The Usefulness of the Hotchkiss-McManus Stain for the Diagnosis of the Deep Mycoses

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The isolation of pathogenic fungi is more difficult than most physicians appreciate and the average hospital bacteriology laboratory approaches the problem in a half-hearted and uninstructed manner. Too often the diagnosis is a "coup de grâce" administered by the pathologist at the clinical pathological conference.

As a matter of fact, a considerable number of deep mycoses must go unrecognized each year despite the provision of adequate material to the pathologist for at least a tentative diagnosis. There are a number of reasons for this state of affairs; the two which are outstanding implicate both the clinician and the pathologist.

Many physicians develop tubular vision when confronted with patients who present the problems of chronic lymphadenopathy, "cold" abscesses, or persistent pulmonary infiltration. Requisitions for laboratory studies on such patients are likely to bear the provisional diagnosis of tuberculosis and to request a search for acid-fast bacilli or evidence of neoplasia. If a series of negative reports is received from the laboratory, the patient may be treated for tuberculosis on clinical grounds or perhaps discharged. All too often the clinician requests cultural studies for fungi as a last resort and somewhat reluctantly.

On the other hand, the pathologists have been equally hampered by tradition. The student is still admonished to use the low power objective of his microscope and a high power cerebral cortex. We realize that pathologists are busy people and particularly those in large hospitals where a great many routine biopsy sections must be read during a day. Most histopathological diagnosis is done, and adequately, with the low and medium power objectives. When such a microscopic examination seems quite compatible with the clinical diagnosis which accompanied the specimen, it is not surprising that both the pathologist and the clinician should be satisfied with the report "chronic granuloma compatible with tuberculosis."

Unfortunately, so much that seems routine is not, and one may be trapped by the obvious. The cases which we present are all examples of pulmonary mycoses which were initially undiagnosed or misdiagnosed. It is our purpose to bring to general attention a particular procedure which greatly reduces the amount of time required for adequate study of a biopsy

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and which reveals fungi in embedded tissues with a clarity not previously obtainable.

The periodic acid-Schiff method is now six years old and similar techniques which are less precise for fungi antedate it by about 15 years. Nevertheless, it is only within the last 18 months that the method has appeared in journals primarily read by clinicians. Some within our acquaintance have tried the method and rejected it because of poor results. We have used this method for over a year in a routine manner. We have not found it to be capricious, but agree that it requires a basic knowledge of microtechnique which any laboratory technician who is adequately trained and sufficiently interested can carry out without particular attention to individual care of each slide.

The Schiff reagent is prepared by decolorizing basic fuchsin with hydrochloric acid and anhydrous metabisulfite. This forms a colorless fuchsin sulfurous acid which will combine with aldehydes. When this combination takes place, the reagent is recolorized by the addition aldehyde and if the aldehyde is relatively insoluble, the substance containing it will be stained varying shades of pink-to red-to purple.

Hotchkiss and McManus, working independently, found that periodic acid could be used to oxidize certain carbohydrates, mucoproteins, and glycoproteins to aldehydes which could then be colored by the Schiff reagent. The pathogenic fungi and some bacteria contain material which, after oxidation with periodic acid, will form colored addition compounds with the Schiff reagent.

In order to be as objective as possible and to illustrate without exaggeration the advantages of the stain in the presented cases, we have selected a method which is not open to the criticisms of either chance or selection. With the exception of our first case, slides were prepared from serial sections and a single section stained by the periodic acid-Schiff method. After the area of involvement was located in this slide, the succeeding slide was stained by a routine hematoxylin-eosin method. The area of involvement was then found on this slide and photographed by appropriate powers of magnification. This area was localized on the slide and the cover slip removed for restaining by the periodic acid-Schiff method. The same area on the same slide was photographed after the application of this stain. This approach obviates the possible criticism, when photographing successive sections, that the area photographed may have contained abundant organisms in one section and not in the other. This is particularly true when dealing with such small organisms as Histoplasma capsulatum. Illustrations are of the same sections and whatever difference exists in obviousness of the etiology is due entirely to the method of staining. It should be remembered that the black and white illustrations cannot adequately reproduce the color contrast between the red organisms and the green background which is so large an advantage of this method.

The circumstances surrounding case one made it impossible for us to follow the procedure just outlined. This patient was the first on whom the method was tried and it was not until six months later that we decided
Figure 1: Pulmonary Moniliasis. (1A) This x-ray film, taken on admission, reveals widening of the supracardiac shadow due to mediastinal lymphadenopathy. (1B) Right pleural effusion was present six weeks before this film was taken. Fluid production was rapid and had to be removed frequently.
to write this article. The finding of enormous numbers of Candida in what was believed to be a piece of bronchus would not have been given so much significance had we not found areas of moniliasis in two of the lymph node biopsies. We were, as a matter of fact, recutting these biopsies serially in a search for Blastomyces and all sections were stained with periodic acid-Schiff reagent.

When the small areas of moniliasis were found, the paraffin block of the expectorated tissue, thought to be bronchus, was sought. It could not be found, and after a careful review of the original section, it was restained with periodic acid-Schiff reagent without first being photographed. To complete the comedy of errors surrounding this case, six months elapsed before it was felt that this paper would be desirable and photography undertaken. To our chagrin, the periodic acid-Schiff stained node sections had faded completely and our efforts at restaining were unsuccessful. The other lymph node biopsies were negative for fungi.

Figure 1A is the chest x-ray film of a 22 year old negro who was admitted to the Charity Hospital at New Orleans in December of 1950. He was admitted with the chief complaints of painful lumps in both supraclavicular regions, pain in the left anterior chest on deep breathing, and paroxysmal cough. He was a saw mill worker who dated his illness to the onset of a painful tumor in the right infraclavicular region which he described as being like a small egg. After taking sul-

FIGURE 2: Pulmonary Moniliasis. Typical pseudomycellium and blastospores may be seen in this high power field taken in an area of fibrous connective tissue and smooth muscle adjacent to a medium-sized vein.
fonamides prescribed by his physician, the swelling subsided moderately and his general sense of well-being improved. Shortly after return to work he developed subscapular pain which was aggravated by movement, together with cervical lymphadenopathy, fever, anorexia, and general malaise. Sulfonamide therapy lessened his discomfort but he soon developed exertional dyspnea and anterior chest pain.

A low grade fever was present upon admission to the hospital but subsided within two days. With the exception of slight temperature elevations following biopsy procedures, he was afebrile until the fifth week of hospitalization. Thereafter a low grade fever was present on at least several days of each week.

The initial laboratory studies revealed moderate anemia with rapid sedimentation rate, normal total and differential white blood cell count, and Mantoux test which was negative at a 1:1000 dilution but positive at 1:100. Cervical node biopsy was negative on smear and culture for acid-fast bacilli and was reported histopathologically as "chronic nonspecific lymphadenitis." No significant abnormalities were found in the sternal marrow.

Two weeks after admission there was considerable increase in the size of the lymph nodes and several firm and tender nodes were palpated in the left axilla and beneath the left pectoralis major. The enlargement of the matted cervical nodes produced the picture of so-called "bull neck." The spleen was not palpable. Wheezing rhonchi became audible. Three weeks after admission an axillary lymph node was biopsied and reported as showing chronic lymphadenitis. Gastrointestinal x-rays, sigmoidoscopy, and bone survey by x-ray were all negative. The electrocardiogram was not outside normal limits. Since it was the opinion of the attending staff that tuberculosis was the probable diagnosis, he was treated with streptomycin and para-aminosalicylic acid.

A left supraclavicular lymph node biopsy carried out four weeks after admission added no new information. At this time the skin tests were repeated: the standard histoplasmin was 2 plus, the blastomyces vaccine 1:1000 and the coccidioidin 1:100 were negative.

FIGURE 3A FIGURE 3B

*Figure 3*: Pulmonary Coccidioidomycosis. (3A) This is the initial roentgenogram which shows the lung abscess in the left upper lobe. The lesion is not yet cavernous. (3B) Eighteen months later, and after a period of healing, the left upper lobe lesion became a very large thin-walled cavity.
Six weeks after admission he became dyspneic, developed both right and left flank pain and complained of pleurisy on the left. Signs of right pleural effusion appeared and the x-ray film shown in Figure 1B was taken.

At this time a fragmented and friable lymph node was removed from the inferior border of the left pectoralis major and was reported: "lymph node showing

**Figure 4A**

*Figure 4: Pulmonary Coccidioidomycosis. (4A) High dry magnification of an H and E. section. The field shown is in the wall of the cavity, close to its open surface. Little can be seen except caseation necrosis. (4B) The Schiff-stained section, with the same magnification, reveals a heavy network of mycelium, some of which is producing arthrospores. This is not the form expected in tissue.*

**Figure 5**

*Figure 5: Pulmonary Coccidioidomycosis: Arthrospores in the caseous cavitory content. This is an oil-immersion photograph of an area in which the mycelium is almost entirely composed of arthrospores. Sputum from such a cavity would be highly infectious.*

**Figure 6**

*Figure 6: Pulmonary Histoplasmosis. This x-ray film was believed to be compatible with the pathologic diagnosis of Hodgkin's disease. It did not change significantly after treatment with nitrogen mustards. Histoplasma capsulatum was found in the pulmonary parenchyma at autopsy.*
acute necrosis with granulomatous reaction. No acid-fast bacilli found on acid-
fast stain.” This was the first node in which Candida were later found by
periodic acid-Schiff staining.

Thoracentesis revealed a straw-colored fluid which was negative culturally for
pyogens and acid-fast bacilli. Subsequent specimens of pleural fluid were sub-
mitted both for culture and study for malignant cells and one specimen was cul-
tured for pathogenic fungi without positive results being obtained by any of
these procedures.

After the patient had received 42 grams of streptomycin he was discharged, but
had to be readmitted within two weeks because of increasing dyspnea. His pleural
effusion had increased and generalized lymphadenopathy was present. All nodes
were tender as was the liver.

On this second admission he was afebrile during the first three weeks of hos-
italization and febrile thereafter until the time of his death eight weeks after
his second admission. An additional lymph node biopsy was done but it, together
with further bacteriologic and microscopic studies of the pleural fluid, were un-
revealing. The biopsy report read: “caseation necrosis compatible with tubercu-
losis.” This was the second node to reveal foci of Candida in the areas of caseation
necrosis. Nitrogen mustard did not influence his clinical course. Two weeks before
his death he developed ascites, facial edema, and dysphagia. A culture of the
sputum taken three weeks prior to death was reported as showing Candida sp.
which was clinically considered to be a contaminant.

One week before death, during a severe paroxysm of coughing, the patient had
a small hemoptysis accompanied by a 1 x 2 x 0.5 cm. of tissue which was saved and
placed in formalin. This was sectioned and believed to show a neoplasm, type
unspecified, probably lymphosarcoma. Permission for autopsy was not obtained.

Nine months after his death the lymph node biopsies were recut and stained by
the periodic acid-Schiff method. As previously stated, two of these and the ex-
pectorated tissue were positive for Candida. Figure 2 is a central portion of the

FIGURE 7A

FIGURE 7B

Figure 7: Histoplasmosis. (7A) This photograph represents a high dry magnifica-
tion of a portion of one of the lymph nodes biopsied in Shreveport and stained
with hematoxylin and eosin. No organisms could be found under oil immersion. The
area at the end of the blood vessel is reproduced in (7B). With the periodic acid-
Schiff stain, numerous H. capsulatum could be found. The reticulo-endothelial
cell at the base of the blood vessel is shown under oil immersion magnification in
order to depict the large number of Histoplasma present.
expectorated tissue after it was restained by the periodic acid-Schiff method. The appearance of the fungus in tissue is so typical a diagnosis of moniliasis can be made although the species of Candida cannot be stated.

Figure 3 is a reproduction of two x-ray films of a middle aged white man who first became ill while residing in Arizona. The initial film was taken during a bout of "influenza" and was interpreted as showing an abscess in the left upper lobe probably coccidioidal in etiology. No fungi were isolated but the coccidioidin skin test was positive. By early 1949 this abscess was allegedly healed and the patient was asymptomatic. After returning to work, he became ill, and in September of 1949 a cavity was again found by x-ray film. At the time of his admission to Charity Hospital in November of 1949 he was producing between 500 and 1,000 milliliters of sputum daily, was febrile, and an x-ray film taken at that time showed a large left upper lobe cavity. Skin tests were negative through a 1:10 dilution of coccidioidin and complement-fixation and precipitin tests were reported as negative by Dr. C. E. Smith. The histoplasmin skin test was negative. Three sputum concentrates and one culture were negative for acid-fast bacilli and three sputa were negative on culture for pathogenic fungi. However, sputa cultured at the medical school laboratories were positive for Coccidioides immitis. A left upper lobectomy performed in March of 1950 was complicated by empyema. One month after operation a massive pulmonary hemorrhage occurred and the patient died.

The left upper lobe was submitted to the pathology department at the time of operation and was reported: "Lung abscess, chronic pneumonia, giant cell type. Bronchitis, chronic. There are numerous intracytoplasmic inclusions suggestive of virus inclusion. While giant cell pneumonia is not frequent in adults, it does occur rarely." The paraffin blocks were recut and sections submitted to periodic acid-Schiff stains. Figure 4 illustrates the findings by hematoxylin-eosin and periodic acid-Schiff staining. It is of special interest that sections through the wall of the cavity and extending into the cavitary content revealed the presence of arthropores. An oil immersion photograph of some of these is shown in Figure 5. It is

**FIGURE 8A**

**FIGURE 8B**

*Figure 8:* Histoplasmosis. (8A) A portion of the first node to be examined at Shreveport showing typical granulomatous reaction with many Reed-Sternberg cells. This H. and E. section revealed no Histoplasma and all concurred in the diagnosis of Hodgkin's disease. The Dorothy Reed cell in the center of the field is shown in (8B) after periodic acid-Schiff staining and oil immersion magnification. It is stuffed with Histoplasma.
difficult to believe that fungi so obvious by the periodic acid-Schiff method could be so indefinite in sections stained with hematoxylin and eosin. The presence of arthrospores within the contents of the cavity is proof that this disease may be contagious. The size of the cavity afforded an environment more suitable to arthrospores than to the tissue-invading forms. Such circumstances must be quite unusual though one can now be sure that man to man transmission is possible.

The third patient was a 63 year old white farmer who was admitted to a hospital in Shreveport, Louisiana in May of 1951 complaining of increasing fatigability and general malaise of two years duration. He had experienced intermittent afternoon fever for 18 months and had lost 30 pounds in the preceding year. For several years he had been aware of a chronic nonproductive cough which had undergone no change. The admission examination was not enlightening except for the presence of prominent lymph nodes in the left axilla. These were hard but neither fixed nor tender. Sputum examination was negative for acid-fast bacilli and fungi on direct smear and one sputum was negative for Mycobacterium tuberculosis on culture. The chest x-ray film was interpreted as being within normal limits. While in the hospital his fever ranged between 100 and 103 degrees F.

On the seventh hospital day biopsy of the left axillary lymph nodes was undertaken and microscopic sections presented the picture of Hodgkin's disease. Smears and cultures of this lymph node were negative for acid-fast bacilli and pyogens. The patient was then transferred to a New Orleans hospital for irradiation therapy. His biopsy slides were reviewed and the diagnosis confirmed. He continued to be febrile, developed hepato-splenomegaly and supraclavicular lymphadenopathy. His total white blood cell count was 9,050 with a differential showing 93 per cent polymorphonuclear neutrophils.

Shortly after admission a left supraclavicular lymph node biopsy was obtained and was regarded as showing only marked necrobiosis with very few viable cells remaining. Those present revealed a granulomatous reaction which was considered nonspecific in the light of a negative acid-fast stain.

This patient was cyanotic, and extensive pulmonary pathology was revealed by

*Figure 9*: Pulmonary Blastomycosis. Extensive fibroproductive infiltration is present bilaterally and a 3 cm. cavity can be seen in the extreme apex on the left.—

*Figure 10*: Blastomycotic Osteomyelitis. The two areas of osteomyelitis, one very small, show no reaction of the surrounding bone. The sequestra are very dense and small, simulating metallic fragments.
x-ray film as shown in Figure 6, and nitrogen mustards were given. Since there was no response to therapy, speculation arose concerning the possibility of this being something other than Hodgkin’s disease. A third lymph node biopsy was then secured from the left axilla. Histopathology reported Hodgkin’s disease with granulomatous areas with necrosis compatible with tuberculosis. Rare acid-fast rods were supposedly seen in a single section. All three biopsy specimens were

**FIGURE 11A**

**FIGURE 11B**

*Figure 11*: Blastomycotic Osteomyelitis. (11A) The low-power H. and E. preparation reveals a chronic type of granulation tissue. (11B) The same field after staining with periodic acid-Schiff reagent. Note the rosette of Blastomyces in a giant cell.

**FIGURE 11C**

**FIGURE 11D**

*Figure 11*. Blastomycotic Osteomyelitis (continued). (11C) High-dry magnification of the H. and E. section shows bodies which are strongly suggestive of Blastomyces. (11D) Hotchkiss-McManus staining of same field. This is the same rosette seen in (11B).
then submitted to the Armed Forces Institute of Pathology which concurred fully with the diagnosis of Hodgkin's disease and tuberculosis.

In addition to the routine cultures for acid-fast bacilli, cultures on Sabouraud's dextrose agar and blood agar were obtained from the third biopsy. Fungus growth was noted by the eighth day and on the 11th day diagnostic tuberculate chlamydospores were seen and Histoplasma capsulatum recognized. No growth occurred on Petragiani's medium by the end of eight weeks. Very careful review of the previous hematoxylin and eosin stained slides failed to show any evidence of histoplasmosis. At this time permission was sought and gained to study the negative tissue blocks with the periodic acid-Schiff stain. Numerous Histoplasma capsulatum were demonstrated in all of the previously examined lymph nodes. In many areas most of the forms were degenerate and present largely as "ghosts"; in other areas perfectly typical and easily recognized intracellular forms were identified.

After Histoplasma were identified in the periodic acid-Schiff stained sections, it was not particularly difficult to locate them in the hematoxylin and eosin stained sections of the same node with the exception of the first nodes biopsied in Shreveport and the lymph node which revealed severe necrobiosis. In neither of these two biopsies were we ever able to recognize with certainty Histoplasma capsulatum in the hematoxylin and eosin preparations. Figures 7 and 8 illustrate the differences in appearance of these sections when stained by the two methods.

Additional positive cultures for Histoplasma were obtained from the bone marrow, blood, and sputum. Two weeks before his death an additional lymph node was removed from the left axilla and histological examination revealed a single focus of Histoplasma in the hematoxylin and eosin preparation. Hotchkiss-McManus stains of the same sections disclosed many organisms. Autopsy revealed generalized histoplasmosis.

The fourth patient was admitted to the Charity Hospital by transfer from another institution in New Orleans. This institution had made a diagnosis by x-ray film of tuberculosis and he was transferred to the tuberculosis unit of Charity Hospital. Ten months prior to admission he had suffered a "cold" with

FIGURE 12A

FIGURE 12B

*Figure 12:* Blastomycotic Lymphadenitis. (12A) The routine H. and E. stain revealed bodies which did not appear to be a part of a granulomatous reaction but their identity did not become apparent until the section was restained with the Hotchkiss-McManus stain (12B). The two fields are identical.
Figure 13: Pulmonary Blastomycosis. (13A) In the multinucleate giant cell several round bodies, clear centrally, may be seen. This is a high-dry magnification of a H. and E. preparation. (13B) A marginal section of the multinucleate giant cell is shown with oil-immersion magnification after staining with the periodic acid-Schiff method. Note the budding Blastomyces.

FIGURE 13C: The planogram shows a cotton-ball density in the right lower lobe. Several lucencies are evident near the center of the lesion.
frequent remissions for short periods of time. Cough, productive of small amounts of white sputum had been present for approximately six months before his admission. Blood streaking of the sputum had occurred on two or three occasions. Hoarseness, which was progressive, began four months before hospitalization and became progressively more severe. Weakness, general malaise, and a 22-pound weight loss occurred over the six month period, but neither chills, fever, nor chest pain had been present. He was examined by his personal physician five months prior to admission. Physical examination together with a chest x-ray film revealed no abnormality. A family history of tuberculosis was present.

The first three sputum concentrates were negative for acid-fast bacilli, but the fourth was reported as positive. A Mantoux test was positive at 1:1000 dilution and the sedimentation rate was elevated. On the basis of the positive sputum concentrate, treatment for pulmonary tuberculosis was undertaken. Figure 9 is a reproduction of an x-ray film taken at this time. There were 16 subsequent sputum smears stained for acid-fast bacilli, all of which were negative. Eight additional sputum concentrates were negative. One sputum culture was negative for Mycobacterium tuberculosis and two cultures were negative for pathogenic fungi.

Laryngoscopy revealed an erosion with granulation tissue over the left true cord. Both cords were injected and inflamed. The larynx was similarly involved. It was the impression of the endoscopist that either laryngeal tuberculosis or carcinoma should be considered. Although there seemed to be some clearing of the chest x-ray film following treatment with bed rest, streptomycin and PAS, there was no change in the patient's hoarseness, weight, or general sense of well being. Because of this and the single positive sputum concentrate, laryngeal biopsies and cultural studies of the sputum for fungi were requested. The initial laryngeal biopsy was reported as showing: "Tissue from larynx showing chronic inflammation, with active lymph follicles." A specimen removed from the left vocal cord at the same time was reported: "Tissue from left cord showing epithelial hyperplasia with acute and chronic inflammation and scattered multinucleated giant cells with peripheral nuclei." Periodic acid-Schiff stains revealed many Blastomyces.

There was no change in his chest x-ray film after its initial improvement, or in his general course following additional therapy with Tibione and pneumoperitoneum.

Nine and a half months after admission he bumped his right leg against a bed-side chair producing a small hematoma. This was quickly followed by a draining sinus and the x-ray film shown in Figure 10 revealed two cystic lesions in the proximal tibial shaft. Two minute sequestra were evident. This was considered to be a tuberculous osteomyelitis and it was incised and drained and the pus cultured for Mycobacterium tuberculosis and pyogens. A portion of the curettings was sent to histopathology. Cultures of the pus were negative, and the curettings were reported by the pathologist: "Chronic debris. No acid-fast bacilli found." Figure 11 shows a hematoxylin and eosin stained section of these curettings and the same section when restained by the periodic acid-Schiff stain.

Thirteen months after admission a prominent and tender pretracheal lymph node appeared and was removed for biopsy. A granulomatous response with caseation necrosis was noted and the pathologist requested sections stained by the Ziehl-Neelsen method. In these sections he noted a few cyst-like bodies with a red staining central granule which were suspected of being Histoplasma. We were given a section of this node to restain by the periodic acid-Schiff procedure. Figure 12 illustrates a section of this node when stained with hematoxylin-eosin and when stained with the Schiff reagent. The periodic acid-Schiff stain of this node prompted us to review the previous biopsies and to culture the sputum for fungi. A laryngocutaneous fistula developed at the biopsy site and pus from this and the osteomyelitis in his right leg were likewise cultured for fungi. Sputum and pus from both areas were positive on culture for Blastomyces dermatitidis.
The last patient was a 56 year old negro hospital employee whose routine chest x-ray film prompted his admission for diagnostic studies. A rounded density was seen in the right lower lobe with no surrounding infiltration. The sharp margins suggested the possibility of a tuberculoma or neoplasm. The planogram which is shown in Figure 13 demonstrated several central lucencies and lung abscess was added to the differential diagnosis.

The patient was entirely asymptomatic and there was nothing of significance in the past history. The physical examination revealed no abnormality and the laboratory studies were within normal limits except for a positive skin test with old tuberculin. Sputum cultures for acid-fast bacilli, fungi, and pyogens were plated and smears were made and stained for malignant cells. Nothing of significance was found.

While these studies were in progress the patient was given large doses of penicillin without effect clinically or radiologically. Right lower lobectomy was performed without difficulty.

The lesion was found to have central ulceration and a portion of the lesion was ground in saline, centrifuged, and the immediately supernatant fluid cultured on blood agar and Sabouraud's dextrose agar. A portion of the material was likewise inoculated into the yolk sac of six chick embryos. Some of the original tissue was further ground and the tissue juice streaked on blood agar, Sabouraud's agar, and Petrik's medium. No fungi were isolated by any of these methods.

Hematoxylin and eosin sections of the embedded lung showed a very large number of organisms most of which were present within multinuclear giant cells. These were quite small, appeared encapsulated, and were thought to be Histoplasma capsulatum. A representative area from such a slide is shown in Figure 13A. Further study of the hematoxylin and eosin preparations lead to considerable doubt as to the nature of the fungus since budding forms were seen which looked more like Blastomyces dermatitidis or Cryptococcus neoformans than they did Histoplasma. The periodic acid-Schiff stain was then applied to this section as shown in Figure 13B. Typical thick-walled and budding forms were found, some of which were dumb-bell shaped with a heavy collar at the point of constriction. These forms were entirely typical of Blastomyces dermatitidis. However, it was of great interest that the majority of the forms did not stain well in the periodic acid-Schiff method. The staining of the walls appeared somewhat granular and slightly diffuse. Many, if not most, of the forms seemed to be undergoing some degeneration. Except for the beautiful demonstration of the budding and elongate forms which appeared quite normal, the general staining of the section by the periodic acid-Schiff method was inferior to that revealed by hematoxylin and eosin preparations.

Our embarrassment at being unable to culture the fungus from the surgical specimen was lessened somewhat by the failure of the hospital laboratory with its numerous cultures of the same specimen. Skin tests with blastomycin were negative following operation. The patient was kept at bed rest without other treatment for four months and then discharged. There were no postoperative complications and no evidence of infection during the period of convalescence.

Although a final diagnosis is not possible without cultural proof, several of our colleagues as well as the Armed Forces Institute of Pathology were in agreement with the diagnosis of blastomycosis. One of us has previously noted that when Histoplasma capsulatum occurs for the most part in multinuclear giant cells, the forms are usually degenerating and either not viable or are culturable with great difficulty. It is possible that this phenomenon may be shared by Blastomyces and that failure in two laboratories to culture the organism is due to the small number of viable forms. There was much in the histopathology of this lesion to suggest that the body was overcoming the infection spontaneously. It is not unlikely that this lesion would have healed without any sort of treatment had it not been discovered by routine x-ray film.
Comment

It is true that with the exception of the first two cases presented the pathologists were able to retrospectively demonstrate the fungi by routine staining procedures when the area in which they were present was demonstrated by the Schiff stain. In the last case presented the organisms were more readily seen in the routine H and E preparation, but were initially misdiagnosed by one of us until their true nature became apparent after staining with periodic acid-Schiff reagent. It is our belief that anyone who will spend a few days learning the method and preparing a representative selection of slides will need no persuasion to convince him of its utility. Its primary advantage is that under low and high dry powers of magnification fungi can be detected which are completely missed by the usual low and high dry powers survey of the biopsy or autopsy specimen. With the staining methods in general use, fungi will be missed occasionally even when the sections are studied under the oil immersion objective. However, many hours can be spent in such a search without covering the area in which the agent is present if the infection is sparse. The color contrast of the periodic acid-Schiff stain immediately attracts one's attention to areas of suspicion and higher power observation will then confirm or disprove the presence of fungi in the area. One of the advantages of this stain is its applicability to sections which have been stained routinely months or even years before.

It is probably true that an etiologic diagnosis of mycotic infections can be made in many instances by using this staining method. However, this will not be generally acceptable, and rather than serving to decrease our efforts at cultural isolation and identification of the fungi, it should increase them.

A number of fungi may be found in sputum, bronchial exudate, or other body secretions and excretions without there being any certainty that their rôle is that of a pathogen. At times these fungi may be found within chronic abscesses or cavities in the lung or they may be seen in the alveolar exudate at postmortem examination. Whether such fungi are present as saprophytes or as pathogens may not be certain unless they can be demonstrated as tissue invaders. The periodic acid-Schiff stain is of special usefulness for this problem. For example, a patient dying from an aspiration pneumonia was found to have Candida in the alveolar exudate when stained with routine methods. A periodic acid-Schiff stain demonstrated these beautifully, but failed to show any evidence of tissue invasion.

SUMMARY

The periodic acid-Schiff stain has many uses, among them being the demonstration of fungi in tissue. Its use for this purpose is increasing but not as rapidly as it should. Besides revealing the fungi with exceptional clarity and detail, the factor of color contrast is very great. Organisms appear in varying shades of red and if light green is used as a background
stain, very rapid survey of stained sections is possible using the low and high power dry objectives.

Two cases are presented in which fungi could never be demonstrated by hematoxylin and eosin stains but were readily found by the periodic acid-Schiff method. Two additional cases are discussed in which the histological diagnosis was not made until biopsy material was stained with the Schiff reagent. A fifth case illustrates how proper identification may be greatly facilitated by this stain.

RESUMEN

El colorante periódico acido-Schiff tiene muchos usos entre ellos que sirve para la demostración de hongos en los tejidos Su uso con este fin está aumentando pero no tan rápidamente como debiera. Además de que revela los hongos con claridad excepcional y con detalle, el factor de contraste de color es muy grande. Los organismos aparecen en tonos diversos de rojo y si el verde claro es usado como fondo de contraste es posible hacer una revisión rápida de los cortes coloreados usando los objetivos secos de bajo y alto poder.

Se presentan dos casos en los que los hongos nunca pudieron ser demostrados por la hematoxilina y eosina pero fueron fácilmente encontrados por el método periódico acido-Schiff. Dos casos más se discuten en los que el diagnóstico histológico no se hizo sino hasta que el material de biopsia fue teñido con el reactivo de Schiff. Un quinto caso ilustra cómo puede facilitarse grandemente la identificación adecuada por este colorante.

RESUME

La coloration acide de Schiff a beaucoup d’usages, parmi lesquels la possibilité de mettre en évidence les champignons dans les tissus. Son utilisation dans ce but es de plus en plus répandue, mais pas autant qu’elle le mériterait. En dehors du fait qu’ainsi se trouvent révélés les champignons avec une précision et une clarté exceptionnelle, il existe un facteur important dû au contraste. Les microbes paraissent dans différentes nuances de rouge, et si on réalise une coloration verte de fond, il est possible d’obtenir avec les objectifs secs à petit et à gros grossissement un examen très rapide des coupes colorées.

Les auteurs rapportent deux cas dans lesquels l’hématoxyline-éosine n’avait jamais pu mettre en évidence les champignons qui furent facilement révélés grâce à la méthode acide de Schiff. Deux observations dans lesquelles on ne put faire le diagnostic histologique avant que les coupes biopiques n’eurent été colorées par le réactif de Schiff sont mises en discussion. La cinquième observation montre comment une identification précise peut être largement facilitée grâce à cette coloration.

REFERENCES