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Fatal Disseminated Histoplasmosis

Anatomic Study of Autopsy Cases

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Summary. Nineteen cases of generalized histoplasmosis, autopsied in four areas of Venezuela, are described. Some of these have been reported previously.

Patients were mainly from a highly-endemic area south of Lake Maracaibo and from the town of Valencia, where sources of infection have not yet been thoroughly explored.

As the lesions were generally not characteristic, no gross diagnosis was made unless smears were properly examined. No cavitary forms were observed.

More tissues and organs were involved than were suspected from gross examination or reported previously except when the Grocott method of staining was used.

Inflammatory reactions in the tissues in the same case may show histiocytic proliferation or be granulomatous or be entirely inconspicuous. Fungi in tissues and organs should be looked for with the Grocott method; they may be overlooked in H and E stained sections. A large number of diseases were associated with histoplasmosis as previous debilitating conditions or as final complications. Causes of death were variable, but most were from organic failure associated with the fungal disease.

The portal of entry of the organisms was the respiratory tract. The numerous acute bilateral pulmonary lesions in this form of histoplasmosis are thought of as multiple primary foci.

Zusammenfassung. Es werden 19 Fälle von disseminierter Histoplasmose beschrieben, die an 4 Stellen in Venezuela seziert worden waren. Einige von ihnen waren schon Gegenstand von Publikationen. Eine Häufung der Fälle wurde in einem schon bekannten Endemiegebiet mit hoher Infektionsrate im Süden des Maracaibosees beobachtet und außerdem in der Stadt von Valencia. In letzterer sind die Infektionsquellen noch nicht festgestellt worden. Die makroskopischen Befunde bei der Autopsie waren meist nicht charakteristisch, sodaß die Diagnose nicht gestellt wurde; außer in Fällen, in denen Ausstriche mit der entsprechenden Technik untersucht wurden. Kavitäre Formen kamen nicht zur Beobachtung. Histoplasmotische Gewebsveränderungen wurden in mehr Organen gefunden, als makroskopisch vermutet und in anderen Kasuistiken, ohne die Anwendung der Grocott-Methode, beschrieben wurde.

Die Gewebsreaktion kann in einer histiocytären Proliferation bestehen, granulomatös sein oder uncharakteristische Merkmale aufweisen. Mehrere dieser Gewebsreaktionen können in einem einzigen Fall vorkommen.

Bei der Suche nach Pilzen im Gewebe sollte die Grocott-Methode angewendet werden. In hämatoxylin- und eosingefärbten Schnitten wurden die Pilze in einer gewissen Zahl von Fällen und in manchen Geweben und Gewebsveränderungen nicht gefunden.

Beachtlich war die Beobachtung zahlreicher anderer pathologischer Befunde und Krankheiten in den Histoplasmosefällen. Es handelte sich teilweise um vorher vorhandene Erkrankungen oder um finale Komplikationen.

Die Todesursache war verschieden; meist handelte es sich um Organinsuffizienzen, die durch die Pilzinfektion verursacht waren.

Die Eintrittspforte des Erregers sind die Atemwege.
Die zahlreichen frischen bilateralen Lungenveränderungen bei dieser Form der Erkrankung können als multiple Primärherde gedeutet werden.

Reports on studies of generalized forms of this deep mycosis based on a large number of necropsy specimens are scarce. The papers by Schulz (1954) and Binford (1955) refer exclusively to cases in the United States of America and were written before the Grocott method (Grocott, 1955) for demonstration of fungi in tissues was in use. Residual foci of the disease in tissues have been studied exhaustively (Straub and Schwarz, 1955; 1960, 1962; Baker, 1964; Salfelder and Liscano, 1965).

Diagnosis of histoplasmosis in tissues may be still difficult (Schwarz, 1968), especially when the inexperienced examiner reviews only H and E sections. Many false-positive diagnoses were made before (Martz *et al.*, 1947; Raftery, 1951; Collier and Winckel, 1952; Wildervauck *et al.*, 1953) and also after the introduction of the use of Grocott's method (Collins, 1957; Rosenbaum *et al.*, 1964; Bank *et al.*, 1965; Gomba and Szokoly, 1967; Correa and Pacheco, 1967; Segal *et al.*, 1969). The comment by Emmons *et al.* (1963) is still valid: that "fatal disseminated histoplasmosis is a relatively rare disease even in endemic areas, and many pathologists with average hospital practice may not have the opportunity to study a case in their postmortem practice".

Therefore, it seemed desirable to review, with modern technics, a collection of necropsy material, with emphasis on organ involvement, morphology of fungi in tissues, tissue reaction, and pathogenesis. As in a previous study on paracoccidioidomycosis (Salfelder *et al.*, 1969) mention is made of which tissues were examined histologically. Further, the study permits discussion of the point of multiple lung foci in primary infection.

Material and Methods

Specimens from nineteen fatal cases of disseminated histoplasmosis in Valencia (8), Mérida (7), Barquisimeto (3) and Ciudad Bolivar (1) were studied. Of these cases, four have been described in previous publications and several others mentioned briefly in a local publication. Case 15 was reported as a fatal laboratory infection in a mycologist (Hartung and Salfelder, 1962); in case 16, generalized histoplasmosis was mentioned as secondary to a disseminated paracoccidioidomycosis (Salfelder *et al.*, 1969); case 17 was published because of erythema nodosum associated with a histoplasmic infection (Salfelder, 1964) and case 18 was described because of differential-diagnostic problems (Salfelder, 1960). The histoplasmosis cases from Valencia seen before 1966 were included in a survey of deep mycoses (Brass, 1966).

One fatal case of histoplasmosis (Mérida, E. 32197) from 1968 was not included, since no autopsy had been done. This 50-year-old Venezuelan agricultural worker from La Palmita, a village in an endemic region south of Lake Maracaibo, showed bilateral pneumonic lesions on X-rays. In a cervical lymph-node biopsy, diffuse proliferation of vacuolic histiocytes, containing numerous *H. capsulatum* with beginning necrosis, was found. The organisms were recognized on H and E and confirmed by the Grocott method. The patient died before the biopsy report was received in the Hospital of El Vigia.

Hepatomegaly and splenomegaly were determined on the basis of the data of Roessle and Roulet (1932). Paraffin sections were stained with hematoxylin and eosin and by the Grocott method. Further, in many cases sections were examined after staining by the Gridley, PAS, Weigerts Fibrin (gram), Goldner, Ziehl-Neelsen, Giemsa, and methylene blue methods.

Cultures were made only in case 15, in which a positive sputum was obtained.

Observations

Clinical Data

As Table 1 reveals, ages of the patients ranged from 2 months to 60 years. The ratio of male to female was 14:5. Among the adults were three agricultural workers, one mason, one housewife, and one mycologist. Patients were principally from rural areas or from the town of Valencia. Duration of symptoms before hospitalization varied greatly. Time of hispitalization was less than 1 week in nine cases and more than 3 weeks in five cases.

Clinical diagnosis of histoplasmosis was made only once, in the case of the mycologist with laboratory infection. Unfortunately, amphotericin B was not available in time for treatment.

Gross Pathology

Table 2 shows that pulmonary lesions were present in 18 cases. In one (case 10), no lesions were mentioned in the autopsy report. The lung lesions did not present a uniform pattern. Multiple nodules of bronchopneumonic or granulomatous character and of different sizes, alone or together with other lesions, were noted in 10 cases. Necrotic foci were noted in another 5 cases, of which three were solitary and two were multiple. These were localized partly in subpleural regions. More extensive pneumonic lesions were seen in five cases, once bilateral and diffuse and once with necrosis. Emphysema and indurations were found in two cases each, petechiae in three cases; in two of these, they were the only pulmonary lesions. Chronic edema and thrombosis of pulmonary arteries were found in one case each. No calcified foci were seen in the lungs. Pleural adhesions were found in five (cases 14, 15 and 18 bilateral and Nos. 4 and 10 unilateral). The mediastinal lymph nodes showed alterations in 11 cases. Solitary or multiple necrotic foci of different sizes, up to 3 cm in diameter, or miliary granulomas were seen in nine cases. In six cases the mediastinal lymph nodes were enlarged: of these, four also showed necrotic foci or granulomas. Four nodes contained foci without enlargement. The abdominal lymph nodes showed gross lesions in ten cases. In six they were only enlarged, in three they showed only necrotic foci or nodules, and in one case both types of lesions were present.

Intestinal lesions were observed in seven cases, in the large bowel alone in five cases and in the ileum and large bowel in two cases. These were generally seen as numerous ulcers in the mucosa. Once a solitary ulcer was noted, and in one other case necrotizing colitis was found.

In the liver, miliary foci were present in six cases, in most of them multiple; in one case only two foci were seen. In one case the liver was not weighed. In nine it was considerably enlarged.

The spleen showed multiple foci in five cases and a single focus in one. In one case anemic infarcts were present. The spleen was not weighed in one case. In twelve, splenomegaly was found.

In nine cases, anemia was observed and in six, jaundice. In the mucosa of the upper respiratory tract, ulcerations were seen in three cases. In the kidneys, miliary nodules were noted in two cases. The adrenals showed necrotic lesions also in two instances. In the brain, petechiae were seen once and once extensive hemorrhage. Thrombosis in the venous sinus of the dura mater, diffuse hemorrhage in the pancreas, and skin nodules were observed in one case each.

Table 1. *Clinical features*

Case No.	Year	Age	Sex	Residence	Duration sympt.	Hosp. days	Clinic. diagn.	Treatment	Other data
1. A. 935 V	1966	2 months	♂	San Diego Carabobo Syndrome	?	18	Hepatosplenic Syndrome	?	—
2. A. 259 V	1956	$2^1/_2$ months	♂	Yagua Carabobo	?	1	?	?	—
3. A. 474 V	1962	5 months	♂	Valencia Carabobo	2 months	28	Leucosis ? Hemolytic anemia, acquired	?	—
4. A. 5,735 B	1961	6 months	♀	Las Piedras Yaracuy	?	31	Pyelonephritis	?	—
5. A. 2,984 M	1965	9 months	♀	El Vigía Mérida	5 months	17	Pneumonia. Cardiac Insuf.	Antibiot.	—
6. A. 427 V	1969	10 months	♂	Valencia Carabobo	Several times ill	2	?	?	—
7. A. 576 V	1968	11 months	♀	San Carlos Cojedes	31 days	1	Hepatosplenic Syndr. Kala Azar ?	?	—
8. A. 3,306 M	1966	1 years	♂	Tucanizon Mérida	8 days	2	Bronchopneumonia Anemia	?	—
9. A. 3,935 M	1968	$1^1/_2$ years	♀	Cuatro Esquinas Zulia	?	41	Ameb. Dys. Int. Paras. Anemia; Bronchopneumonia	Antianemic. Antiparasit.	—
10. A. 2,380 M	1962	4 years	♂	Sta. Bárbara Zulia	1 month	13	Ameb. Dys. Anemia	Antianemic. Blood Transf.	—

Table 1. Continued

Case No.	Year	Age	Sex	Residence	Duration Sympt.	Hosp. days	Clinic. Diagn.	Treatment	Other data
11. A. 17,563 B	1968	4 years	♂	Yucurito Portuguesa	2 months	4	Tuberculosis ?	Antibiot.	—
12. A. 558 V	1962	5 years	♂	Valencia Carabobo	7 days	1	Encephalitis ?	?	—
13. A. 71 V	1968	16 years	♂	Valencia Carabobo	$3^1/_2$ years	3		Steroids	Treated also in USA
14. A. 48CB	1961	41 years	♂	Ciŭdad Bolivar Bolívar	3 months	31	Chron. Hepatitis	?	—
15. AS. 75 M	1960	45 years	♂	Mérida Mérida	50 days	13	Histoplasmosis	Sulfonamides	Ital. immigr. Lab. Infect.
16. A. 4,473 B	1960	46 years	♂	Araure Portuguesa	3 months	4	Paracoccidioido-mycosis	?	—
17. A. 2,191 M	1961	50 years	♀	Jají Mérida	9 days	2	Bronchopneumonia	?	—
18. A. 1,295 M	1956	52 years	♂	Sta. Bárbara Zulia	22 days	10	Bronchopneumonia	Antibiot.	Ital. immigr.
19. A. 783 V	1958	60 years	♂	El Palido Carabobo	21 days	24	Extrarenal Uremia	?	—

AS = case from Tuberculosis Sanatorium, B = case from Barquisimeto, CB = case from Ciudad Bolivar, M = case from Mérida, V = case from Valencia.

Table 2. *Gross*

Case No.	Lungs	Med. Ly Nod	Int. Tract	Abd. Ly Nod
1.	Micronod. Bronchopn. bil.	—	Necrotiz. Colitis	—
2.	Small nod. and Petechiae bil.	—	—	Enlarged
3.	Bronchopn. Nod. and atelectasia unilat. Chronic edema	—	—	—
4.	Subpleur. Necrot. focus 0.7 cm	Necrot. focus 0.3 cm	Numer. ulcers large bowel	Mil. nod.
5.	Subpleur. Necrot. focus 4:3 cm Confl. Pneumon. contralat.	Necrot. foci 2 and 3 cm	Numer. ulcers ileum a. large bowel	Necrot. foci
6.	Petechiae bilat.	—	Ulcers large bowel	Necrot. foci
7.	Petechiae bilat.	—	—	—
8.	Confluent Bronchopn. and mil. foci, bilat.	Necrot. foci 1 cm	—	—
9.	Subpleur. Necrot. focus 0.2 cm Necrotiz. Bronchopn. contralat.	Necrot. foci 1 cm	—	—
10.	—	Necrot. focus 0.3 cm	Numer. ulcers necros. large bowel	—
11.	Multiple foci 0.3 cm bilat.	Enlarged	Numer. ulcers ileum large bowel	Enlarged
12.	Bul. and interstit. Emphysema	—	—	Enlarged
13.[a]	Mil. foci bilat.	Enlarged	—	Enlarged
14.	Indurat. and Necrot. foci sup. lobe. Mil. foci bilat.	—	—	—
15.	5 necrot. foci 0.5 cm bilat. Mil. granulom. Art. Thromb. Hemorrhag. Infarcts	Mil. foci Englarged	—	—
16.	Emphysema. Dif. Pneumon. in one lobe	Necrot. foci Enlarged	Ulcers large bowel	Necrot. foci. Enlarged
17.	Subpleur. Indurat. left sup. lobe	Mil. foci Enlarged	—	Enlarged
18.	Dif. Pneumonia bilat. Mil. bilat. foci	Necrot. foci Enlarged	—	Enlarged
19.	Mil. foci bilat.	—	—	—

[a] Brain not examined. [b] Diagnosis made by smears. Weight of liver and spleen in g.

pathology

Liver	Spleen	Other organs	Gross Diagn.
80	130 (2.75)	Anemia	Bronchopneumonia
185	135 (3.3)	Brain Petechiae Jaundice	Visceral Leishmaniasis
320	155 (6.35)	Anemia	Bronchopneumonia ? Hepatosplenomegaly
214 Mil. nod.	56 (3.0) Mil. nod.	Anemia	Histoplasmosis[b]
326	43 (7.0)	Anemia	Tuberculosis Bronchopneumonia
360	70 (5.9)	Dif. Hemorrh. Pancr.	Primary intestinal tuberculosis
404	150 (9.2)	Anemia Jaundice	Visceral Leishmaniasis or Tuberculosis
370	56 (6.5) Subcaps. Nod. (0.2 cm)	Anemia	Histoplasmosis[b]
350	50 (4.6)	Thromb. sin. ven. sag. sup.	Histoplasmosis ?
590	85 (14.0)	Anemia Jaundice	Dysenteric Colitis
165	160	Phar. and lar. ulcers Anemia	Histoplasmosis[b] Bronchopneumonia
380 2 Nod.	35 (9.75)	Necrot. foci left adrenal	Tuberculosis ?
3,400 Mil. foci	1,800	Trach. bifurcat. ulc. Anemia Jaundice	Malign. Lymphoma
1,770	1,210 Anemic infracts	Jaundice	Tuberculosis
Mil. foci	Mil. foci	Mil. foci kidney	Histoplasmosis Pulm. Infarcts
1,772	335	Ulc. Oral cav. a. trachea Jaundice	Paracoccidioidom. and Visceral Leishmaniasis[b]
1,325 Mil. foci	70	Skin nod. sup. extrem.	Tuberculosis
1,925 Mil. foci	350 Mil. foci	Mil. foci kidneys	Tuberculosis Bilat. Pneumonia
1,300	210	Gen. Arterioscl. Old cerebr. inf. Necr. c. adren.	Amyloidosis, Uremia Tuberculosis

( ) = Body weight in kg.

Table 3. *Tissues examined*

Organs Cases	1	2	3	4	5	6	7	8	9
Skin				×					
Tonsils				×					
Trachea									×
Larynx									
Sal. gland									
Lungs	×+	×+	×+	×+	×+	×+	×+	×+	×+
Med. Ly. No.	×+	×+		×+	×+		×+	×+	×+
Esophag.	×								×
Stomach									
Small bowel				×+		×	×+	×	×
Duod.								×+	×
Jejun.								×+	×
Ileum								×+	×
Large bowel	×+			×+	×+			×+	×+
Apend.									×+
Col. asc.								×+	×+
Col. transv.								×+	×+
Col. desc.								×+	×+
Sigmoid								×+	×+
Rectum								×+	×+
Abd. Ly. Nod.		×+			×+	×+	×+	×+	
Liver	×+	×+	×+	×+	×+	×+	×+	×+	×+
Spleen	×+	×+	×+	×+	×+	×+	×+	×+	×+
Pancreas	×	×+		×	×	×+		×+	×
Myocard.		×+	×+	×	×		×+	×	×
Kidneys		×+	×	×	×			×+	×
Testes									
Brain	×+	×+		×	×				×
Spinal cord					×				×
Venous Sinus									×+
Thyr. gland.				×	×				×
Adr. gland.					×+			×+	×
Spin. Col.	×+			××+					×+
B. M. femur		×+	×+				×+		
Organisms H. E.	+	+	+	+	+	+	+	+	−

× = examined, + = positive.

Gross diagnosis was histoplasmosis (in the mycologist with the positive sputum) in one case; histoplasmosis three times, based on examination of smears from the cut pulmonary surface; and once "on suspicion". Tuberculosis alone or with other diseases was assumed in seven cases, in one a primary intestinal infection. Diagnosis of pneumonia alone or together with other diseases was made in five cases and of visceral leishmaniasis alone or with other conditions in three. One case each was diagnosed as hepatosplenomegaly, malignant lymphoma, paracoccidioidomycosis and dysenteric colitis or amyloidosis.

histol./mycotic lesions

10	11	12	13	14	15	16	17	18	19	Total Posit.	Ex/
							×			2/—	
	×+					×+		×+		4/3	
						×+				1/1	
						×+				1/1	
	×							×		2/—	
×+	×+	×	×+	×+	×+	×+	×+	×+	×+	19/18	
×+	×+		×+		×+	×+	×+	×+		14/14	
										2/—	
						×+				1/1	
×+	×+					×+				6/4	
	×+									3/2	
										2/1	9/8
	×+									3/2	
×+						×				7/6	
										1/1	
										2/2	
										2/2	
										2/2	
										2/2	
										2/2	
×+	×+		×+		×					9/8	
×+	×+	×+	×+	×+	×+	×+	×	×+	×+	19/18	
×+	×+	×	×+	×+	×+	×+	×+	×+		18/17	
×	×		×		×			×	×	13/3	
×	×+	×	×	×	×	×+	×+	×+	×	17/7	
×	×+	×		×+	×+	×+	×	×+	×	15/7	
	×					×+				2/1	
	×+					×+				7/4	
	×									3/—	
										1/1	
	×+					×		×	×	7/1	
×	×+	×+			×	×+	×	×+	×+	11/7	
			×					×+		5/4	9/8
						×+				4/4	9/8
−	+	−	−	−	−	+	−	−	−	10	

Organs Involved

As Table 3 reveals, many tissues were not previously examined histologically, including skeletal muscle, great blood vessels, genital organs with the exception of testes, and the pituitary and thymus glands. The skin, trachea, larynx, salivary glands, esophagus, stomach, testes, and venous sinus of the dura madre were examined in only one or two cases; tonsils and spinal cord in only three, four or five; intestinal tract, abdominal lymph nodes, brain, thyroid gland, and bone marrow in six to nine instances. In 11 cases, the adrenal gland was reviewed, and

Table 4. *Tissue reactions*

Tissue reaction[a] classifications	Case numbers	Total of cases
Histolocytic	3	1
Histio-Necrotic	2; 11	2
Necrotic-Epithelioid	14	1
Histio-Nec-Epi.	1; 4; 5; 9; 10	5
Histio-Nec-Epi. with giant cells	6; 16	2
Histio-Nec.-Epi. c. g. cells y sclerohyalinosis	18	1
Histio-Nec. giant cells	8	1
Histio-Epi. y giant cells	7	1
Tubercul. granul.	12; 13; 15; 17; 19	5

[a] Recorded even if found only in a single organ.

in 13 to 19, lungs, mediastinal lymph nodes, liver, spleen, pancreas, myocardium, and kidneys.

Tissues not involved (in addition to those not examined) included skin, salivary glands, esophagus, and spinal cord. In all the other organs examined, mycotic lesions were found. In sequence of absolute frequency, they were observed in the lungs and liver (18), spleen (17), mediastinal lymph nodes (14), abdominal lymph nodes, bone marrow, intestinal tract (8), adrenal glands, kidneys, myocardium (7), brain (4), and tonsils and pancreas (3 cases). The highest relative frequency was found in the mediastinal lymph nodes, followed by the lungs, liver, spleen, bone marrow, intestinal tract, tonsils and adrenals.

In three (cases 8, 9 and 11), in which different parts of the small and large bowel were examined, diffuse and extensive involvement was found, with the exception of case 9, in which the entire small bowel was spared but there was a diffuse involvement of the large bowel. In the cases in which both small and large bowels were examined, the colon was more often involved.

Histoplasmotic Tissue Reaction

Tissue reactions varied considerably. For purposes of classification, nine groups were recognized (Table 4). Most frequent were histiocytic proliferation with necrosis, followed, in decreasing order, by epitheloid cell reaction and tuberculoid granuloma formation.

In one case of tuberculoid granuloma were seen simultaneously necrotic foci without cellular reaction, and in another case calcifications were observed. Necrosis was not always found in lymph node lesions even when present in the lungs, and vice versa.

Histoplasmic alterations of blood vessels were found in 13 cases, nine times in the lungs, five times in the spleen, and once each in the liver and in the adrenal gland. Although generally found in venous walls, in 3 (cases 2, 15 and 17) the lesions were in arteries (Fig. 1), once with organized thrombosis. The lesions in the

vein walls consisted of cell infiltrations and thrombotic accumulations of nucleated blood cells attached to the wall. Often a striking similarity to the classic "Venentuberkel" of Weigert was seen. Frequently, histoplasmotic inflammation in the neighborhood of blood vessels apparently had invaded the vessel wall.

Lungs

The lesions in the 18 cases of lung invasion were of three types: a) Multiple bilateral pneumonic foci of different sizes (11), b) Inflammatory edema (3), and c) Cases with distinct granulomatous reaction (4).

a) In this group, in eight cases one or several larger pneumonic foci, mostly with multiple smaller ones, and in three cases only disseminated smaller foci, were seen; these ranged from submiliary to supermiliary size.

The pneumonic areas were not sharply circumscribed, with alveoli filled with large (Fig. 2) and smaller histiocytic cells which sometimes resembled epitheloid cells. In the interalveolar spaces were seen mostly mononuclear cells in variable amounts. Necrosis of different sizes in single or in all foci were seen in eight cases (Fig. 3).

In the neighborhood of the necrotic areas, epitheloid cells occurred in variable amounts. In four cases no necrosis was observed. Sometimes perifocal edema was seen. Scattered single giant cells of the Langhans type were seen in only three cases.

b) In three cases (1, 11 and 18) a diffuse bilateral pneumonia was observed. The alveoli showed an edematous content which resembled that of an alveolar proteinosis with few histiocytic elements (with Grocott-positive organisms) (Fig. 4). In addition, one specimen showed interstitial cell infiltrates; one, miliary necrotic foci; and one, small necrotic foci.

c) In four cases (13, 15, 17 and 19) lesions with tuberculoid granulomas were seen (Fig. 5); in case 15, five larger bilateral necrotic foci and a dissemination of miliary and submiliary granulomas in both lungs. In case 17, a large pneumonic area with necrotic foci and tuberculoid granulomas outside of this area were found. A dissemination of submiliary and miliary tuberculoid granulomas with necrosis was seen in case 13 and 19.

No scar tissue or calcifications were found in the lung tissue.

Lymph Nodes

In the mediastinal lymph nodes, histiocytic proliferation prevailed (11 cases) ranging from small local to diffuse general involvement. These regions of histiocytic proliferation appeared at low power in H and E-stained sections as clearer zones (Fig. 6). In two cases the histiocytes had a vacuolic appearance. Only in two cases was no necrosis found. The necrotic foci were of different sizes and consisted of homogeneously red-stained masses (in H and E); sometimes nuclear detritus in great amount was recognized. They were situated in the regions of histiocytic proliferations. In three cases epitheloid cells were seen in the periphery of the necrosis. Scattered and single giant cells were present in two cases (Fig. 7). In two, accumulations of numerous histiocytes were also seen in dilated lymph spaces (Fig. 8).

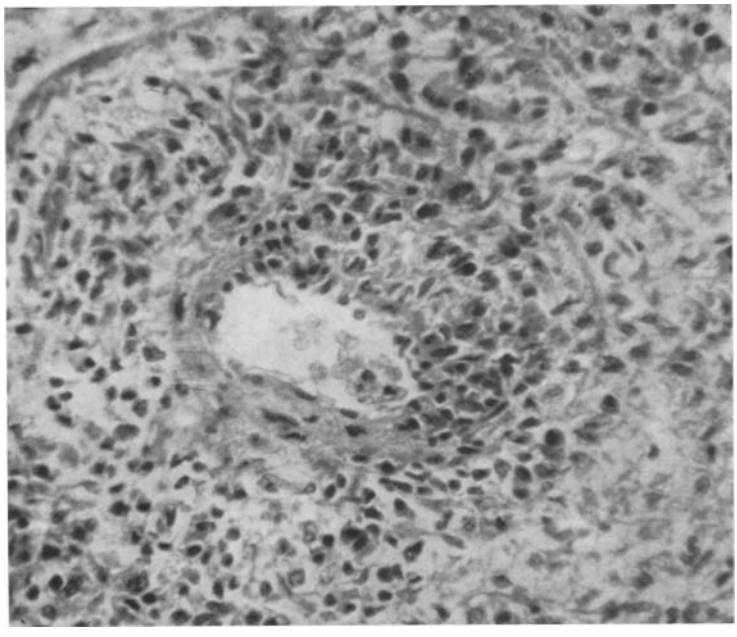

Fig. 1. Histoplasmotic arteritis. Perivascular cellular infiltration with diffuse invasion of arterial wall. Org. not visible at this magnif. Case 2; Lung; H and E; ×300

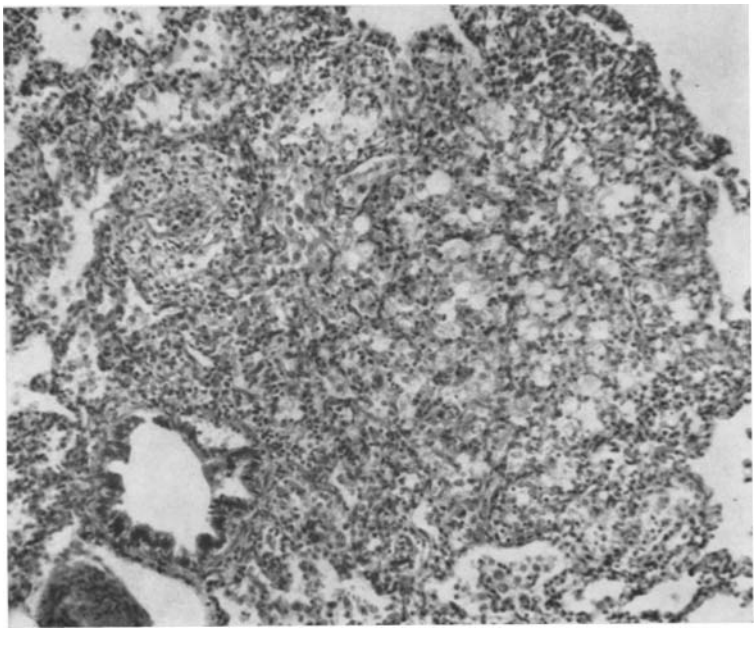

Fig. 2. Pneumonic focus with numerous vacuolized histiocytes. Case 2; H and E; ×85

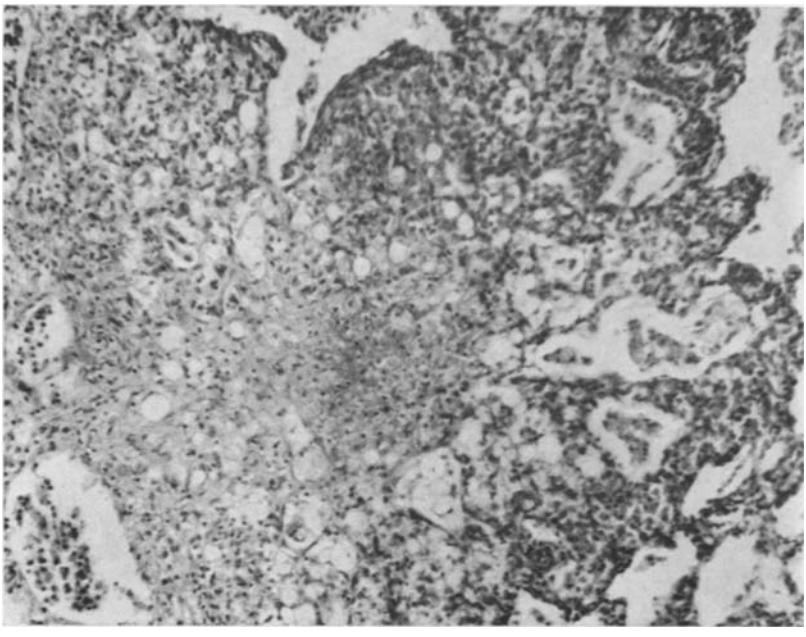

Fig. 3. Pneumonic focus with necrosis. Case 2; H and E; ×85

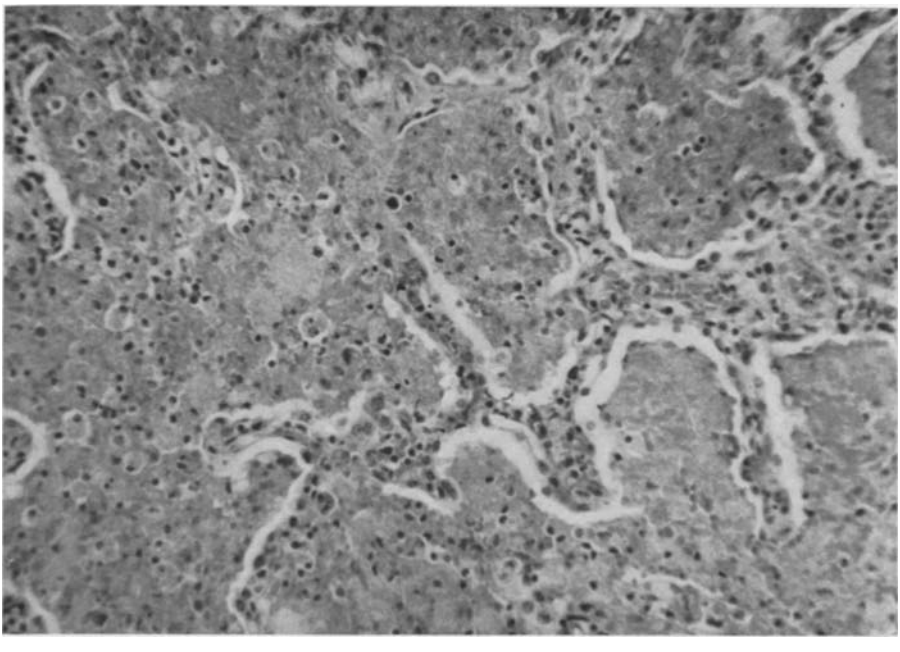

Fig. 4. Diffuse pneumonia with proteinaceus edema and few intraalveolar cells. Case 11; H and E; ×190

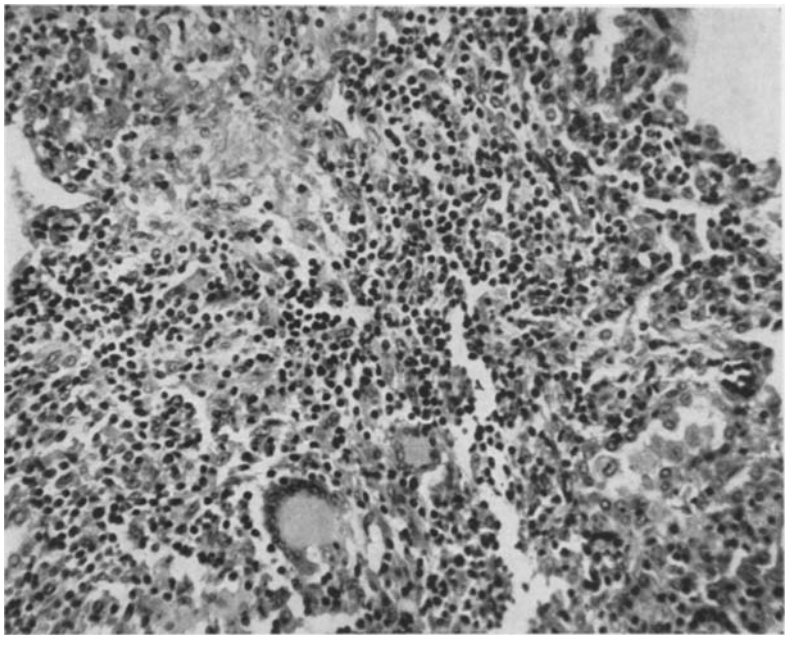

Fig. 5. Epitheloid cell granulomas, giant cells and lymphocytes. Case 13; Lung; H and E: ×190

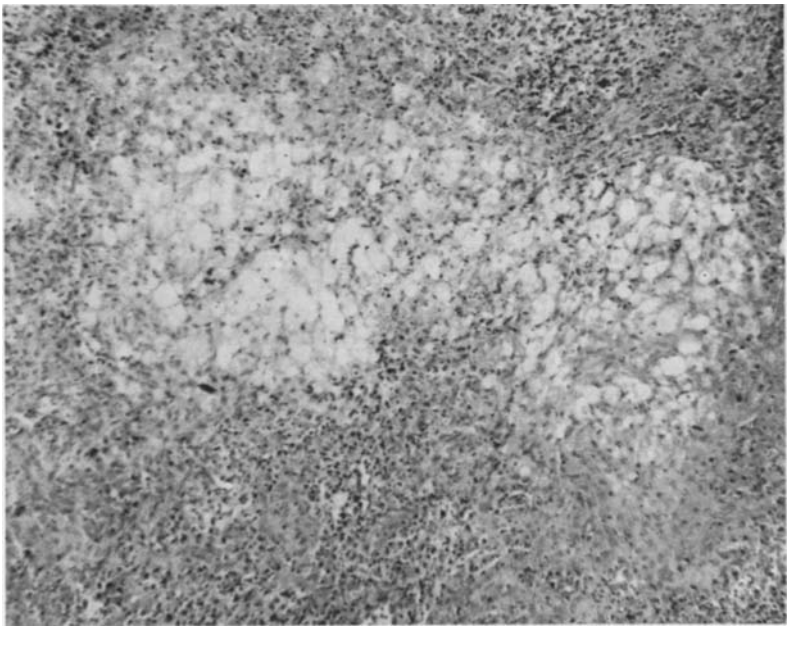

Fig. 6. Circumscribed vacuolized histiocytes with beginning necrosis. Org. not vis. at this magnif. Case 1; Med. lymphnode; H and E; ×85

Tuberculoid granulomas were found in cases 15 and 17, the first containing also necrotic foci. Only in one (case 18), extensive fibrosis and hyalinization with necrosis was detected. Necrosis involved bronchial walls with destruction of mucosa.

The lesions in the abdominal, usually mesenteric, lymph nodes consisted of proliferated histiocytes and necrosis.

Liver

In case 17, small cell nodules in the parenchyma, mentioned in the autopsy report, were not found on reexamination of a few slides. In 11 cases, a clear-cut proliferation of Kupffer's cells was seen (Fig. 9); in four, this was the only manifestation of histoplasmosis in this organ.

Small cell nodules in the parenchyma, consisting of histiocytes and epitheloid cells, once with a giant cell, were found in eight cases (Fig. 10). Submiliary and miliary necroses surrounded by epitheloid cells were seen in two cases, once (No. 18) alone. Cellular infiltrates were found in the portal fields four times, in one case with a small necrosis. Scattered single giant cells (Fig. 11) were seen in four cases, tuberculoid granulomas in two, and necrotic, partly calcified, foci with epitheloid cells in one case.

Spleen

Histiocytic proliferation in larger regions or in a diffuse form was found in seven cases. In a few cases, these alterations were easily seen because of the lighter appearance, in contrast to the darker normal spleen parenchyma or because of the vacuolic aspect of the histiocytes. Smaller histiocytic nodule, twice with necrosis, were found in another five cases. Submiliary or miliary necrotic foci alone were observed twice; in two other cases, scattered single giant cells. Tuberculoid granulomas of different sizes were present also in two cases (15 and 17). In case 14, the thrombotic accumulations of histiocytes containing organisms in veins were the only manifestation of histoplasmosis.

Bone Marrow

Mostly, more or less numerous, scattered macrophages containing organisms were found. In one case, macrophages were accumulated in small granulomatous nests. In another case, small necrotic foci surrounded by epitheloid cells were observed, and in yet another, in addition to scattered organisms containing macrophages, giant cells were seen. These were found singly or in small groups. No tuberculoid granulomas were detected.

Gastro-intestinal Tract

Lesions were seen in the mucosa and submucosa; only in one case were there tissue alterations extending deeply into the muscular layer reaching the serosa.

The cellular infiltration in these sites consisted of lymphocytes, small histiocytes, and occasionally a few plasma cells and eosinophilic leukocytes. These were found inside and outside of lymph follicles and were of variable intensity. In some cases and at some sites they were minimal and their histoplasmotic nature could be diagnosed only when organisms were found in Grocott-stained sections. In a total of six cases, superficial and mostly multiple necroses or ulcerations were seen in the mucosa.

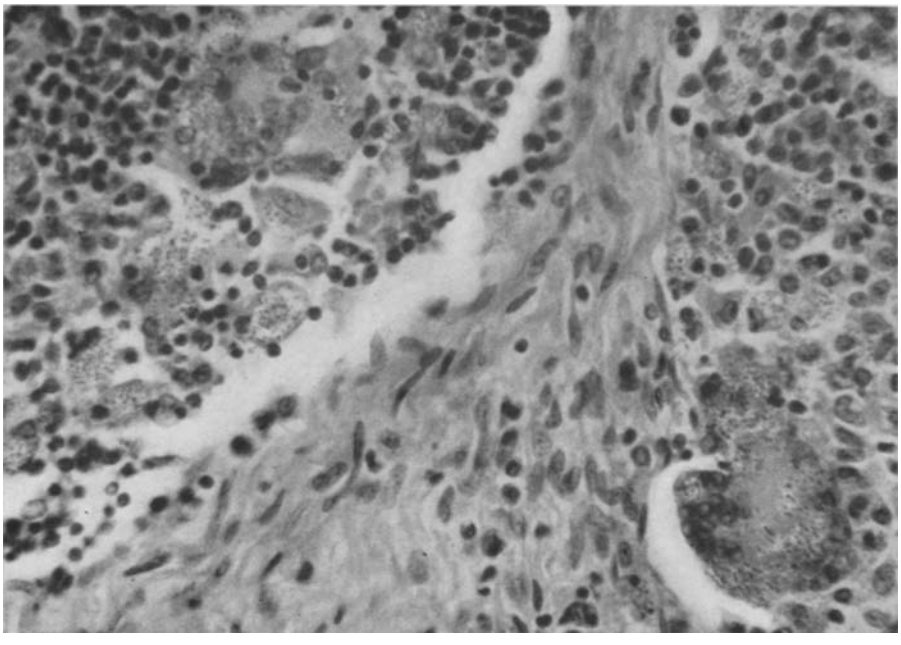

Fig. 7. Organisms in histiocytes and giant cells. Case 7; Med. lymphnode; H and E; ×275

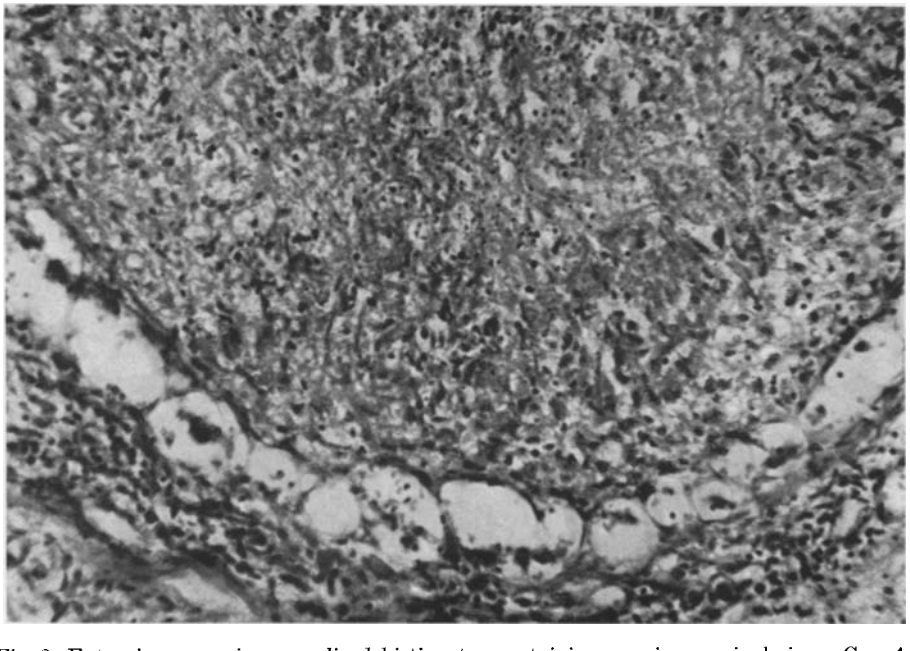

Fig. 8. Extensive necrosis; vacuolized histiocytes containing org. in marginal sinus. Case 4; Med. lymphnode; H and E; ×190

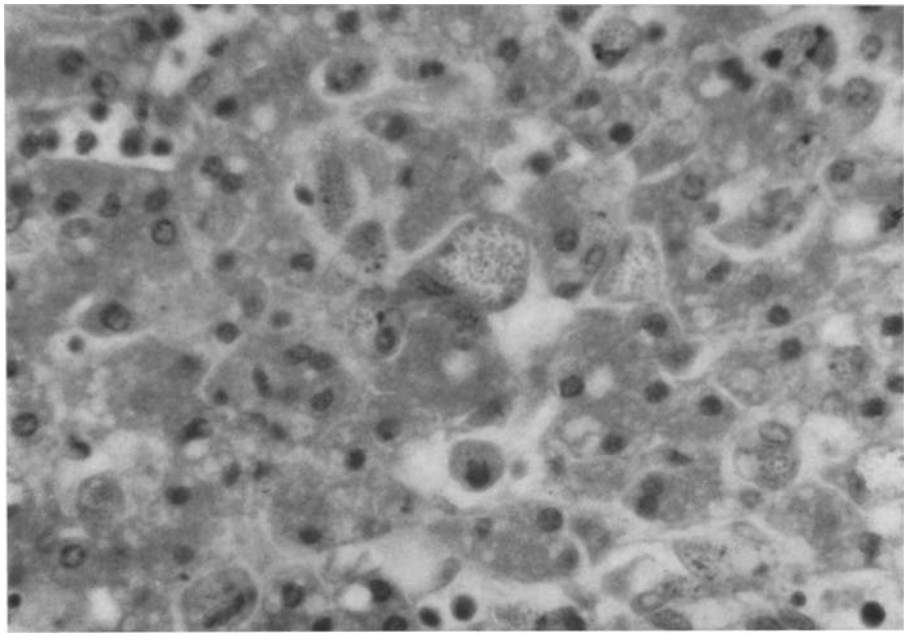

Fig. 9. Proliferation of Kupffer's cells containing org. Case 3; Liver; H and E; ×450

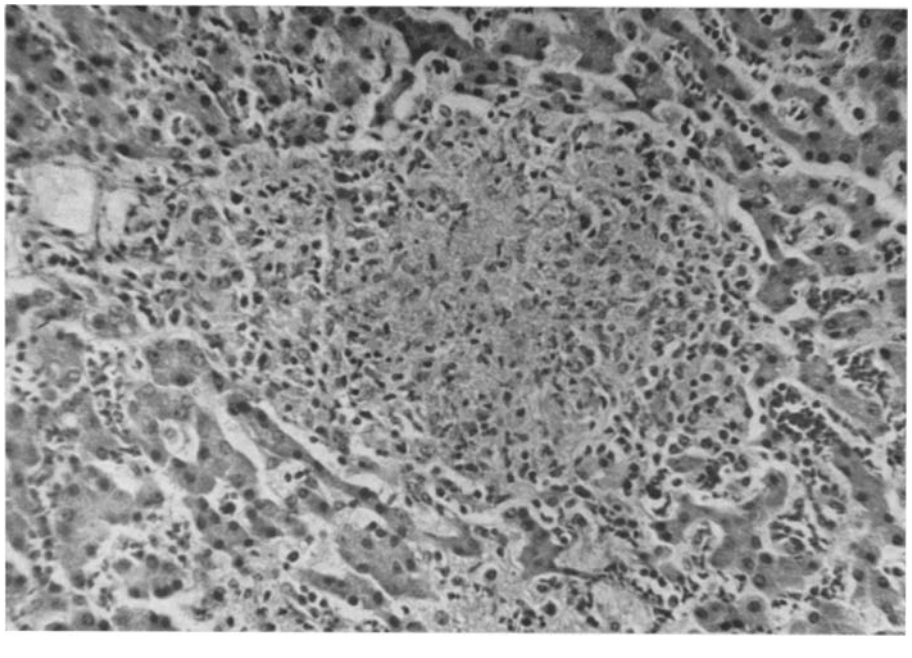

Fig. 10. Epitheloid cell granuloma and beginning necrosis. Case 9; Liver; H and E; ×190

Adrenal Glands

Lesions were found in the cortical and medullary zones. They ranged from few and scattered organisms containing histiocytes to lympho-histiocytic infiltrates of different sizes without necrosis and beginning necrosis of regions with accumulations of histiocytes. In two cases (12 and 19) necrotic lesions of different sizes with granulomatous reaction were seen, one with epitheloid cells and single giant cells surrounding the necroses and the other with calcification of the necrotic material.

Kidneys

In most instances of involvement of these organs, small interstitial cellular infiltrates were found, consisting of lymphocytes and mononuclear cells. Only in one case necrosis was detected in one of these infiltrates. In another case, circumscribed necrosis in a glomerulus was seen, with periglomerular cell reaction. In one case a few large cells could be seen in glomeruli in H and E-stained sections. Formation of small granulomas with single giant cells was found also in only one case. The lesions found in H and E sections were more or less inconspicuous. Organisms in glomerular capillaries and in the infiltrates were clearly seen only in Grocott-stained preparations.

Heart

Clear-cut histoplasmotic tissue reaction was seen in the myocardium only in two cases, once as multiple small lymphohistiocytic infiltrates in the parenchyma and perivascular spaces (Fig. 12) and once as a small circumscribed necrosis.

In a case with concomitant Chagas' myocarditis, the inflammatory reaction apparently was due to this condition. In the other cases, single and few histiocytes or endothelial cells of capillaries were seen containing organisms only with difficulty in H and E-stained sections (Fig. 13). No lesions or organisms were found in the endocardium or epicardium.

Brain

In two cases, small perivascular hemorrhages were found and in one of these also small necrotic foci (Fig. 14). In the remaining cases, only organisms containing single macrophages or endothelial cells were seen in the brain tissue and, in one case, in the leptomeninges. No lesions were seen in the choroid plexus. An old embolic cerebral infarct in case 19 was not examined histologically.

Tonsils

Extensive lymphohistiocytic infiltrates, in one instance present also in the retrotonsilar muscle tissue, were seen in two cases. In both of these the mucosa was in some places necrotic or ulcerated. Almost no cellular reaction and only fungi containing macrophages were seen in the third.

Pancreas

Small lympho-mononuclear infiltrates were found in the septal interstitium, once also in the parenchyma. Case 6 showed diffuse hemorrhages as well. In another case, small recent necroses were seen at the site of infiltrates.

Thyroid Gland

One of the small interstitial and inconspicuous cell infiltrates in the one case with histoplasmotic involvement showed a small necrotic area.

Testes

Apparently the few organisms situated in scattered macrophages in the interstitium and inside some tubules did not provoke the considerable orchitis found in case 16, which was more probably the consequence of Chagas' disease.

Sagittal Superior Sinus of the Dura

Scarce lymphocytic infiltrates were seen in the wall with formation of a recent thrombus at this site. Organisms were present in the wall but not found in the thrombotic masses.

Fungi in Tissues

Yeastlike organisms could be seen in H and E-stained sections in only 10 cases; in some, only in small numbers and after prolonged examination, although it was known through Grocott staining that the tissues contained organisms. They were not seen in 9 cases. They were visible with H and E, especially when present in large numbers. The hematoxylin-positive fungus cells are small granules of a more or less uniform size, not sharply outlined (Fig. 15); the surrounding halo which gave origin to the denomination "capsulatum" is seen only occasionally. The organisms were seen with the H and E staining method especially in lungs, lymph nodes, liver, and spleen. The tissue lesion with fungus cells could be seen within the histiocytic proliferations and necroses. In small cell nodules, granulomas, and nonspecific cell infiltrates, they were found only exceptionally. In necroses they may be easily confused with granular chromatin material of destroyed tissue cell nuclei.

In sections or smears stained with the Grocott method, the fungus cells (Fig. 16) appear as round or oval, 2 to 5 μ corpuscles, with slight variations in size, which stain uniformly black. The wall of the cell often appears a little darker and is sharply outlined. Single budding was seen rather frequently; multiple budding may also occur but is difficult to recognize clearly. Formation of chains of 3 to 5 organisms could be observed too, the latter sometimes resembling pseudohyphae. True hyphae were not seen. Fungus cells of crescent shape, like a compressed ball, occur occasionally.

The organisms were generally inside of histiocytic elements and mostly present in clusters (Fig. 17). Histiocytes, predominantly in lymph nodes, occasionally contained large numbers of fungus cells and had a vacuolic aspect, especially when the organisms had fallen out. Only exceptionally were they found inside giant cells. They were present in necrotic lesions in variable amounts. Often they were more numerous in the necrosis surrounding granulation tissue in a garland-like fashion (Fig. 18); they were less numerous in cell nodules with epitheloid cells or in tuberculoid granulomas. Often, in dense accumulations of fungus cells, fungus-cell detritus was seen as a "dusty" black granular material. Clear-cut fungemia was seen in 10 cases, the fungus cells situated in macrophages within

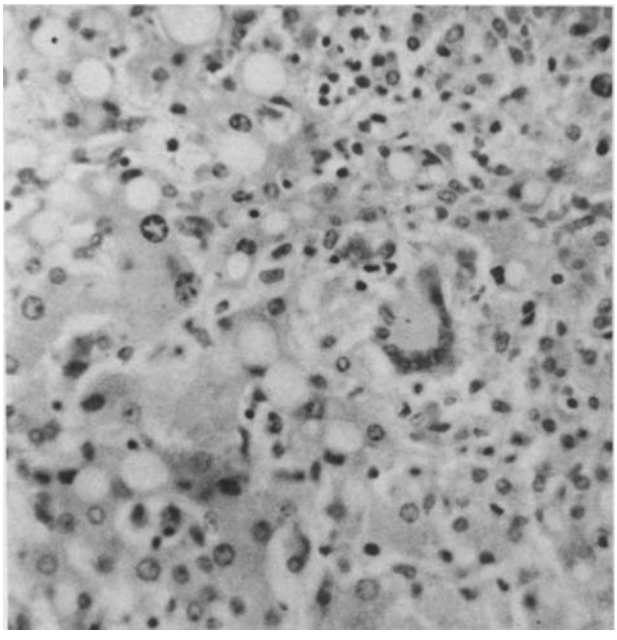

Fig. 11. Inconspicuous cell. infiltr. and giant cell. Case 16; Liver; H and E; ×300

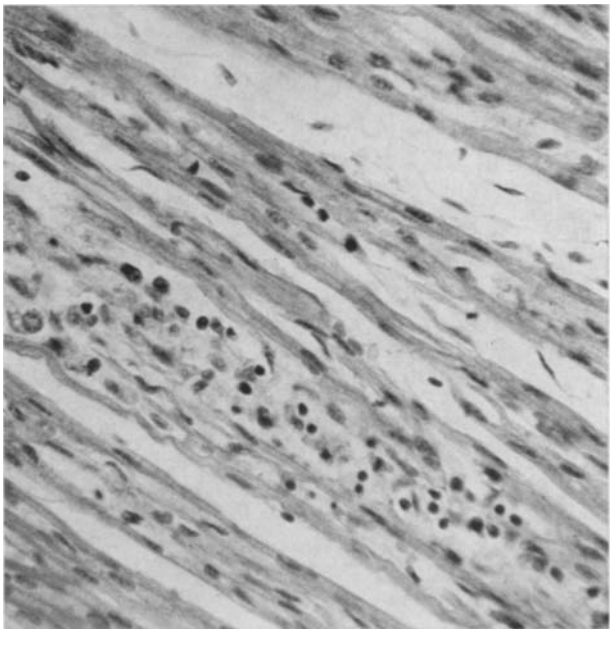

Fig. 12. Small lympho-histiocytic infiltrate. Org. not vis. at this magn. Case 2; Myocardium; H and E; ×300

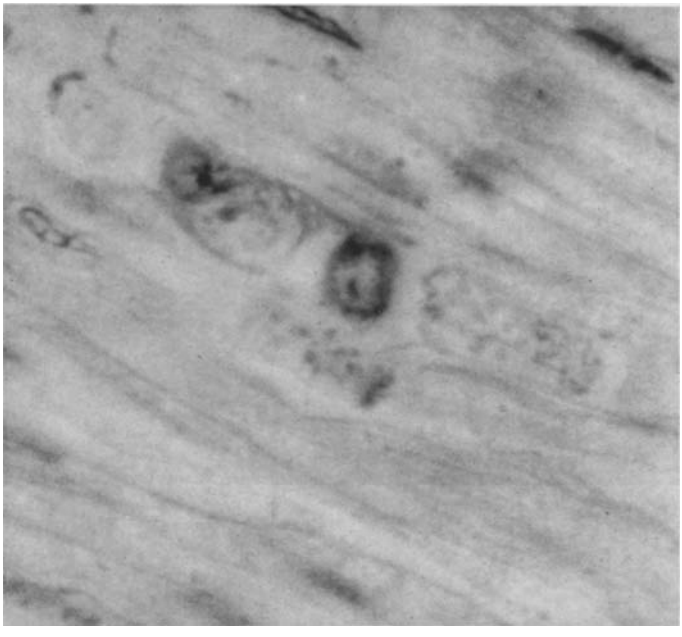

Fig. 13. Clusters of org. in macrophages situated between muscle fibers. Case 2; Myocardium; H and E; ×1,150

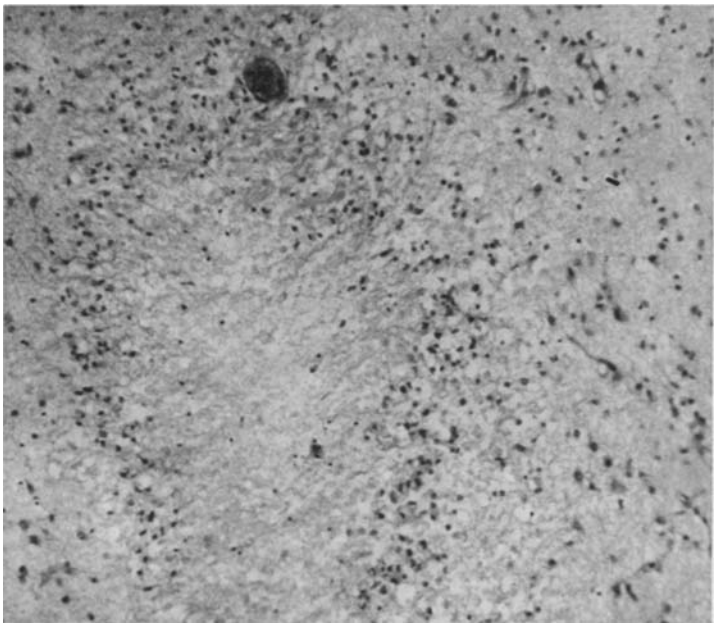

Fig. 14. Small infarct surrounded by cells. Few org. outside of this lesion, however not vis. in H and E. Case 2; Brain; H and E; ×115

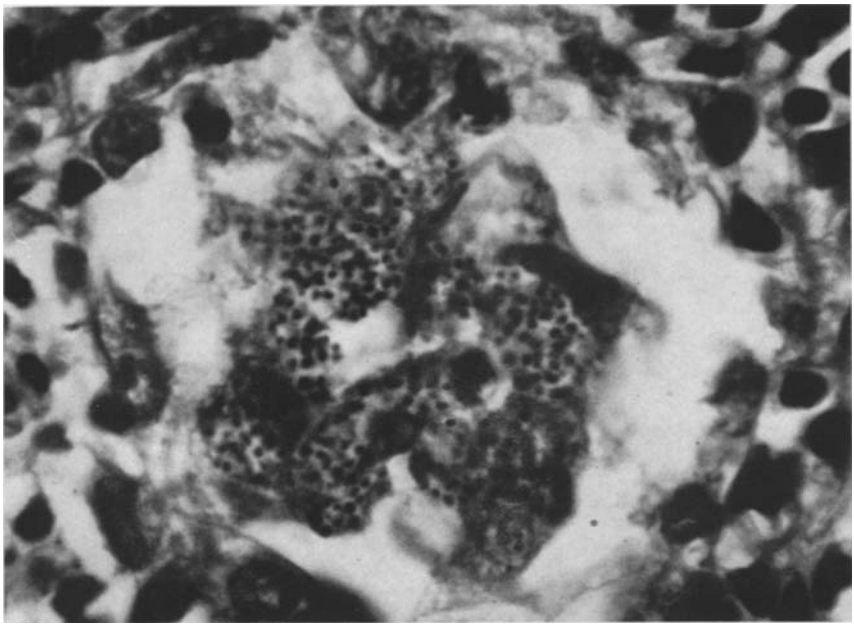

Fig. 15. Org. inside of histiocytes in alveolus, clearly vis. in H and E. Case 5; Lung; H and E; ×1,150

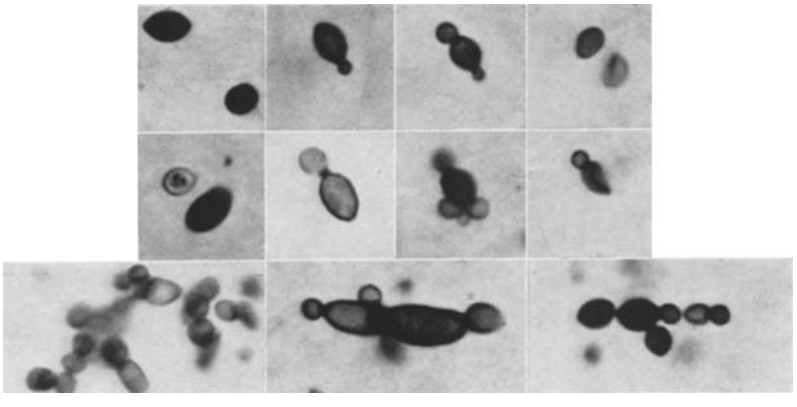

Fig. 16. Variations in form, size and shape of yeastlike cells of Histopl. caps. in tissue. Case 11; Lung and lymphnode; Grocott; ×1,150

both large and small blood vessels, sometimes in large numbers. In the fungemia of capillaries it was occasionally difficult to differentiate between fungus containing macrophages and endothelial cells.

An exceptional finding was fungus cells in one case inside of renal and in another inside of seminiferous tubules.

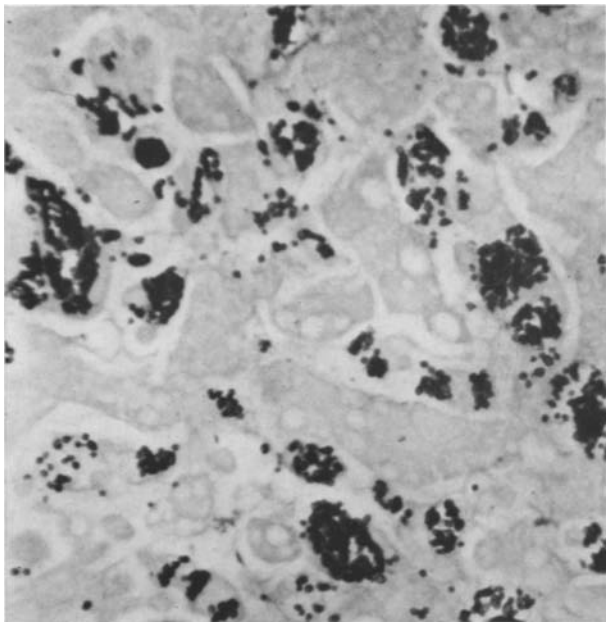

Fig. 17. More numerous and larger org. vis. in Grocott-stain. Case 3; Liver; Grocott; ×450

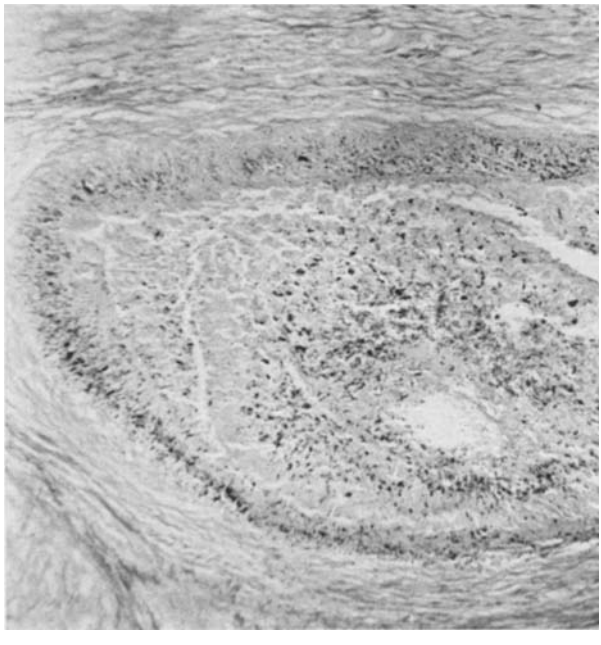

Fig. 18. Org. in necrotic center; accum. in a garland-like fashion in peripheral granulation tissue with fungus cell debris. Case 12; Adrenal gland; Grocott; ×60

Associated Diseases

In 11 cases, other lesions not containing fungi nor due to *H. capsulatum* were found. *Candida* sp. was seen twice, once (case 1) in the esophagus and colon attached to the mucosa, without lesions due to this fungus, and once (case 12) in the lungs, in which no histoplasmotic lesions were seen and a purulent bronchitis with focal pneumonia was present. The two latter conditions, apparently, were not due to *Candida*. The cells of *Candida* could be differentiated clearly from those of *H. capsulatum*.

Foci of variable sizes of bacterial pneumonia were found in seven cases. Some of these contained no fungus cells; in others, large ones, small histoplasmotic foci were also present. The bacterial pneumonia was characterized by a leukocytic and sometimes fibrinous intraalveolar exudate; bacteria were seen occasionally in smears and sections, but not confirmed by culture.

A circumscribed jejunitis was observed in one case (8). Fungus cells were not present and a leukocytic exudate was found.

A few small nests of *Pneumocystis carinii*, stained with the Grocott method, were found in one case (9); no foamy alveolar content was seen at these sites in H and E-stained sections.

Recent necrotic foci in the adrenal gland of one case (10) did not contain fungi; apparently the necrosis was due to a concomitant venous thrombosis in this organ.

A malignant lymphoma with localization in several organs was seen in one case (13). The patient had been treated with large doses of corticosteroids and had been hospitalized in the USA also.

Hepatitis with Councilman bodies was seen twice (case 13 and 14).

Chronic pulmonary tuberculosis was found in one case (14) with acid-fast bacilli in the sections. In this case, hepatitis, bacterial pneumonia, and a moderate interstitial focal myocarditis was found as well.

Case 16 represents a rarity: In addition to the generalized histoplasmosis were found also a generalized paracoccidioidomycosis and Chagas' disease, with nests of leishmanias in heart-muscle fibers, and myocarditis and parasites inside seminiferous tubules with orchitis. In both organs with leishmanias, scattered organisms of *H. capsulatum* were also present. The case was mentioned in a previous study (Salfelder *et al.*, 1969) in which only the double fungus infection was described. Reexamination of sections showed the Chagas' disease only recently. In this case were found also thymoma, intestinal lipomas, and dysenteric colitis.

Case 19 showed also several pathologic conditions besides the histoplasmosis. In addition to generalized amyloidosis, bilharziasis of the liver, generalized arteriosclerosis, multiple emboli, and pulmonary and cerebral infarcts were found.

Comments

In the majority of cases cultures had not been made; however, there is no doubt about diagnosis. With the Grocott method the yeast cells of *H. capsulatum* can be distinguished in tissues clearly from *Toxoplasma gondii* and leishmanias and also from other fungi.

Clinic

Two of our 19 patients were Italian immigrants. In our country, histoplasmosis occurs frequently in these people (Angulo, 1961). Regarding age and sex, the data in this series are in accord with those of previous studies (Schulz, 1954), with a definite peak in infants and in people over age 40 and a heavy predominance of the male sex. Histoplasmosis is endemic in Venezuela, the rate of histoplasmin sensibility varying in different parts of the country. No surprise was the finding of many patients coming from a highly endemic area in the south of Lake Maracaibo, where the organisms have been isolated from soil (Capretti *et al.*, 1961). But the town of Valencia and the State of Carabobo, where 7 patients had lived, was not known as an especially important endemic region. No attempts to determine sources of infection have been made there. Duration of disease could not be elucidated precisely by the data of the clinical histories. Short hospitalization in nine cases certainly contributed to not having established clinical diagnoses.

Gross Pathology

Regarding gross pathology, there are few precise data in the reports on larger series. Schulz (1954) laments the lack of accuracy of autopsy data in his review of 120 cases in the literature, which applies also to histologic features and the number of tissues and organs examined. On the other hand, this is understandable in view of the fact that most of the described cases were found in routine autopsies in which grossly no histoplasmosis or generalized disease was suspected.

Lymph node enlargement, hepato- and splenomegaly, and anemia were frequent, but nevertheless were not always noted. In addition, associated diseases often camouflaged gross pathology. Gross diagnosis could be made only when smears were examined by the Grocott method. This technique, however, takes several hours. In smears stained with H and E or Giemsa, the organisms can easily be confused with leishmanias or toxoplasmas.

Organ Involvement

It must be emphasized that in our series, organs or tissues were not systematically examined histologically. However, involvement of the principal organs was more frequent than, for example, in Schulz's (1954) study, probably due to the use of Grocott's method, involvement being defined also when organisms were present in tissues without reaction.

Rare localizations of histoplasmotic lesions, mentioned by Schulz (1954) and Binford (1955), as involvement of endocardium, skin, bladder, prostate, salpinx uterina, esophagus, gallbladder, parathyroid glands, skeletal muscle, and middle ear, were not found in this material, since these sites were not examined histologically.

Comparing gross and microscopic features, histoplasmotic lesions were detected by microscopy often in grossly unsuspected organs and sites. This was particularly true in different areas of the intestinal tract, when detailed examinations were done, in the lymph nodes, bone marrow, adrenals, and myocardium.

The same is true with localization in the eyes. While experimentally, ocular lesions could be elicited (Salfelder *et al.*, 1965), until now histologic alterations in spontaneous disease have been reported but once (Hoefnagels and Pijpers, 1967).

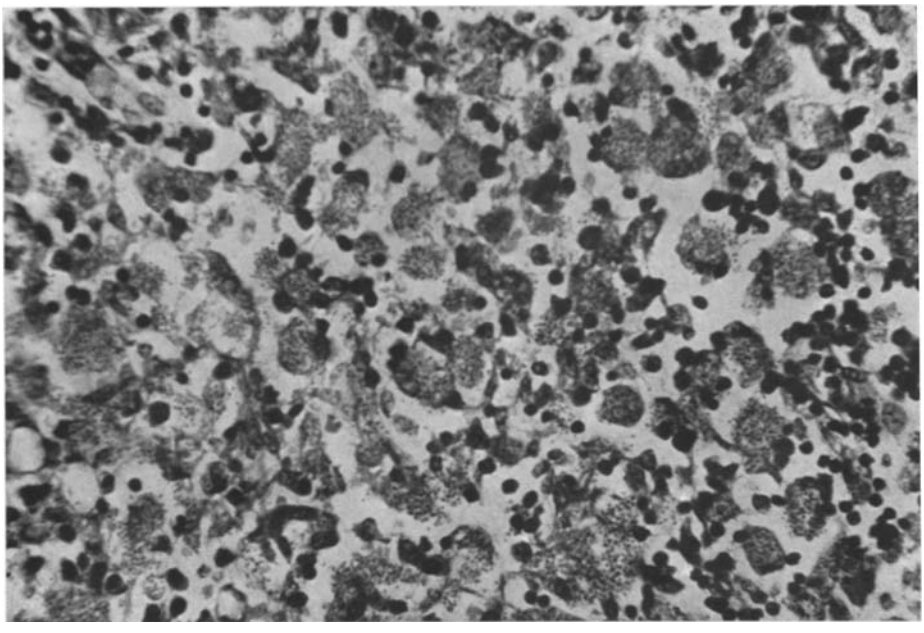

Fig. 19. Diffuse histiocytic reaction with num. intracell. org. Case 5; Pararectal lymphnode; H and E; ×300

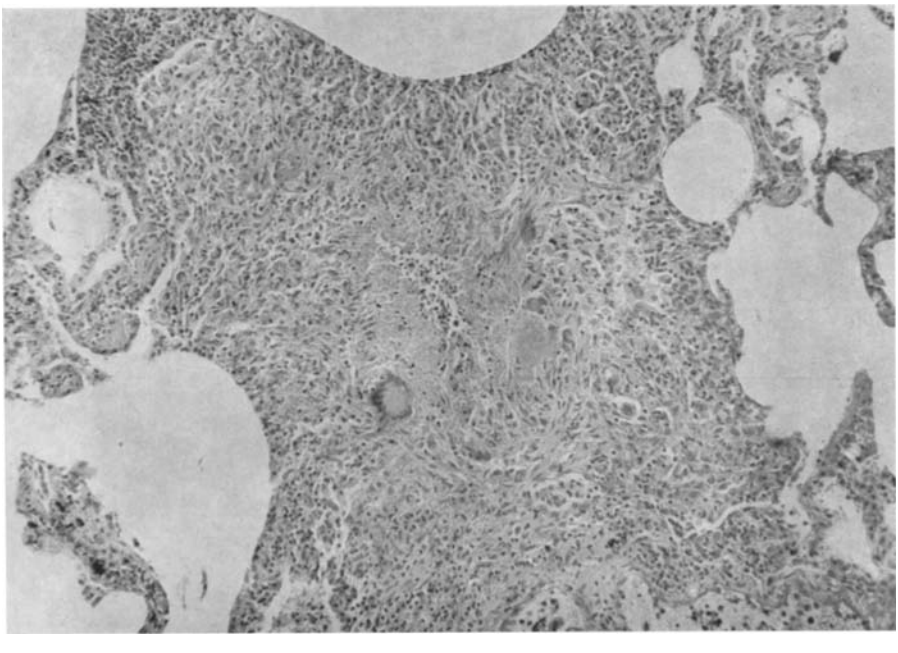

Fig. 20. Tuberculoid granuloma. Org. not vis. in H and E. Case 19; Lung; H and E; ×100

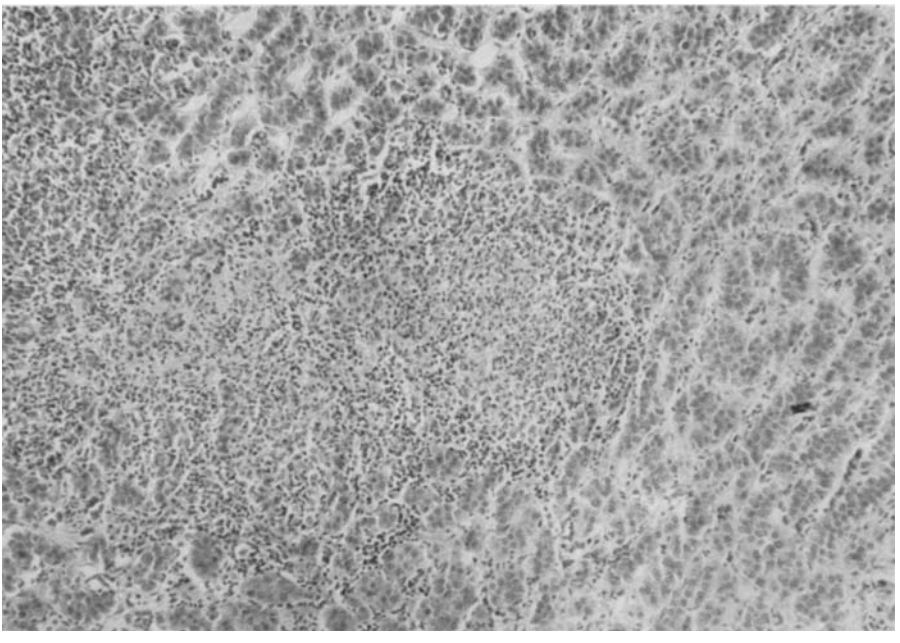

Fig. 21. Inconspic. cell. infiltr. with beginning necrosis. Org. not vis. in H and E. Case 19; Adrenal gland; H and E; ×75

Tissue Lesions

Three main types of tissue reactions can be distinguished in this series. First, the predominant and more or less exclusive histiocytic proliferation (Fig. 19), which occurs generally in reticuloendothelial tissues and in the lungs; it can be seen diffusely or in foci. When numerous organisms are present, they are easily seen inside of histiocytes in H and E-stained sections.

Second, formation of cellular nodules, which may show epithelioid cells and become granulomas which cannot be distinguished from tubercles (Fig. 20).

Finally, the histoplasmotic lesions may be constituted by inconspicuous cell infiltrates with few histiocytes (Fig. 21); they occur generally in myocardium, kidneys, pancreas, thyroid gland, occasionally in adrenal glands and in the intestine.

These protean types of reaction may be present in one organ or in one patient alone or may be mixed.

Necrotic lesions of different sizes can be observed in all three types or, in exceptional cases, may be the only alterations.

In addition to the venous lesions in human histoplasmosis (Schulz, 1954) and in animals (Straub and Schwarz, 1960; Akbarian *et al.*, 1964) arterial histoplasmotic alterations also were seen in our series. These consisted of arteritis or thromboarteritis, the latter with formation of pulmonary infarcts. In veins, true thrombi were observed, thrombotic accumulations of parasitized macrophages attached to the wall, or subendothelial histiocytic proliferations (Schulz, 1954).

The vascular alterations occurred in vessels surrounded by histoplasmotic tissue alterations or with no pathologic lesions in their vicinity.

The diffuse edematous histoplasmotic pneumonia with scarce intraalveolar-cell exudate apparently has not been described before.

No brain abscesses as mentioned by Binford (1955) or Emmons *et al.* (1963) were observed, nor granulomas in the brain or meninges as described by Schulz (1953, 1954); neither necrotizing lesions in the kidneys or granulomas in the myocardium were observed in our series.

Organisms

Organisms could not be seen in all cases and organs in H and E-stained sections. Emmons *et al.* (1963), on the contrary, affirm that they can be recognized with ease in the disseminated form of the disease. The same authors state further that in well-fixed tissue, a single tiny nucleus may be observed, a morphologic feature not confirmed in our material. However, at high-power, irregularly distributed chromatinic material could be seen in many fungus cells. Great variations in size and shape and well-developed hyphae (Emmons *et al.*, 1963) were not seen in our autopsy cases, but single budding was frequent and pseudohyphae were observed. Numerous budding fungus cells of *H. capsulatum* in tissues have been seen only occasionally by Schwarz. This is probably due to the fact, that Schwarz generally examines residual lesions where the organisms are not so active as in our generalized forms of the disease. Fungus-cell detritus in accumulations of numerous organisms was rather frequent, a finding also noted and commented on in other deep-fungus diseases (Salfelder *et al.*, 1969).

Schaumann bodies, frequently observed in experimental histoplasmosis of hamsters (Okudaira *et al.*, 1961; Salfelder and Schwarz, 1965), were not seen in this series; they were considered earlier as large forms of *H. capsulatum* and occur only in hamster tissues.

For the study of *H. capsulatum* in tissues, the statement of Schwarz (1968) seems apt: that this should be done with the Grocott method in the same way as in the case of acid-fast bacilli, for which special staining methods are available. H and E does not suffice either for acid-fast bacilli or fungi. Schwarz referred particularly to the organisms in necroses, but as seen above, this is valid also for fungus confirmation in other kinds of lesions.

Differentiation between fungemia, i.e. the presence of organisms in macrophages of the blood-vessel content, and organ involvement, i.e., confirmation of fungus cells in endothelial cells or perivascular histiocytes, was sometimes difficult in the case of capillaries with the Grocott method; this may have caused some slight inaccuracies in our report.

Associated Diseases

The frequency with which other diseases are associated with histoplasmosis serves to make clinical and gross diagnosis more difficult. Previous reports on fatal histoplasmosis do not contain similar data, but we have found the same true in paracoccidioidomycosis (Salfelder *et al.*, 1969).

The therapeutic implications of these observations do not need to be stressed, but lamentably diagnosis must come before therapy.

The presumed bacterial pneumonia was diagnosed on the basis of visible bacteria and/or leukocytic and fibrinous exudate, the two latter not being observed in pure histoplasmotic lesions. Bacteria, however, were not confirmed by culture. Whether the bacterial infection took place simultaneously with the fungus infection or was a final event cannot be elucidated. Pneumonias have been found also in spontaneous canine histoplasmosis, due either to worms or of an obscure nature (Straub and Schwarz, 1960).

Candidiasis and pneumocystosis were only parasitisms.

In the case of malignant lymphoma, debilitation by this disease and by the prolonged treatment with corticosteroids presumably favored generalized fungus disease, as is known with other deep mycoses.

The same is true for such other severe conditions as tuberculosis, paracoccidioidomycosis, Chagas' disease, amyloidosis, and bilharziasis. In addition, other debilitating factors, such as anemia from intestinal parasites and nutritional deficiencies, are common in the rural population of this country.

In the case of simultaneous triple infection at autopsy (case 16), paracoccidioidomycosis apparently was first. As to Chagas' disease and histoplasmosis, it was difficult to decide which of these infections preceded the other. Details of this case will be dealt with separately.

Cause of Death

In 15 of our 19 cases, histoplasmosis or conditions considered sequelae of this fungus disease were the cause of death. In the remaining four cases, histoplasmosis, considered secondary to severe previous disease or diseases, played a secondary role as cause of death. In eight cases, histoplasmosis alone was the cause of death. In three cases (1, 11, 18) a diffuse histoplasmotic edematous pneumonia had apparently caused pulmonary insufficiency. In three others (2, 7, 10) jaundice might indicate hepatic failure, but could be attributed also to hemolytic anemia (toxic or immunologic mechanisms). In one (case 6), pancreatic hemorrhages could be accused as the direct cause of death, and in one (case 4) no special organ insufficiency was found.

In five cases (3, 5, 8, 9, 12) bacterial pneumonia was possibly an important complication and eventually the direct cause of death; in one of these (case 12) histoplasmotic lesions in the adrenal glands presumably had produced in addition insufficiency of these organs. Clinically, signs of adrenal failure were not expressly mentioned, but can be assumed (Emmons *et al.*, 1963).

In one case (No. 15) shock due to pulmonary infarcts caused by histoplasmotic thromboarteritis was assumed as a fatal sequela; in another (No. 17) allergic shock with edema of the glottis and erythema nodosum was assumed. In case 19, generalized histoplasmotic infection caused death together with amyloidosis, arteriosclerosis, multiple thrombosis and embolism, infarcts and bilharziasis, as in three other cases with malignant lymphoma, tuberculosis and paracoccidioidomycosis, and Chagas' disease.

Pathogenesis

The portal of entry of the infective agent in histoplasmosis has definitely been determined to be the lungs (Straub and Schwarz, 1955). That in one case of this

series pulmonary lesions were not described at gross examination apparently is due to oversight. Frequency or predominancy of involvement of one site or the other should not be considered definitive as to the portal of entry. Despite the presence of intestinal lesions, assumed by Emmons *et al.* (1963) as evidence of the gastrointestinal tract as the entry portal, residual foci of benign histoplasmosis in endemic areas are found only in the lungs and/or mediastinal lymph nodes; further primary intestinal histoplasmosis could not be produced experimentally (Salfelder and Sethi, 1967).

In the majority of cases it is assumed that the infection is recent and belongs to the primary form of disease with immediate hematogenous dissemination. The few cases with tuberculoid granulomas and the single one with calcified necroses may not represent, primary, or postprimary infection.

In only one case, probably endogenous, reinfection can be considered. The fibrohyaline hilar lymph node lesions with necrosis suggest a previous, older infection, while all necrotic foci in other tissues are more recent. An overwhelming, massive hematogenous spread from the older lymph node lesions can be assumed. Since the lymph node lesions extended to the bronchial wall with necrosis of these structures, a bronchogenic dissemination could have been responsible for the diffuse histoplasmotic edematous pneumonia.

While histiocytic reaction and widespread lesions were predominant in infants and granulomatous lesions with less organ involvement more often present in adults, the "infantile" type of generalized disease was seen also in adults. Primary lung histoplasmosis has been compared with the primary complex in tuberculosis (Schulz, 1954). This comparison is valid only as regards the obligatory involvement of lymph nodes in this stage of evolution. In contrast to the primary tuberculous complex with a solitary lung focus as the rule, there is evidence, in our series, of multiple pulmonary foci in primary infection. In 18 cases no calcified lung or mediastinal lymph-node foci were found. The multiple and bilateral histoplasmotic lesions were of variable sizes, showing necroses of many different sizes or no necrosis at all. Basically, however, they can be considered as more or less of the same age of evolution. There is no need to interpret one or several larger foci which eventually show necrosis as solitary primary foci and the others as the result of hematogenous spread.

The formation of multiple lung foci in primary infection may be attributed to larger infectious doses, with inhalation to different sites in both lungs. Also, the formation of necroses can be due to a larger number of inhaled fungal elements at the respective sites or differences in multiplying capacity.

It seems logical that the inhalation of smaller amounts of fungi toward fewer sites leads to solitary or less numerous and smaller primary foci frequently found at routine autopsies in endemic areas and also clinically (Procknow, 1967). Multiple residual lung lesions were considered as primary by Straub and Schwarz (1962) in humans and were found also in dogs (Straub and Schwarz, 1960, 1962) without evidence of hematogenous dissemination in human and animal cases. — Multiple primary foci have been produced also experimentally (Procknow *et al.*, 1960; Salfelder and Sethi, 1967; Farrel and Cole, 1968). Further, the multiple foci in the acute epidemic form of the disease are recognized as primary (Silverman *et al.*, 1955; Lehan and Furcolow, 1957; Rubin *et al.*, 1959; Schwarz *et al.*, 1960)

and multiple histoplasmomas in some instances can be interpreted also in this way. In larger series of fatal disseminated histoplasmosis this has not yet been done. Apparently, earlier writers who found no single primary foci in autopsy material were confused about the diagnosis of primary histoplasmotic infection.

At the site of fungus invasion, the histiocytic proliferation is remarkable, crowding and replacing normal tissue. That these cells multiply after having engulfed organisms (Emmons *et al.*, 1963) seems not proven. Necrosis found in areas with histiocytic accumulation is probably due to interference with nutrition rather than to a direct histolytic action (of organisms) on the tissue (Emmons *et al.*, 1963). Granulation tissue with epithelioid and giant cells forms after onset of necrosis, or formation of granulomas takes place without previous necrosis. In the granulomas, necrosis may develop secondary to destruction of parasitized histiocytes.

Vascular lesions of different types with thromboembolic processes may lead or contribute to formation of necrosis, infarcts, and hemorrhages, previously observed by Schulz (1954). Histoplasmotic alterations of arteries and veins can be produced by invasion of fungi from surrounding lesions or progressive growth and extension of the latter toward the vessel wall. In other instances, the involvement of blood vessels is due to circulating parasitized macrophages becoming attached to the vascular wall.

The frequent fungemia and the vascular lesions make it clear that dissemination is generally hematogenous. While mediastinal lymph node involvement is always the result of lymphogenic spread, the abdominal lymph nodes may, in the absence of intestinal lesions, also be involved due to hematogenic or retrograde dissemination.

Intracanalicular extrapulmonary spread seems rare. In one case, bronchogenic dissemination by invasion of main bronchi was suspected. But, in contrast to tuberculosis, intestinal lesions are more probably the result of hematogenous spread.

References

Akbarian, M., Salfelder, K., Schwarz, J.: Cultural and serological studies in experimental canine histoplasmosis. Antimicr. Ag. Chemother. 656—666 (1965).

Angulo Ortega, A.: Las formas circunscritas de histoplasmosis pulmonar (Histoplasmomas). Mycopathologia (Den Haag) **15**, 217—230 (1961).

Baker, R. D.: Histoplasmosis in routine autopsies. Amer. J. clin. Path. **41**, 257—470 (1964).

Bank, S., Trey, C., Gans, I., Marks, I. N., Groll, A.: Histoplasmosis of the small bowel with "giant" intestinal villi and secondary protein-loosing enteropathy. Amer. J. Med. **38**, 492—501 (1965).

Binford, C. H.: Histoplasmosis. Tissue reaction and morphologic variations of the fungus. Amer. J. clin. Path. **25**, 25—36 (1955).

Brass, K.: Las micosis profundas. Jornadas "Dr. Ramón Arcay Tortolero". Hospital Central Valencia, Dic. pp. 31—88.

Capretti, C., Salfelder, K., Romero, A.: Histoplasma capsulatum en el suelo de nuestro ambiente. I. Examenes micológicos. Mycopathologia (Den Haag) **17**, 55—70 (1962).

Collier, W. A., Winckel, W. E. F.: Beiträge zur geographischen Pathologie von Surinam. 6. Histoplasmose bei Säugetieren in Surinam. Leeuwenhoek J. Microbiol. Serol. **18**, 349—356 (1952).

Collins, D. C.: Histoplasmosis in the aged. J. Amer. Geriat. Soc. **5**, 27—31 (1957).

Correa, W. M., Pacheco, A. C.: Naturally occurring histoplasmosis in guinea pigs. Canad. J. comp. Med. **31**, 203—206 (1967).

Emmons, C. W., Binford, C. H., Utz, J. P.: Medical mycology. Philadelphia: Lea and Febiger 1963.

Farrel, R. L., Cole, C. R.: Experimental canine histoplasmosis with acute fatal and chronic recovered cases. Amer. J. Path. **53**, 425—445 (1968).

Gomba, S., Szokoly, V.: Generalisierte tödliche Histoplasmose. Zbl. allg. Path. path. Anat. **110**, 90—93 (1967).

Grocott, R. G.: A stain for fungi in tissue sections and smears using Gomori's methenamine-silver nitrate technic. Amer. J. clin. Path. **25**, 975—979 (1955).

Hartung, M., Salfelder, K.: Histoplasmose mit tödlichem Ausgang als Berufserkrankung bei einem Mykologen. Int. Arch. Gewerbepath. Gewerbehyg. **19**, 270—289 (1962).

Hoefnagels, K. L. J., Pijpers, P. M.: Histoplasma capsulatum in a human eye. Amer. J. Ophthal. **63**, 715—723 (1967).

Lehan, P. H., Furcolow, M. L.: Epidemic histoplasmosis. J. chron. Dis. **5**, 489—503 (1957).

Martz, J., Rambo, O., Smith, M., Lynch, J.: Case of histoplasmosis. J. Pediat. **31**, 98—99 (1947).

Okudaira, M., Schwarz, J., Adriano, S. M.: Experimental production of Schaumann bodies by heterogeneous microbial agents in the golden hamster. Lab. Invest. **10**, 968—982 (1961).

Procknow, J. J.: Pulmonary histoplasmosis in a farm family-fifteen years later. Amer. Rev. resp. Dis. **95**, 171—188 (1967).

— Page, M. I., Loosli, C. G.: Early pathogenesis of experimental histoplasmosis. Arch. Path. **69**, 413—426 (1960).

Raftery, A.: Subclinical histoplasmosis. Gastrointestinal histoplasmosis of children. J. Amer. med. Ass. **145**, 216—219 (1951).

Roessle, R., Roulet, F.: Maß und Zahl in der Pathologie. Berlin: Springer 1932.

Rosenbaum, A. E., Schweppe, H. L., Rabin, E. R.: Constrictive pericarditis, pneumopericardium and aortic aneurysm due to Histoplasma capsulatum. New Engl. J. Med. **270**, 935—938 (1964).

Rubin, H., Furcolow, M. L., Yates, J. L., Brasher, C. A.: The course and prognosis of histoplasmosis. Amer. J. Med. **27**, 278—288 (1959).

Salfelder, K.: Zur Differentialdiagnose der Histoplasmose. Z. Tropenmed. Parasit. **2**, 453—469 (1960).

— Fatal case of erythema nodosum and histoplasmosis. Mycopathologia (Den Haag) **22**, 315—321 (1964).

— Doehnert, G., Doehnert, H. R.: Paracoccidioidomycosis. Virchows Arch. Abt. A. Path. Anat. **348**, 51—76 (1969).

— Liscano, T. R. de: Lesiones histoplasmóticas autópsicas como índice epidemiológico de la enfermedad en los Andes Venezolanos. Mycopathologia (Den Haag) **26**, 19—30 (1965).

— Schwarz, J.: Experimental cutaneous histoplasmosis in hamsters. Arch. Derm. **91**, 645—656 (1965).

— — Akbarian, M.: Experimental ocular histoplasmosis in dogs. Amer. J. Ophthal. **59**, 290—299 (1965).

— Sethi, K. K.: Experimental intestinal histoplasmosis of hamsters. Mycopathologia (Den Haag) **32**, 153—162 (1967).

Schulz, D. M.: Histoplasmosis of the central nervous system. J. Amer. med. Ass. **151**, 549—551 (1953).

— Histoplasmosis; a statistical morphologic study. Amer. J. clin. Path. **24**, 11—26 (1954).

Schwarz, J.: Pathology of histoplasmosis. Pathology Annual, vol. **3**, p. 335—366. New York: Appleton-Century-Crofts 1968.

— Baum, G. L., Floyd, H.: Pathogenesis of "epidemic" histoplasmosis. Ann. N. Y. Acad. Sci. **89**, 47—58 (1960).

Segal, C., Wheeler, C. G., Tompsett, R.: Histoplasma endocarditis cured with amphotericin. New Engl. J. Med. **280**, 206—207 (1969).

Silverman, F. N., Schwarz, J., Lahey, M. E., Carson, R. P.: Histoplasmosis. Amer. J. Med. **19**, 410—459 (1955).

Straub, M., Schwarz, J.: The healed primary complex in histoplasmosis. Amer. J. clin. Path. **25**, 727—741 (1955).

— — General pathology of human and canine histoplasmosis. Amer. Rev. resp. Dis. **82**, 528—541 (1960).

— — Histoplasmosis, coccidioidomycosis and tuberculosis: A comparative pathological study. Path. et Microbiol. (Basel) **25**, 421—477 (1962).

Wildervauck, A., Winckel, W. E. F., Collier, W. A.: Tropical eosinophilia combined with histoplasmosis. Docum. med. geogr. trop. (Amst.) **5**, 67—71 (1953).

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