# THE ETIOLOGY OF DISCRETE SPLENIC AND HEPATIC CALCIFICATIONS IN AN ENDEMIC AREA OF HISTOPLASMOSIS

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Calcifications of the spleen have been occasionally recorded during roentgenologic examination or at necropsy. An excellent review was published by Gray.[1] As with other early investigators,[2] he believed tuberculosis to be the most common cause of such lesions. In 1945, Christie and Peterson[3] and Palmer[4] reported convincing evidence of a relationship between the existence of nontuberculous intrathoracic calcifications and histoplasmin sensitivity. One year later, High[5] suggested that the splenic calcifications found in the course of routine roentgen examinations of the chest might represent sequelae of histoplasmosis. The same author also noted intrahepatic calcification in 3 instances.

In 1955, we reported that calcific lesions in the spleen were found in larger numbers and with much greater frequency in an endemic area of histoplasmosis than in other geographic locations. With the Gridley stain, organisms morphologically consistent with *Histoplasma capsulatum* were demonstrated in 19 of 40 examples of "typical" intrasplenic lesions. The latter were characterized by rounded configuration, a laminated structure, and were present in numbers exceeding 5 per affected spleen. The feature of lamination was somewhat variable.[6] A comparison of about 100 cases each from New York, Rotterdam, and Cincinnati revealed "typical" splenic lesions in 2.7 per cent, 2 per cent, and 44 per cent, respectively.[6] This was the first demonstration of a morphologic indication of etiology. Subsequently a relationship between skin sensitivity to histoplasmin and the roentgenographic appearance of splenic calcifications was established.[7] Subsequent studies[8–15] have provided abundant evidence that multiple concentric splenic calcifications may be considered highly suggestive of past infection with Histoplasma.

In 1957, Young, Bills and Ulrich[16] reported that in 46 necropsies, neither acid-fast organisms nor fungi were demonstrable in smears or cultures taken from small splenic lesions or calcifications. In only a single case were organisms resembling *H. capsulatum* found in micro-

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scopic sections. More recently, however, utilizing the Grocott silver stain, Young[17] has found Histoplasma-like organisms in 10 per cent of the lesions encountered.

The present study affords a better means of evaluation since primary pulmonary complexes were examined and shown to be of histoplasmic origin. This was the case in each instance in which splenic and hepatic calcifications were encountered. Thus the potential diagnostic value of the demonstration of such foci in an endemic area of histoplasmosis becomes evident.

## Material and Methods

Ninety-two consecutive necropsy examinations were performed in adults during the summer and fall of 1959 at the Jewish Hospital, Cincinnati. The patients died of a variety of disorders; in no instance was the cause of death tuberculosis, histoplasmosis or another granulomatous disease. The spleens, lungs, intrathoracic lymph nodes, and, in a few cases, the livers also were examined roentgenographically after removal from the body. By this means, in 43 instances discrete splenic calcifications were demonstrated. In 11 of these, discrete calcific foci were encountered in the liver as well.

Because of incomplete preservation of tissue, 13 of the 43 cases were excluded from this study. All primary pulmonary complexes found in the remaining 30 cases and almost all extrathoracic lesions were examined microscopically, when necessary after decalcification with 5 per cent nitric acid. Paraffin sections were stained with hematoxylin and eosin, and by the van Gieson, Kinyoun,[18] Gridley,[19] and Grocott[20] procedures. It was often necessary to recut sections and to stain them by the Grocott procedure before fungi could be found.

## Results

### *Incidence of Splenic and Hepatic Calcifications*

Among the 92 cases, there were 60 with intrathoracic calcific lesions (65.3 per cent). All of these were identified as histoplasmosis by microscopic demonstration of the organism in the individual foci. In these 60 cases, there were 43 (71.7 per cent) with splenic and 11 (18.3 per cent) with hepatic (calcific) lesions. In no instance were there hepatic lesions unaccompanied by splenic foci. Intrathoracic lesions were manifest in all.

Table I

THE INCIDENCE OF VISCERAL LESIONS OF HISTOPLASMOSIS AT NECROPSY

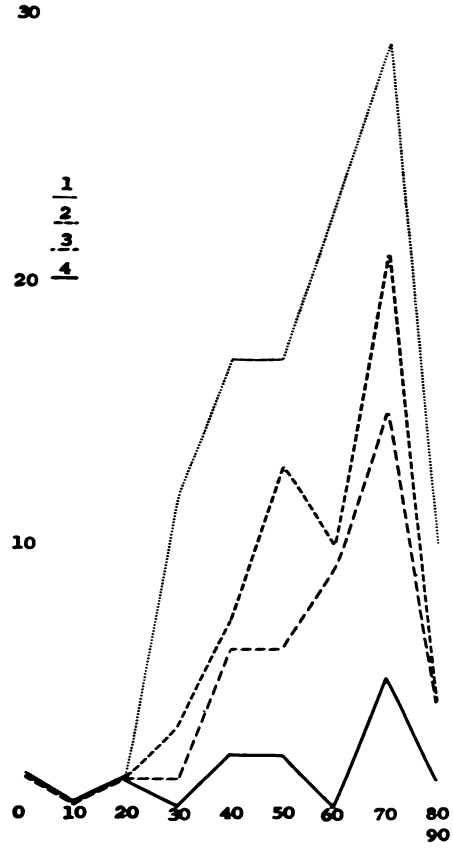
| Lesion | White | | Colored | | Total |
|---|---|---|---|---|---|
| | Male | Female | Male | Female | |
| Pulmonary | 30/42* (71.4%) | 23/37 (62.2%) | 3/6 (50%) | 4/7 (57.2%) | 60/92 (65.3%) |
| Splenic | 21/42 (50%) | 18/37 (48.6%) | 1/6 (16.7%) | 3/7 (42.9%) | 43/92 (46.8%) |
| Hepatic | 5/42 (11.9%) | 5/37 (13.5%) | 0/6 | 1/7 (14.3%) | 11/92 (12%) |

* Numerator, cases with calcified histoplasmic lesions; denominator, total number of cases examined.

The incidence of these processes in relation to race and sex is shown in Table I. The age distribution closely followed the over-all pattern of the pulmonary lesions and the necropsy population (Text-fig. 1).

### *Gross Observations*

Most of the splenic lesions were spherical, 1 to 3 mm. in diameter, and gray to grayish yellow in color. Frequently a distinct lamination was noted with a solid nucleus surrounded by concentric, calcified deposits. The lesions were scattered throughout the parenchyma and on occasion lay in the splenic capsule (Fig. 1). Although the size of the lesions was usually uniform in a single spleen, occasionally there was wide variation, with both large (5 to 10 mm.; maximum 35 mm.) and small lesions in the same organ. In most instances there was dense mineralization and the lesions were hard to cut without decalcification.




TEXT-FIGURE 1. Age distribution of histoplasmic lesions in necropsy material (autumn, 1959). 1. Total number of necropsies. 2. Cases with pulmonary histoplasmic lesions. 3. Cases with splenic histoplasmic calcifications. 4. Cases with hepatic histoplasmic lesions. Age groups are arranged from 0 to 9, 10 to 19 years, etc.

In 5 cases, calcification was not a feature and the lesions appeared as centrally deposited, grayish yellow, caseous material surrounded by a fibrous capsule (Fig. 3).

The hepatic lesions (Fig. 2) were more frequently distributed in the capsule or subcapsular regions than in the parenchyma. The macroscopic and roentgenographic appearances were essentially similar to those of the splenic foci.

When the splenic lesions were conspicuously calcified, this was also the case in the pulmonary foci, the regional lymph nodes and the hepatic lesions. When splenic lesions were not calcified, mineralization was also absent from the other locations.

### *Histologic Observations*

The histologic features of the splenic and hepatic lesions were essentially as described in our previous report.[6] Several types were recognized:

*The "Early" Lesion.* This was characterized by a loose, fibrous capsule surrounding a central zone of acidophilic coagulation necrosis without significant calcification (Fig. 6). Fibroblasts, small numbers of lymphocytes, and occasional epithelioid cell granulomas accompanied by foreign body giant cells were enclosed within a fibrous capsule (Fig. 7). Even at this stage, many collagen fibers appeared in the central necrotic zone extending from the fibrous capsule.

*The "Old" Lesion* (3 types).

1. In this form a fibrous capsule consisting of dense hyaline tissue of varying thickness surrounded a central necrotic zone (Figs. 4, 5 and 8). Calcification was generally marked in the inner half of the capsule and bone formation was noted occasionally. The central necrotic area exhibited basophilic staining and was often concentrically banded. Cholesterol clefts were frequent in the capsule and in the central necrosis.

2. Here, the core was composed principally of loose connective tissue associated with foreign body granuloma formation and cholesterol clefts.

3. These were completely hyalinized nodules without central cores.

The foregoing 3 forms either represented sequential developmental stages or were the result of tangential sectioning of certain lesions. "Early" lesions were never found in the presence of "old" ones. Although in a given case or section some lesions appeared to be necrotic and others completely fibrotic, serial sectioning revealed that most of the fibrotic lesions were actually tangential sections of a capsule surrounding an active process.

Organisms resembling *H. capsulatum* were demonstrated in the splenic foci in 26 of the 30 cases. The organisms were regularly observed

in or immediately adjacent to the center of necrotic portions. They were never found in the peripheral zone of necrosis, in granulomas or in the fibrous capsule. The number of organisms was usually small. In general, the "early" lesions contained much larger numbers than the "old" ones. They appeared singly but occasionally formed small clusters (Fig. 9). The organisms were oval and measured 2 to 3 by 2 to 5 $\mu$. Distorted, faintly stained bodies were occasionally seen among the typical organisms. There were no unusual spore forms, and acid-fast bacilli were not demonstrable.

The interrelationships among the pulmonary lesions, the organisms in the splenic lesions, and the number of splenic lesions are tabulated in Table II. Regardless of the number of primary pulmonary lesions,

TABLE II

RELATIONSHIPS BETWEEN PRIMARY PULMONARY AND SPLENIC LESIONS

| Pulmonary lesion of histoplasmosis * | Organisms in splenic lesions | No. of splenic lesions: Few † | Several † | Many † | Subtotal | Total ‡ |
|---|---|---|---|---|---|---|
| Single primary focus | + | 1 | 4 | 10 | 15 | 17 (88.2%) |
| | − | 1§ | 1§ | | 2 | |
| Multiple primary foci | + | 1 | 3 | 6 | 10 | 12 (83.4%) |
| | − | 1§ | 1§ | | 2 | |
| "Epidemic" type [13] | + | | | 1 | 1 | 1 (100%) |
| | − | | | | | |
| Subtotal | + | 2 | 7 | 17 | 26 | |
| | − | 2 | 2 | | 4 | |
| Total | | 4 | 9 | 17 | | 30 |
| Per cent ‡ | | (50%) | (77.7%) | (100%) | | |

* *H. capsulatum* demonstrated in each instance.
† Few, 1 to 2; several, 3 to 5; many, 6 or more.
‡ Percentage of cases with organisms demonstrated.
§ Fibrotic hyalinized lesions.

splenic lesions were invariably multiple. Actually in 26 of the 30 cases with pulmonary complexes (87 per cent) there were multiple splenic calcifications. The number of demonstrable organisms increased with the number of foci; this held true in both the spleen and liver. Sequential sections were often necessary for the conclusive demonstration of organisms (Table III). The Grocott stain was most successful in demonstrating organisms, except in a few instances in which "fibrotic" foci were

TABLE III

IDENTIFICATION OF *Histoplasma capsulatum* ON RECUTTING OF TISSUE BLOCKS

| Organ | No. of cases | First observation | | | Second observation | | | Third observation | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | + | − | % | + | − | % | + | − | % |
| Lung | 37 | 34 | 3 | 91.9 | 37 | 0 | 100.0 | | | |
| Lymph node | 36 | 31 | 5 | 86.1 | 32 | 4 | 88.9 | 35 | 1* | 97.2 |
| Spleen | 30 | 22 | 8 | 73.3 | 24 | 6 | 80.0 | 26 | 4* | 86.7 |
| Liver | 8 | 5 | 3 | 62.5 | 5 | 3 | 62.5 | 5 | 3* | 62.5 |

+ = organisms demonstrated; − = organisms not demonstrated.
* Only fibrotic hyalinized lesions.

observed. Recutting of these cases was not helpful, presumably because the necrotic center had been passed; thus the sections were representative of capsule rather than the active process itself.

## DISCUSSION

In 1939, Reichle and Work[2] found that among 452 individuals necropsied at the Cleveland City Hospital, calcified splenic nodules were found in the spleens of 73, the livers of 47, and the kidneys of only 2. The inoculation into 20 guinea pigs of material from these lesions in 14 cases yielded positive growth of acid-fast organisms in only 3 instances. They concluded, with little justification, that calcified nodules were indeed of tuberculous nature. There was no morphologic description given of the organisms observed. In 1944, Gray[1] reported splenic calcifications in 63 of 111 unselected necropsies. In 8 of the cases there was active systemic tuberculosis; no tubercle bacilli were demonstrated in splenic foci in these individuals. Crushed material from 2 cases was injected into guinea pigs; necropsy 8 weeks later revealed no tuberculosis. In spite of this, the author concluded that the miliary splenic calcifications represented tuberculosis.

In the present group, organisms morphologically consistent with *H. capsulatum* were observed in 86.7 per cent of the calcified splenic lesions. The organisms were demonstrated in all cases in which the area of central necrosis was included in the section. Unusual forms of Histoplasma (hyphae,[21-23] globular forms,[21-24] tuberculate chlamydospores,[23] large forms,[25-29] or flagellated forms[30]) were not encountered. Schulz,[31] in a statistical review of histoplasmosis, showed a high incidence of lesions in the liver, spleen, and lung. The comparatively low incidence of hepatic calcifications in the present group of cases probably reflected incomplete roentgenographic examination.

The lack of cultural growth does not necessarily exclude histoplasmosis. Several investigators[8,32,33] have attempted culture of old histo-

plasmic granulomas with uniformly negative results. The organisms in these residual foci of histoplasmosis are presumably nonviable.[21,33,34] This explains why Young and co-workers[16] and others failed to obtain growth from calcified splenic lesions.

The Grocott stain proved to be more suitable for the demonstration of *H. capsulatum* than the Gridley stain. Positive and negative errors in the recognition of organisms were more readily avoided. Some lesions contained only a small number of organisms, and careful and repeated microscopic examinations of multiple sections through the central necrotic area, with suitable staining, were often necessary in order to demonstrate spores.

Zeidberg, Dillon and Gass[35] reported the reversion of positive histoplasmin skin tests to negative reactions. This phenomenon, which we have confirmed,[7] is most prominently observed in individuals over 40 years of age. The persistence of sensitivity to histoplasmin has been explained as an expression of a continued, active, first infection or of reinfection.[35] However, many older persons living in the endemic area of histoplasmosis react negatively to histoplasmin.[7]

Multiple discrete splenic and hepatic calcifications demonstrable by roentgenogram should be diagnostic of past infection with *H. capsulatum*. This assumes potential importance in the clinical evaluation of individuals with negative reactions to histoplasmin. The present investigation clearly indicates that in an area where histoplasmosis is endemic, calcified hepatic and splenic lesions may be presumed to indicate histoplasmosis. This presumption may be considered even more strongly if more than 5 calcified foci are observed in a given spleen and if the center of the lesions has been or is the seat of necrosis.

An explanation of the pathogenesis of these lesions is not easy and not wholly clear. There can be little doubt that the *H. capsulatum* is distributed to the spleen and liver by way of the blood stream, most likely during transitory fungemia. At this point, however, conclusive knowledge ends. One may speculate that the hematogenous dissemination results in a "filtering" of individual organisms, some of which are destroyed and others proliferate, resulting in central necrosis and permanent stigmas in the form of calcified foci. It is equally possible that the splenic lesions may result from particulate embolism with intrasplenic vascular trapping of small conglomerates of organisms with or without accompanying thrombosis.

There is evidence that transitory asymptomatic fungemia may occur. We have observed positive blood cultures of *H. capsulatum* in children without symptoms of septicemia; these patients have recovered with or without therapy. Similar experiences have been reported by others.[36]

The histoplasmic basis for these splenic (and hepatic) calcifications seems evident. It is disquieting, however, that large and numerous lesions of this nature may occur without clinical manifestations. On the other hand, splenic infarcts, even of large size, may also be asymptomatic.

## Summary

Splenic and hepatic calcifications are often observed in an endemic area of histoplasmosis. Among 92 consecutive necropsy examinations in adults in the Cincinnati area, there were 43 cases with discrete, often multiple splenic calcifications, and 11 with hepatic calcifications.

The splenic calcifications in 30 cases and similar hepatic lesions in 8 were examined microscopically. In all of the lesions with active or healed central necrosis, organisms resembling *H. capsulatum* were demonstrated morphologically by special stains. The presence of multiple discrete splenic and hepatic calcifications appears to be a characteristic sequel of histoplasmosis and presumably develops asymptomatically during the period of primary infection.

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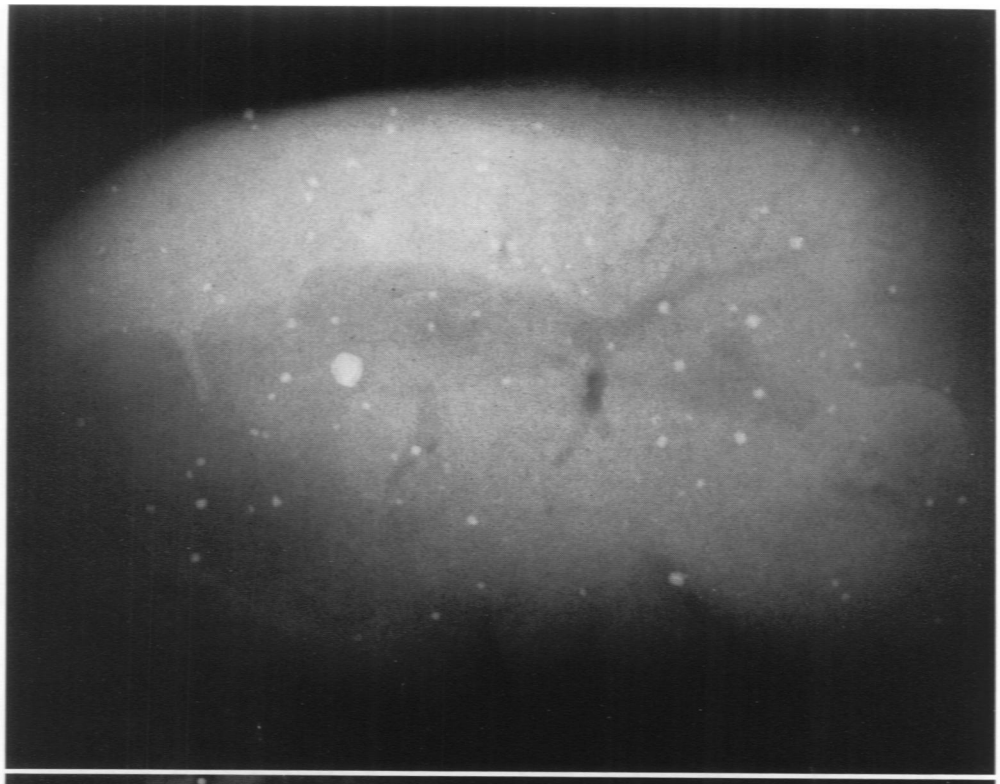
[ *Illustrations follow* ]

## Legends for Figures

Except where indicated, photomicrographs were prepared from sections stained with hematoxylin and eosin.

Fig. 1. Roentgenogram of the spleen removed at necropsy from a 41-year-old white man. Many calcifications may be seen; the largest one has "typical" concentric rings.

Fig. 2. Roentgenogram of the liver removed at necropsy from a 51-year-old white man. Numerous calcifications are visible.



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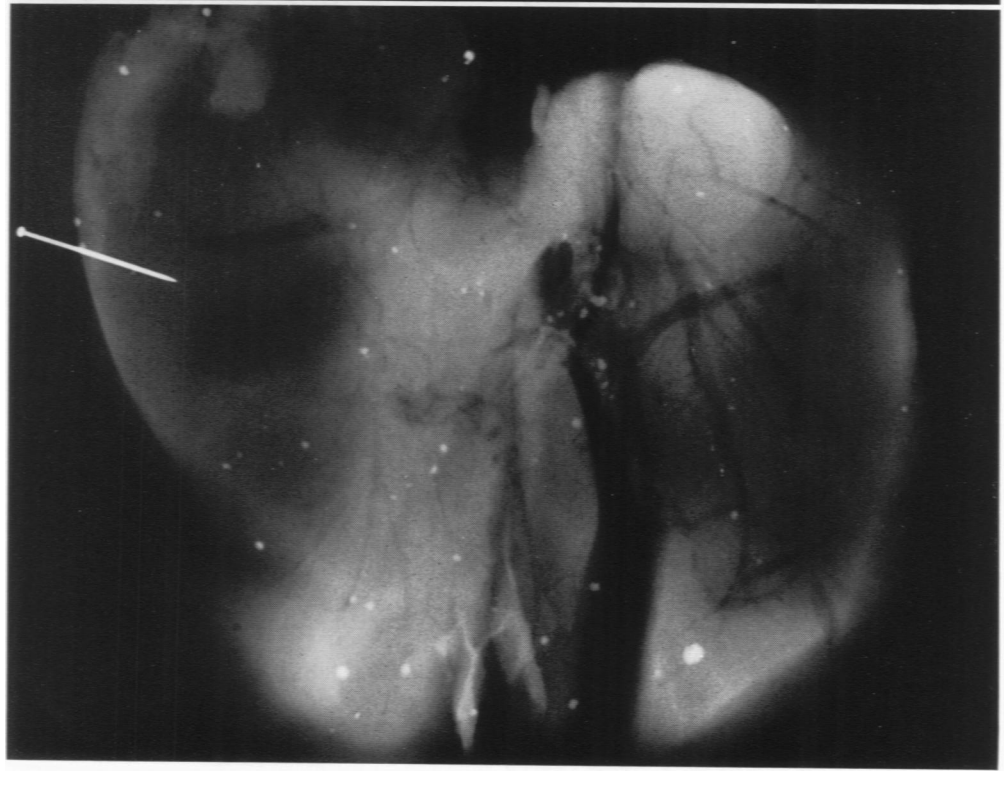

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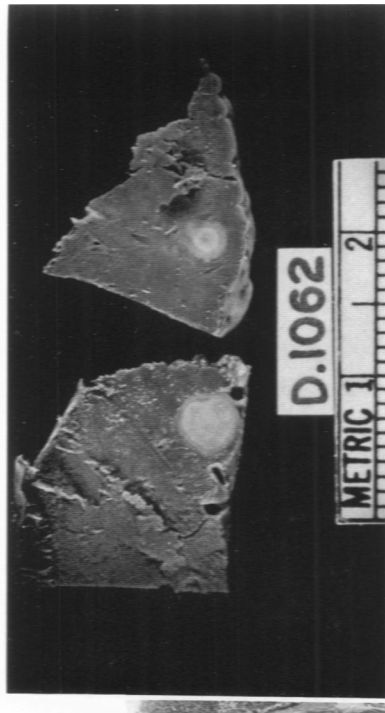


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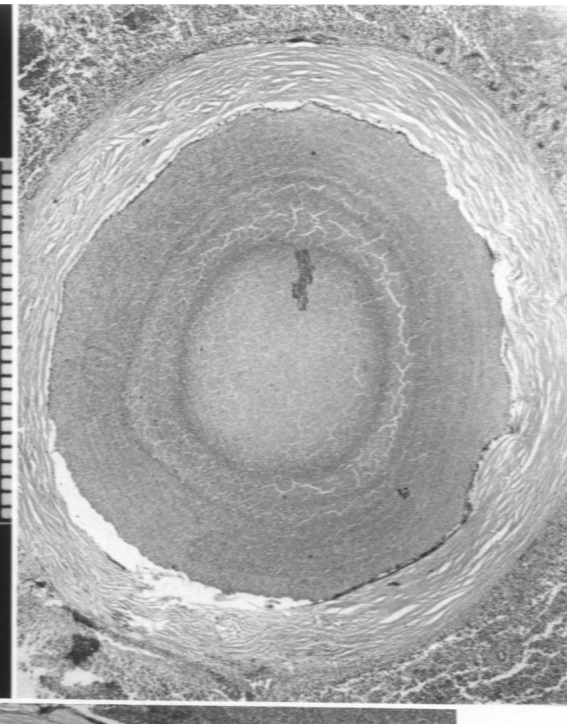

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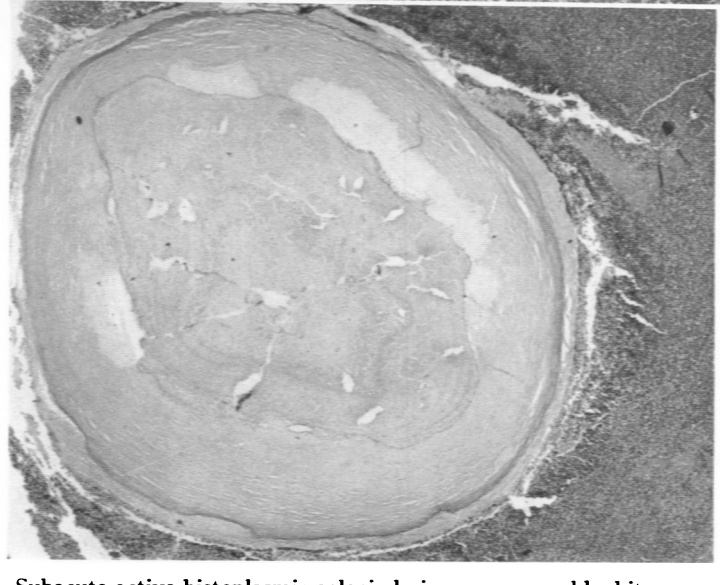

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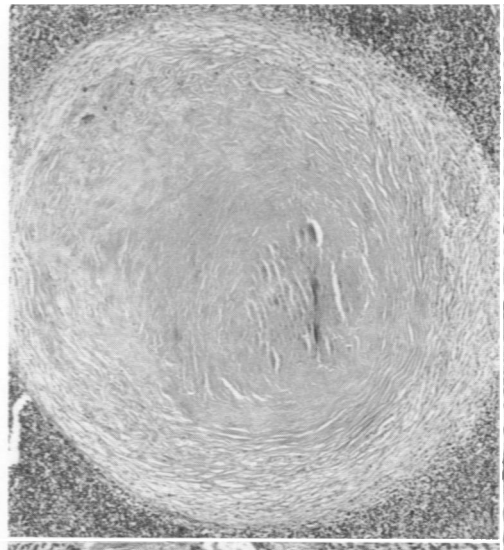
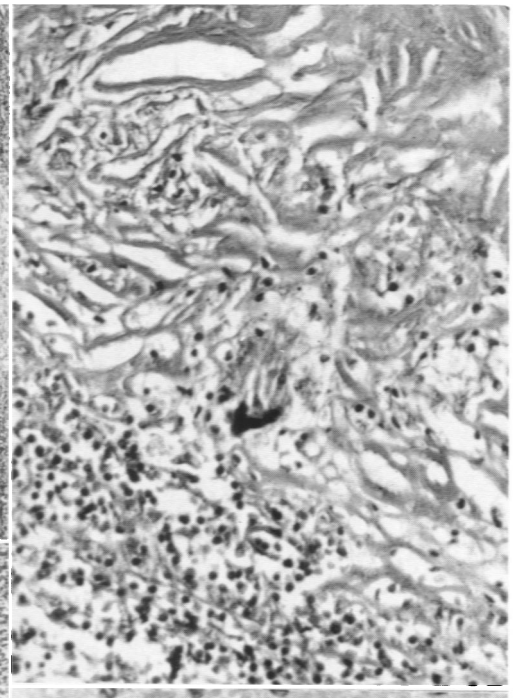
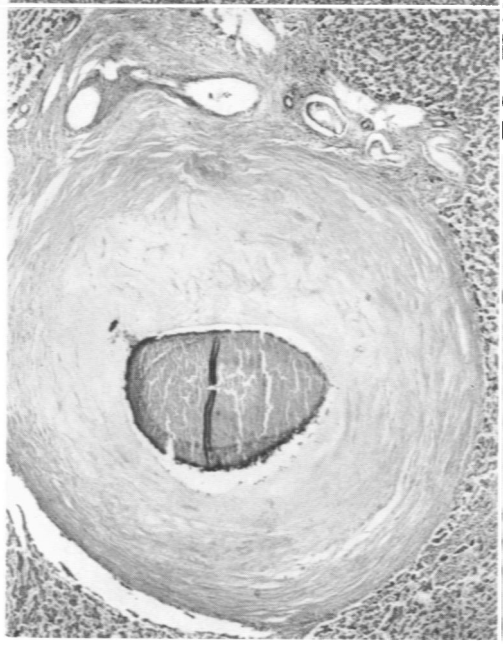
FIG. 3. Subacute active histoplasmic splenic lesions, 59-year-old white woman. Two foci of caseous necrosis are surrounded by a fibrous capsule.

FIG. 4. Advanced splenic lesion, 35-year-old white man. A fibrous capsule surrounds the calcified central necrosis in which concentric bands of mineral deposits are visible. × 35.

FIG. 5. Old splenic lesion, 48-year-old white man, showing a hyalinized fibrous capsule. The focus is heavily calcified. Note the similarity of calcific bands to Liesegang rings, occurring in gels. × 30.







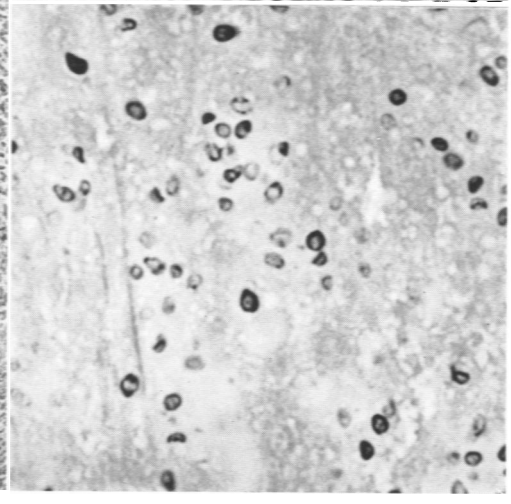


FIG. 6. A still active nodule in the spleen, 76-year-old white man. Noncalcified central coagulation necrosis is surrounded by a loose fibrous capsule. × 50.

FIG. 7. Early splenic lesion, same case as shown in Figure 3. A giant cell and epithelioid cells are demonstrated in the fibrous capsule. Central necrosis may be seen in the right upper corner. × 200.

FIG. 8. Old hepatic lesion from the same case shown in Figure 2. The centrally necrotic and calcified focus is surrounded by a thick, hyalinized fibrous capsule. × 35.

FIG. 9. Splenic focus, same case as shown in Figure 7. Numerous yeast cells which are morphologically representative of *H. capsulatum* are demonstrated in the center of the caseous necrosis. Grocott stain. × 600.