (ANGULO, 1948; DOMINGUEZ, 1961; FETTER *et al.* — review of 40 cases — 1967), nor of the pancreas (EMMONS *et al.*, 1963) were observed in our material.

Besides granulomas in solid viscera, a mixture of tissue reactions prevailed in almost all lung, lymph-node, and partial adrenal lesions, which makes specific classification impractical. The absence of calcifications was noteworthy. Only ANGULO showed pulmonary lesions with calcifications in one case; intraabdominal calcifications (ROCHA *et al.*, 1966) were not proved anatomically as paracoccidioidal. Neither was "specific" vasculitis seen, as it commonly is in generalized experimental and human histoplasmosis (AKBARIAN *et al.*, 1964; SALFELDER *et al.*, 1965), in the form of Weigert's "Venentuberkel".

Fibrosis and scar formation in the lungs led to sclerotic vascular lesions and narrowing of arteries, the cause of frequently observed cor pulmonale in our series. Paracoccidioidal pneumonia with numerous intraalveolar leukocytes and intraalveolar granulomas, intraalveolar carnification, and hyaline membranes, as observed in our material, are not common features. We hesitate to speak of allergic reactions (BUENGELER, 1942; FIALHO, 1960) in this disease.

As previously mentioned, organisms in tissues were best seen using Grocott's procedure (GROCOTT, 1955). It is amazing that silver methods were not used earlier since BUENGELER reported their usefulness as early as 1942. Because of the varying size of the fungus cells, they must be differentiated mainly from *B. derm.*, *C. imm.*, and *C. neof.*, with poor capsule formation, and *H. caps.* In one of our cases, fungus cells showed morphologic similarities with *Pneumocystis carinii.* The multiple budding is so characteristic that diagnosis can be based on histologic grounds, cultures not being absolutely necessary. Diagnostic problems arise only when few organisms are present in tissues and when "steering wheel" forms are not seen. The occurrence of hyphae is exceptional, they were found in only one of our cases, in a necrotic zone with breakdown of tissue.

Fungemia (BUENGELER, 1942), with numerous organisms in blood vessels and lying free in tissues without reaction, was not seen in our series. This finding is common in other deep mycoses, as, for instance, in cryptococcosis (SALFELDER, 1969).

In accumulations of large numbers of fungus cells in the tissue, the Grocott-positive dustlike particles represent fungus-cell detritus, which has been described also in other deep mycoses (SALFELDER and SCHWARZ, 1967; SALFELDER, 1969). These dustlike structures alone in the tissues, however, cannot be taken as diagnostic of fungus (SWEANY, 1960). The birefringency of fungus cells of *P. bras.* which appear also as Malta crosses in sections of paraffin-embedded material with polarized light is due to artefacts in the preparation of the slides (SALFELDER, 1968).

The high incidence of associated diseases as observed in our material, as in the series of BRASS (1966) was amazing, and makes clinical and gross diagnosis difficult, requiring examination of all tissues to make certain the diagnosis and to ascertain the extent of each condition. While in our three cases of P. with tuberculosis it was impossible to tell which infection had taken place first, in the case of P. and generalized histoplasmosis the tissue reaction of the latter, consisting of histiocytic proliferation and necrosis without formation of granulomas, suggested that they had developed more recently than the paracoccidioidal lesions.

Various portals of entry of the fungus elements are believed to exist, and traumatic lesions are especially supposed to facilitate the entrance of the agents into the organism. Since in the two first cases (LUTZ, 1908) the oral cavity was involved and paracoccidioidal lesions were subsequently observed mainly at this site, the general agreement is that the mucosa of the upper digestive and respiratory tract is the portal of entry (BUENGELER, 1942; FIALHO, 1960; AZULAY, 1963). Observations of dental paracoccidioidal granulomas (BOGLIOLO, 1949; FONSECA, 1957; POLLAK and RODRIGUEZ, 1957) also have pointed to this site as possible portal of entry.

The frequent observation of intestinal involvement has given rise to the belief by some that the intestinal tract is the portal of entry, a theory which can hardly be maintained in view of the rare involvement of this site as seen in autopsy series. The question arises as to why infection in paracoccidioidomycosis should be completely different from what has been accepted generally in other deep mycoses, as in North American blastomycosis, coccidioidomycosis, histoplasmosis and cryptococcosis? As early as 1956, GONZALEZ OCHOA assumed that the lung is the portal of entry, and other recent authors are of the same opinion (LONDERO and FABRICIO, 1966; MACKINNON, 1968).

Several data give support to this assumption. Examination of 100 dental (apical) granulomas rendered positive results only in patients with pulmonary paracoccidioidomycosis (POLLAK and GARCIA LOPEZ, 1961). In systematic histologic examinations of dental granulomas, no paracoccidioidal granuloma was ever found (Mérida, unpublished data). Thus paradental P. can be interpreted as a secondary lesion; properly looked for lung lesions will always also be discovered. In autopsy series, frequency of lung lesions is high (BRASS, 1966; FIALHO, 1960; ANGULO, 1948); in our small autopsy material, pulmonary lesions were always observed. The same was true in all our biopsy cases with the exception of one, in which no pulmonary X-ray lesions were seen. Further, unilateral lung involvement (BRASS, 1966) and exclusive lung involvement (BRASS, 1966 and our series) can hardly be interpreted as secondary involvement.

Unfortunately, there are no other ways to prove primary lung paracoccidioidomycosis, since primary complexes as in tuberculosis and histoplasmosis, residual lung and/or lymph-node foci, do not form in P. and since calcifications do not occur or are extremely rare. If calcifications exist, it must be determined whether they are of histoplasmic origin. Our case with old pulmonary scars showing extensive hyalinosis and dust pigment did not contain fungi and can, therefore, not be interpreted as paracoccidioidal. *The almost exclusive occurrence of the disease in certain male age groups of the rural population points to an occupational*

exposure which suggests inhalation of the fungus. It is hoped that further studies will bring forward more evidence of the portal of entry.

The hematogenous route of dissemination after primary infection seems to be the most favored in P. Dissemination by the lymphatic route does not occur so frequently and uniformly as in tuberculosis and histoplasmosis, and there is little evidence of the canalicular route of dissemination to the digestive tract.

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Dr. K. Salfelder (Prof. Titular)
Instituto de Anatomía Patológica de la Universidad de Los Andes
Apartado 75
Mérida/Venezuela, S. A.