circulating anticoagulant may be present,[29] and this also may depend on an altered immune mechanism. Thrombocytopenia is common in SLE,[27] and this has been attributed to an immune mechanism. The accumulated evidence suggests that the serum factor with affinity for nuclei is an anti-nuclear antibody, and that it is probably another manifestation of the altered immune mechanism in this disease.

SUMMARY

A method utilizing the fluorescent antibody technique was employed to study a factor with affinity for calf thymic nuclei in the serum of patients with systemic lupus erythematosus and other diseases. An attempt was made to quantify the amount of serum factor by means of titre determinations. High titres (up to 1:2048) were encountered in the serum of patients with multiple clinical manifestations of SLE and a history of a positive L.E. cell test. The titre of the anti-nuclear substance was not always related to the degree of fluorescence observed.

The sensitivity of the method was compared with the results obtained with L.E. cell preparations performed on the same sample of serum. In 39 cases of patients who at some time had a positive L.E. cell preparation, the L.E. cell preparation was positive in 20 at the time of study, whereas 35 cases showed significant titres of anti-nuclear antibody. Positive results were also seen in three cases of possible SLE and in five cases with diseases seemingly related to SLE. One apparently "false positive" result was obtained with the serum from a patient with multiple myeloma and macroglobulinæmia.

High serum titres were found in 13 cases showing active disease, but also in 12 cases either with slight activity or in remission. In only two cases was there evidence of a low titre associated with active disease. The changes in serum titre with remission or exacerbation of the diseases are illustrated by several cases.

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CRYPTOCOCCOSIS: A REPORT OF FIVE CASES

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CRYPTOCOCCUS NEOFORMANS (*Torula histolytica*) is a yeast-like non-mycelial organism, unique in possessing a thick mucoid capsule. It is widespread in nature and has been isolated from soil, fruit, raw milk and pigeon excreta. Infection by this organism was first described by Busse[1] in 1894, and now over 300 cases have been reported.[2] There is a widespread distribution of the disease with no distinctly endemic areas such as occur with histoplasmosis and coccidioidomycosis. The majority of reported cases are from the United States of America and from Australia. There may not be an increased incidence of the disease in these areas but rather a greater clinical awareness.

The organism is pathogenic to a wide variety of animals including horses, dogs, cats, monkeys, guinea-pigs and dairy cattle. Rabbits and birds resist infection, possibly because of their higher body temperature. In man, the usual primary infection is in the respiratory tract. Experimental evidence,[3] however, suggests a possible portal of entry in the alimentary tract, and occasional infections have followed local inoculation.[4] The organism has a low virulence, and no cases of direct transmission to man have been reported. However, extensive outbreaks of cryptococcal mastitis in epidemic proportions have occurred in dairy herds. The majority of patients with cryptococcosis are between 30 and 60 years of age, but the disease has been described in patients of all ages. Males are affected slightly more frequently than females.

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The infection may remain localized at the site of entry but more frequently it disseminates, showing a definite predilection for the central nervous system. Thus by far the commonest clinical syndrome results from meningo-encephalitis, the onset of which is often insidious, although a history of upper respiratory or pulmonary infection may be obtained. Frontal headache is usually the first complaint and may initially be intermittent but becomes increasingly severe and continuous. Vomiting is a frequent complaint and there may be vertigo and faintness. Examination usually reveals an expanding intracranial lesion with few localizing signs, and there is often evidence of meningeal irritation. Fever is slight or absent.

Diagnosis is made by examination of the cerebrospinal fluid. This is usually under increased pressure and clear, with slightly increased protein and reduced sugar and chloride concentrations. There are usually between 300 and 700 white blood cells per mm., lymphocytes predominating.

The organism can be seen in direct examination of the spinal fluid by using the India ink technique. This outlines the mucinous capsule as a transparent halo around the cell, a spherical structure between 4 and 7 microns in diameter. Budding forms help in identification. The organism grows on most common bacteriological media, both at 37° C. and at room temperature, forming visible mucoid colonies in two or three days. One source of confusion is the presence of encapsulated, non-pathogenic cryptococci in air, sputum and skin, usually classified as *Crytococcus neoformans* var. *innocuous*, but these strains are unable to grow at 37° C.

Clinically there is usually progressive deterioration until death supervenes in a few weeks or months. However, infection may remain static. Beeson[5] reported one case of a young woman whose illness lasted for 16 years from diagnosis till death. Although the brain is the commonest site of infection, other systems may be involved. Cawley, Grekin and Curtis[6] in reviewing 120 cases of cryptococcosis noted that 13 had cutaneous manifestations of the disease. Bone lesions are present in about 10% of cases[7] and are usually multiple and widely disseminated. Clinical diagnosis is often difficult and is usually made by biopsy or culture. Joint involvement is rare and occurs secondarily to bone disease. Any tissue, however, may be involved in the widely disseminated forms of the infection, although clinical manifestations are unusual.

The incidence of cryptococcosis is much higher in patients with malignant diseases of the reticuloendothelial system,[8] such as Hodgkin's disease, leukæmia, lymphosarcoma and multiple myeloma. This parallels the higher incidence of other infections, such as tuberculosis and brucellosis, in these disorders, and probably represents a lowered body resistance. In one such case,[9] cryptococcosis, histoplasmosis and tuberculosis occurred simultaneously in a patient with Hodgkin's disease.

The clinical and pathological features of cryptococcosis are illustrated by the following case reports of patients seen during various stages of their disease at the Jewish General Hospital, Montreal, in the past five years.

CASE REPORTS

CASE 1.—D.L., a 20-year-old French Canadian girl, was admitted to hospital in July 1946, complaining of a painless lump in the neck of three months' duration. A chest radiograph revealed slight mediastinal enlargement, and a diagnosis of atypical Hodgkin's disease was made after biopsy examination of a cervical node. In the succeeding 10 years she had nine hospital admissions during which the diagnosis of Hodgkin's disease was confirmed by repeated biopsies. X-ray therapy, nitrogen mustard, T.E.M. and blood transfusions were administered. In January 1957, she complained of recent weight loss, and x-ray examination of the chest revealed for the first time patchy infiltrations in the right lower and left upper lung fields. In February 1957, she complained of a sore throat and cough; a superficial 3-mm. ulcer was noted on the soft palate. This healed in three days. She was found to be diabetic and insulin therapy was commenced. In April 1957, she complained of difficulty in breathing and of continuous left shoulder pain. She expectorated copious white sputum and had a fever of 102° F. Chest radiography revealed further infiltration in the lung fields. Yeasts were noted on a smear of the sputum, and pneumococci and monilia were reported on culture. Culture for *Mycobacterium tuberculosis* was negative. The dyspnœa was partially relieved by bed rest, use of bronchial dilators, chemotherapy and ACTH. By May 1957, she was again complaining of weakness and dyspnœa, and the chest radiograph showed diffuse infiltration. She continued to cough and had intermittent fever. Her 15th and final hospital admission was on June 18, 1957. In addition to the previous findings she was now anæmic and very ill. She complained bitterly of right frontal and temporal headache and of nausea. She became disoriented, drowsy and eventually comatose, and died on July 10, 1957.

At autopsy the body weighed 88 lb. Enlarged, fleshy but discrete lymph nodes were found in the mesenteric, axillary, tracheo-bronchial, mediastinal, retroperitoneal and peripancreatic regions. The spleen weighed 260 g. and was adherent to the surrounding structures. Section showed nodular brown lesions obliterating the normal architecture. Tumour masses were found in the stomach, and another mass had partially obstructed the sigmoid colon. Nodular, yellow-brown rubbery masses were seen in all lung lobes, especially in the peribronchial sites. Yellow areas were noted in the thoracic and lumbar vertebral bodies. No abnormality was seen in the meninges, but after fixation scattered cystic areas were noted throughout the brain substance. These varied between 1 and 3 mm. in diameter and were most numerous in the basal ganglia.

Microscopically, typical changes of Hodgkin's disease were found in all lymph nodes examined and also in the spleen, liver, stomach, sigmoid colon and vertebral bone marrow. The solid masses in the lungs also represented lymphomatous infiltration. In addition to this widespread neoplastic disease, a widely dissemin-

Canad. M. A. J.
Sept. 10, 1960, vol. 83

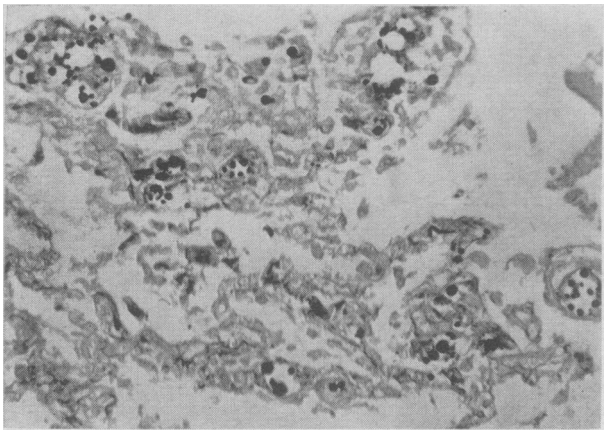

Fig. 1.—Case 1. Lung with budding cryptococci in alveolar septa. Gömöri's methenamine-silver stain, × 200.

ated infection by *Cryptococcus neoformans* was noted. The small cysts in the brain contained masses of the encapsulated organisms, and the meninges were also involved. Characterisically there was virtually no reaction to the organisms. The lungs (Fig. 1) revealed widespread involvement both in relation to and separate from the neoplastic infiltrates. The organism was also found in lymph nodes, kidney, bone marrow and the thyroid gland. In the kidney the organisms were localized in glomeruli (Fig. 2), where focal destruction was evident.

CASE 2.—E.R., a 53-year-old Hungarian woman, was first admitted on June 15, 1959, complaining of a 24-lb. weight loss, anorexia and fatigue of three months' duration. She had had an inguinal node biopsy in Hungary in 1952, and had received a course of x-ray therapy. Examination in June 1959 revealed anæmia, hepatosplenomegaly and an enlarged axillary node.

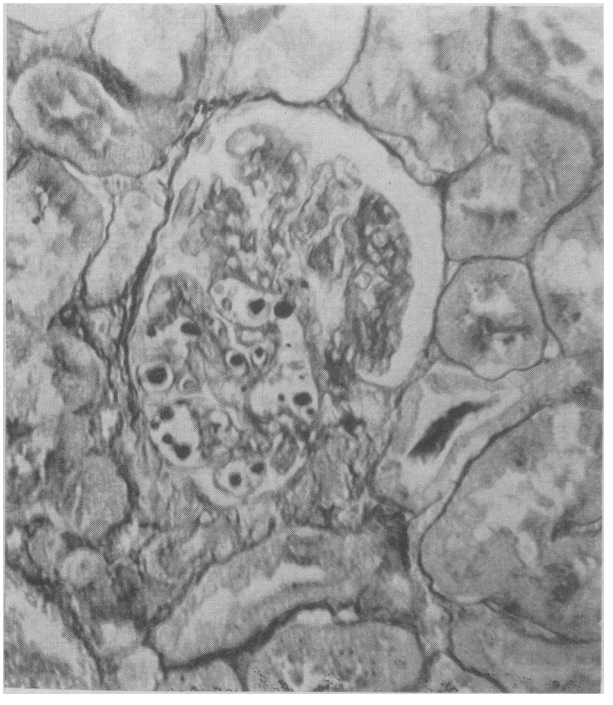

Fig. 2.—Case 1. Renal glomerulus with cryptococci within capillary loops. Gömöri's methenamine-silver stain, × 200.

Chest radiography revealed a large pleural effusion on the left with widening of the mediastinum. Hodgkin's disease was diagnosed by axillary node biopsy. She was given nitrogen mustard and x-ray therapy, and her condition improved slightly.

She was readmitted on December 12, 1959, complaining of persistent and progressive vomiting for ten days, associated with constant occipital headache and dizziness. Left ptosis but no other neurological abnormality was found. No abnormal masses or lymph nodes were palpated. A chest radiograph revealed an area of pulmonary consolidation in the left base. Her condition steadily deteriorated; she became drowsy and disoriented, and died in coma on December 25.

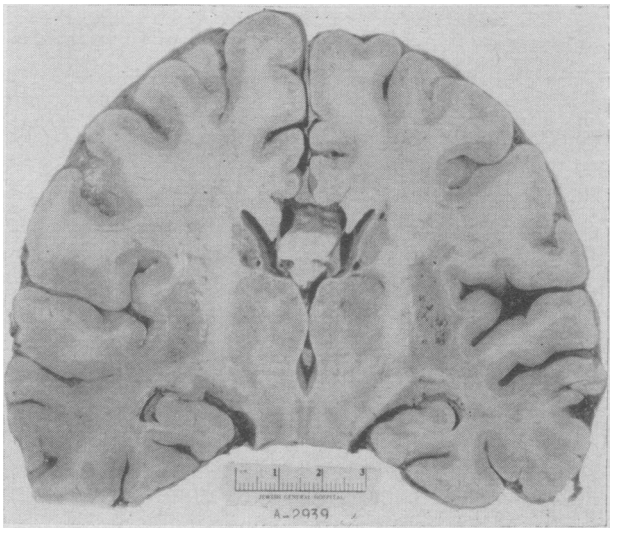

Fig. 3.—Case 2. Clusters of small cystic lesions in the putamen on the right and in the caudate nucleus on the left.

At autopsy the body weighed 110 lb. There was 500 c.c. of clear fluid in the right pleural cavity. Enlarged matted mediastinal and peripancreatic lymph nodes were found. The spleen weighed 210 g. and was firm and nodular. A tumour mass was found at the hilum of the left lung. The meninges were slightly pigmented but not grossly thickened. Section of the brain after fixation (Fig. 3) revealed numerous clusters of small cysts in the basal ganglia, bilaterally. Microscopical study showed Hodgkin's disease involving

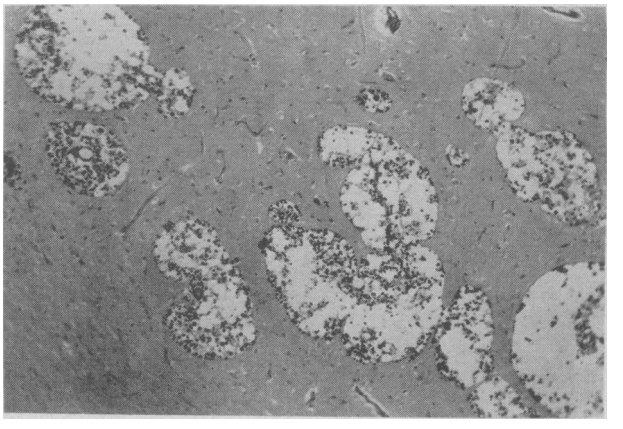

Fig. 4.—Case 2. Brain with expanding colonies of cryptococci. Note the absence of inflammatory reaction. Gömöri's methenamine-silver stain, × 150.

spleen, lymph nodes and lung. *Cryptococcus neoformans* was demonstrated in large numbers in the brain cysts (Fig. 4), the meninges, lungs, liver and spleen. The organisms occurred singly or in variably sized clusters, pushing aside the normal tissues. Virtually no inflammatory response was present in any of these situations.

CASE 3.—S.A., a 63-year-old white woman, was first admitted to the Jewish General Hospital in 1944, at which time she was found to have polycythæmia vera. For eight years the condition was controlled by repeated phlebotomies. In 1953, she received x-ray

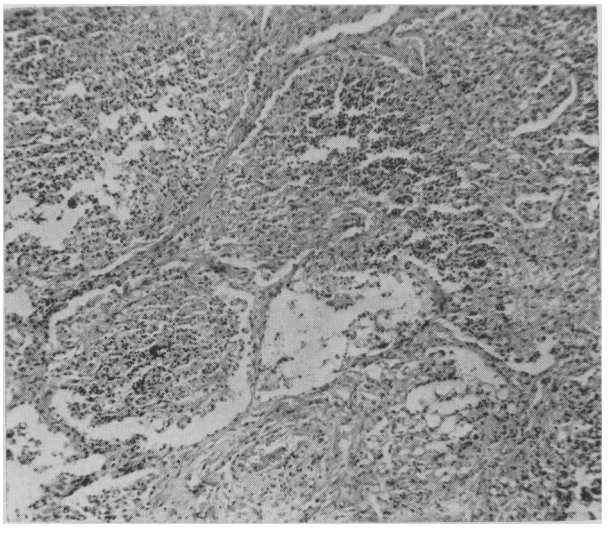

Fig. 5.—Case 3. Lung with destructive granulomatous inflammation. Note the typical cystic lesions in the lower half of the field. Hæmatoxylin, phloxine and saffron, × 150.

therapy to an enlarged spleen, and later the same year she was discovered to have a staghorn calculus in the right kidney with an associated acute pyelonephritis. She also had hypochromic anæmia, which improved on iron therapy. She was admitted to hospital again in October 1956, with obstructive jaundice due to a stone in the common bile duct. She was treated conservatively because she was considered a poor surgical risk. Jaundice persisted for the next year and then cleared spontaneously. In July 1957, a chest radiograph revealed poorly defined densities in the lingula of the left lobe and in the right upper and lower lobes. The parenchymatous infiltrations were not present on a previous film seen four months earlier.

She was admitted to the Jewish Hospital of Hope in October 1957, complaining of nausea and vomiting of several weeks' duration. She was cachectic and had signs of congestive heart failure. There were no signs of neurological disease. A chest radiograph now showed multiple mottled shadows throughout both lungs with conglomeration in the right lower and left mid-lung fields. Her condition steadily deteriorated and she died on November 14, 1957.

Autopsy was performed at the Pathological Institute of McGill University. The body weighed 70 lb. The heart showed left ventricular hypertrophy. There was generalized arteriosclerosis. The lungs were riddled with firm white nodules. The gallbladder contained two large calculi, and the common bile duct was obstructed by a stone. The spleen weighed 1100 g. and the organ was firm. A staghorn calculus occupied the right renal pelvis. The brain weight 1056 g. Numerous small cysts were found scattered throughout the brain stem, cerebellum, and cerebral hemispheres, being most marked in the basal ganglia. Microscopical study revealed generalized cryptococcal dissemination. The organisms had produced severe destructive lesions in the lungs with marked granulomatous inflammatory response (Fig. 5). In the necrotic areas masses of cryptococci could be seen. Similar destructive lesions were noted in the adrenal gland and the organisms were found in the lymph nodes and kidneys. The brain cysts contained masses of organisms. Moderate gliosis was present in the surrounding tissue. In addition to the fungal infection there was hyperplasia of the bone marrow and myeloid metaplasia of the spleen. Bilateral acute and chronic pyelonephritis was also present. *Cryptococcus neoformans* was grown on Sabouraud's medium from lung and kidney tissue.

CASE 4.—J.T., a 74-year-old white man, was admitted to the Jewish General Hospital in August 1954, complaining of weakness and fatigue. He was found to have herpes zoster. In December 1954, he developed bilateral glaucoma secondary to inactive irido-cyclitis. He did not respond to conservative treatment, and bilateral cyclodiathermy was performed. A chest radiograph revealed scoliosis and increased markings on the right side. He was transferred to the Jewish Hospital of Hope in February 1955 for convalescent care. On admission he was co-operative, and apart from impaired vision, examination was negative. His condition rapidly deteriorated. He became increasingly noisy and difficult to control. He complained of severe headaches which were somewhat eased by codeine. His temperature ranged betweeen 99° and 100.4° F. He died in sleep on March 29, 1955.

Autopsy was performed at the Pathological Institute of McGill University. The body weighed 97 lb. The heart was not enlarged. There was slight pulmonary œdema but no other signs of congestive heart failure. The right adrenal gland was markedly atrophic while the left showed compensatory hyperplasia. The right kidney was atrophic too, weighing only 50 g., while the left was enlarged, weighing 355 g. The brain weighed 1360 g. but its appearance was not described. Microscopical study revealed pyelonephritis on the right. The meninges over the cortex were thickened and œdematous, and contained numerous cryptococci. Cystic spaces filled with the organisms were noted in the cerebellum, especially in the vicinity of small blood vessels. There was virtually no inflammatory response. Giant cells forming small granulomas were encountered in the lungs.

CASE 5.—B.V., a 54-year-old white woman, had six admissions to the Jewish General Hospital during 1959 for uræmia due to chronic pyelonephritis with acute exacerbations. She had mild cardiomegaly, atrial fibrillation and mild diabetes mellitus. Her last admission was on January 7, 1960, when she complained of sore throat and nose bleeds. She was very noisy and apprehensive. Physical examination was unrevealing but the urine contained pus. The blood urea nitrogen value was 232 mg. per 100 c.c. and the hæmoglobin value was 54%. The serum carbon dioxide combining power was 11 mEq./l. and the potassium was 7.4 mEq./l. She was extremely difficult to control and was thought

Canad. M. A. J.
Sept. 10, 1960, vol. 83

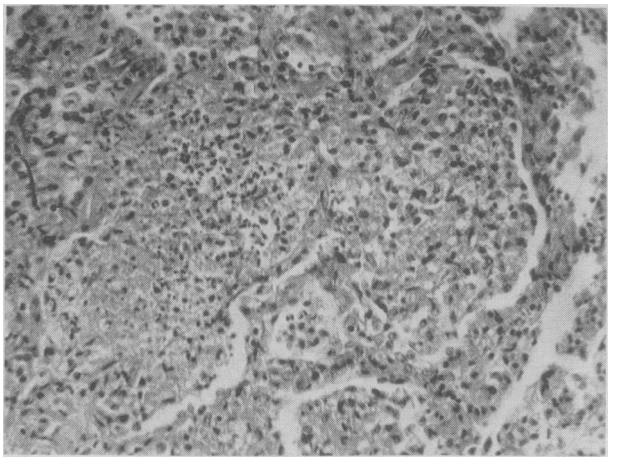

Fig. 6.—Case 5. Lung with granulomatous inflammation in alveolar spaces. Hæmatoxylin, phloxine and saffron, × 200.

to have organic psychosis probably due to her uræmic state. She died suddenly on January 9.

At autopsy the body weighed 141 lb. The right kidney weighed 60 g. and the left 80 g. Both were severely scarred, and section showed small abscesses scattered about the parenchyma. The heart weighed 500 g. and showed left ventricular enlargement. The lungs were œdematous. The central nervous system was not examined. Microscopical study revealed chronic pyelonephritis with superimposed acute inflammation. There was slight non-specific myocardial fibrosis. Section of the left lower lobe of the lung revealed several minute granulomas. These filled alveolar spaces but left the septa intact. There was a central necrotic area with surrounding epithelioid and polymorphonuclear cell reaction (Fig. 6). Masses of characteristic encapsulated, budding yeasts were seen (Fig. 7). No other evidence of cryptococcal infection was apparent.

DISCUSSION

Five cases of cryptococcosis have been described. The first two had been treated for progressive Hodgkin's disease while the third had had polycythæmia vera. These are diseases of the reticuloendothelial system which tend to lower resistance to infection. Two of the five patients were diabetic. Diabetes is associated with a higher incidence of infections, including mycoses, such as mucormycosis, which is found with increased frequency in diabetic patients.[10] Steroid therapy was used in the first patient and may have lowered the normal resistance. The infection was widely disseminated in the first four cases. It seems probable that infection started in the lungs, from where it presumably spread by way of the blood stream. The organisms grew to form small cystic lesions pushing the normal tissues aside, but only in Cases 3 and 5 was there a significant inflammatory reaction, the organisms being characteristically inert. Clinical features are accordingly late and indefinite. The most striking feature is severe headache and vomiting, and personality changes. Clinical diagnosis was not made in any of these cases, but examination of the cerebrospinal fluid could, in at least four instances, have revealed the typical encapsulated budding yeasts.

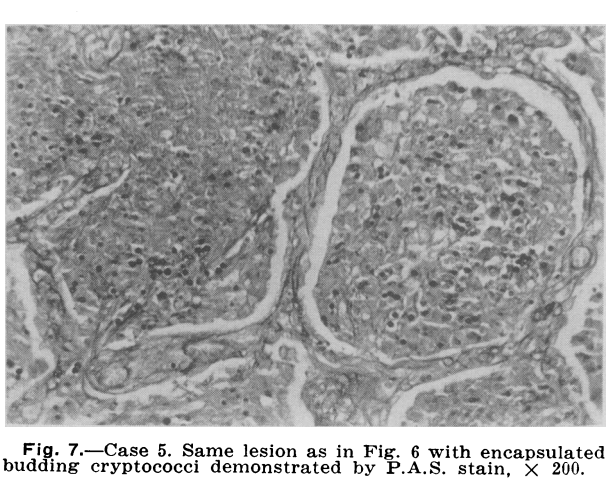

Fig. 7.—Case 5. Same lesion as in Fig. 6 with encapsulated budding cryptococci demonstrated by P.A.S. stain, × 200.

The occurrence of five cases of cryptococcosis in a single Canadian general hospital in a period of five years emphasizes that this diagnosis should be considered when severe vomiting, headache or development of personality changes appears in debilitated patients and especially in those with Hodgkin's disease or allied disorders. Treatment to date has been unsatisfactory, but a new drug, amphotericin B, has been used with some success.[11]

SUMMARY

Five patients with cryptococcosis seen during a five-year period have been described. Two had Hodgkin's disease and one had polycythæmia vera. The fourth was malnourished and the fifth had chronic renal failure. Two had diabetes mellitus. Four of the patients had disseminated infection, including characteristic meningo-encephalitis. Certain clinical and pathological features of this disease are illustrated.

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