Cryptococcal meningitis associated with steroid therapy

T. S. WILSON, W. A. FLEMING, F. L. J. ROBINSON, AND B. NICHOLL

From the Bacteriology Department, The Laboratories, Belfast City Hospital, the Department of Microbiology, Queen's University, Belfast, and the Northern Ireland Fever Hospital, Purdysburn, Belfast

SYNOPSIS Two patients on prolonged steroid therapy developed meningitis due to *Cryptococcus neoformans*. The first responded satisfactorily to treatment with amphotericin B, both initially and again following relapse. The second died shortly after treatment was begun. Pathogenicity studies suggest that the strain isolated from the fatal case was the more virulent.

Cryptococcal meningitis probably occurs more often in Britain than is generally appreciated, and this possibility should be remembered when investigating patients with obscure forms of meningitis; if not, then the correct diagnosis may not be made. Attention is drawn to the increasing number of recently reported cases of this disease which have been associated with long-term steroid therapy.

Infections due to the yeast-like fungus *Cryptococcus neoformans* are relatively common in some parts of the world, but they are seldom seen by clinicians in the British Isles. Cryptococcosis (torulosis) was first described in Britain by Smith and Crawford (1930) and since then about 30 additional cases have been reported, most of these having meningeal involvement. There have undoubtedly been other unreported cases (Symmers, 1967); nevertheless, the total number of cryptococcal infections diagnosed in Britain remains small.

In this paper we describe two patients in Northern Ireland who developed cryptococcal meningitis while receiving prolonged therapy with corticosteroid drugs. We also present the results of laboratory studies on the cryptococcal strains which were isolated, and of some epidemiological investigations.

Case Reports

CASE 1

A 40-year-old housewife was admitted to the Northern Ireland Fever Hospital on 11 November

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1967 with suspected tuberculous meningitis. She had complained of intermittent headache and vomiting for two weeks and had been investigated in a peripheral hospital before her transfer. She had a previous history of sarcoidosis, confirmed by biopsy of a left cervical gland in August 1961. Steroid therapy had been started then and was continued in varying dosage throughout the next six years. At the time of admission she was receiving prednisone, 10 mg, tid.

On examination she was pale, alert and lively, and had no difficulty answering questions quickly and accurately. There were definite meningeal signs. The only other abnormal findings were a rather deformed and thickened nasal bridge and active sarcoid lesions of the right cheek and right angle of the mouth. She had an intermittent low-grade fever.

Several examinations of cerebrospinal fluid between 11 November and 27 November showed high protein and low sugar levels, and an increased cell count (Table I). Direct microscopic examination of these specimens did not reveal tubercle bacilli. From the sample of 27 November a few yeast-like colonies were isolated after 48 hours' incubation on Sabouraud's medium, and these were subsequently identified as *C. neoformans*. This finding was confirmed in a further sample on 30 November and at this time occasional capsu-

lated yeast cells were seen in an Indian ink preparation of the cerebrospinal fluid deposit. Examination of Lowenstein-Jensen cultures of previous specimens also showed growth of the yeasts.

Therapy with amphotericin B, given intravenously in 5% dextrose, was begun on 30 November. An initial dose of 12.5 mg daily was increased to 25 mg and then to 50 mg daily. However, this dose could not be maintained

Date	Protein (mg per 100 ml)	Sugar (mg per 100 ml)	WBC (per c mm)	Polymorphs (%)	RBCs (per c mm)
11.11.67	98	14	257	35	—
13.11.67	98	13	254	40	31
19.11.67	90	Trace	405	85	10
22.11.67	168	Trace	294	70	270
27.11.67	272	Trace	470	80	42
30.11.67	210	Trace	295	—	3
Amphotericin B 30.11.67-31.1.68					
5.2.68	164	35	36	75	760
14.3.68	96	45	35	80	—
25.5.68	108	33	98	65	16
Amphotericin B 29.5.68-18.6.68					
1.7.68	90	45	5	—	155
11.2.69	42	50	2	—	—

Table I Cerebrospinal fluid findings in case 1

Fig. 1 Blood urea level and dosage of amphotericin B in case 1 (first course of treatment).

because of nausea and vomiting and a rising blood urea level (Fig. 1). Dosage was therefore reduced to 25 mg daily and later to 25 mg on alternate days, the latter being well tolerated. Simultaneously the dosage of prednisone was gradually decreased from 10 mg tid to a maintenance level of 2.5 mg bid.

The patient's clinical condition steadily improved, and amphotericin B was discontinued on 1 February 1968 after a total course of 1 gram, given over 63 days. Examination of cerebrospinal fluid on 5 February still showed a raised protein level and high cell count (Table I), but *C. neoformans* was neither seen microscopically nor cultured. The patient was discharged from hospital on 8 February. At review on 14 March she was well, although the findings in cerebrospinal fluid were still abnormal (Table I).

She was readmitted on 25 May with a two-week history of a head cold and intermittent headaches, but without meningeal signs. Examination of cerebrospinal fluid revealed a raised protein level and increased cell count (Table I), and *C. neoformans* was again isolated on culture. A second course of amphotericin B, begun on 29 May, was well tolerated. A total of 1 gram was given over 20 days as 50 mg daily, despite a maintained rise in blood urea. This remained around 90-110 mg/100 ml, but it returned to 26 mg/100 ml within two weeks of ceasing therapy. No episodes of phlebitis occurred. Occasional nausea and rigors seemed to be associated with the speed of infusion. During each course of amphotericin B it was noted that there was a temporary loosening of the finger nails and desquamation of the skin of the fingers and palms. Prednisone therapy was discontinued on this occasion immediately after admission. The patient's condition improved rapidly, and, although cerebrospinal fluid protein was still raised on 1 July (Table I), the leucocyte count had returned to normal and the fluid was sterile on culture. She was therefore allowed to return home.

Fig. 2 Indian ink preparation showing heavily capsulated and budding yeast cells in cerebrospinal fluid (case 2).

Seven months later the patient attended for review. Since her discharge from hospital she had enjoyed good health, although she had new sarcoid lesions on the lateral aspects of both upper arms. The protein, sugar, and cell counts in cerebrospinal fluid were now normal. However, *C. neoformans* was again isolated, though only two colonies were cultured from 5 ml of spinal fluid. Blood urea was 28 mg/100 ml. As she seemed to be controlling the meningeal infection satisfactorily in the absence of steroid therapy it was thought that a further course of treatment was not indicated.

In May 1970, almost two years after her relapse, this patient remains very well, and she has had no further recurrence of meningeal symptoms. There has been no significant change in the sarcoid condition, despite the cessation of steroid treatment.

CASE 2

A 53-year-old unemployed man was admitted to the Belfast City Hospital on 9 January 1967 during an asthmatic attack. He had a previous history of psoriasis and asthma, and had been in hospital on several occasions between 1964 and 1966 for treatment of these conditions. Steroids had been given intermittently during this period, and continuous low-dosage therapy was started in mid-1966.

The asthma responded to an increased steroid dosage, but attempts at withdrawal always resulted in a recrudescence of psoriasis. As a result he received prednisone continuously from January 1967 until February 1968, the dose varying from 15 to 40 mg daily. Several complications of steroid therapy developed during this time, including Cushing's syndrome, oral candidiasis, spinal osteoporosis, and diabetes mellitus.

He was quite well in early 1968, but in mid-February some headache and dizziness was followed by a 'flu-like' illness, with fever and cough. His condition changed little over the next four days, but he then had an epileptiform fit and showed signs of chest infection. During the following two weeks he had periods of drowsiness and mental confusion, and the chest signs never completely cleared, despite two courses of antibiotics. A second epileptiform fit occurred on 6 March, with further confusion and disorientation. Subacute meningitis was suspected, and cerebrospinal fluid taken on 8 March showed protein 380 mg/100 ml, sugar 30 mg/100 ml, and white cells 30/c mm (mainly lymphocytes). Microscopic examination of the unstained deposit in the counting chamber showed many yeast-like cells of varying size. These resembled erythrocytes, but a few were definitely budding, and large capsules were easily demonstrated in an Indian ink preparation (Fig. 2). A presumptive identification of *C. neoformans* was made, and this was later confirmed after culture of the fluid. A

heavy growth of capsulated yeasts was obtained after 48 hours' incubation on both blood agar and Sabouraud's medium, growth occurring at 37°C and at room temperature.

The patient's condition deteriorated rapidly and he became semicomatose. Blood urea rose from 54 to 118 mg/100 ml over the next few days, causing delay in starting amphotericin B therapy. An initial dose of 10 mg by intravenous infusion was given on 11 March but the patient died the following day. Permission for a postmortem examination was refused.

Laboratory Investigations

C. neoformans was isolated from the cerebrospinal fluid of both patients. For purposes of identification we refer to strain 1 (isolated from case 1) and strain 2 (from case 2).

Eleven samples of cerebrospinal fluid from the first patient were examined between 11 November 1967 and 11 February 1969 (Table I). Yeasts were recognized microscopically in only one of these (30 November 1967), when a few poorly capsulated cells were seen in an Indian ink preparation. *C. neoformans* (strain 1) was isolated by culture from several specimens, but in every instance only a scanty growth of the organisms was obtained. For example, culture of 5 ml of the cerebrospinal fluid of 11 February 1969 (1 ml on each of five plates) yielded only two colonies of cryptococci. Attempts to isolate cryptococci from the skin and nose of this patient were unsuccessful.

In contrast, the only sample of cerebrospinal fluid from the second patient contained many yeast cells. These closely resembled erythrocytes in the counting chamber, though a few showed budding. Study of Indian ink and stained preparations confirmed that they were heavily capsulated yeasts (Fig. 2), and it was estimated that there were about 240/c mm of cerebrospinal fluid. Culture of the fluid yielded a heavy growth of *C. neoformans* (strain 2).

The isolates from both patients grew in 48 hours on blood agar, Sabouraud's dextrose agar, and malt agar. Both were spherical yeasts with the morphological, cultural, and physiological characteristics of *C. neoformans*. These included capsules (wider in strain 2 than strain 1), growth at 25°C and 37°C, urease production, and assimilation of glucose, sucrose, and maltose.

Both strains were pathogenic for mice on intracerebral injection of 1×10^6 cells. Strain 2 appeared to be the more virulent, killing all of six mice within seven days of inoculation, while only three out of six mice inoculated with a similar number of cells of strain 1 died within 14 days. The last animal was killed after 24 days, and *C. neoformans* was isolated from its brain and spleen.

Retrospectively, three separate attempts were

made to isolate *C. neoformans* from the hospital environment of case 2. Altogether 27 samples were examined, consisting mainly of pigeon droppings collected from sites such as windowsills, fire escapes, balconies, a flat roof, and an attic near the patient's ward. Thirteen swabs were also taken in and about the ward itself. Suspensions of the samples were plated out on Sabouraud's medium containing chloramphenicol or later Littman's agar with chloramphenicol was also used. The swabs were inoculated directly on to the same media. The plates were incubated at 37°C for at least seven days, and yeast-like colonies were tested for urease production. Yeasts capable of splitting urea were further examined for carbohydrate assimilation and mouse pathogenicity tests. *C. neoformans* was not isolated from any of these samples or swabs.

We also examined the pigeon excreta from a few domestic pigeon lofts in and near Belfast using the same methods. Three out of 21 of these samples were positive for *C. neoformans*, all three positive samples being from the same loft.

Discussion

EPIDEMIOLOGY AND MYCOLOGY

Although most of the systemic mycoses occur in tropical and subtropical areas, cryptococcosis has been reported from many countries, both tropical and temperate. Its epidemiology was obscure until Emmons (1955) reported the isolation of *C. neoformans* from pigeon nests and excreta in the United States. The fungus has since been recovered from pigeon droppings and pigeon habitats in many parts of the world, and it is only occasionally isolated from other sources. In Britain *C. neoformans* has been found in pigeon excreta in London (Randhawa, Clayton, and Riddell, 1965; Partridge and Winner, 1966) and in Glasgow (Evans, 1968), but a lower percentage of samples has been positive than in America and elsewhere. Previous attempts to isolate *C. neoformans* in the Belfast area were unsuccessful (Mackenzie, 1968).

The source of infection in these two patients remains uncertain. The first, a housewife living in a small country town, had no direct contact with pigeons. The other, a resident of Belfast, had been in hospital for 13 months before his terminal cryptococcal illness, and it seemed possible that he had acquired the infection during this time. Parts of the hospital were frequented by large numbers of pigeons, and an attic over a landing just outside the patient's ward contained a thick layer of pigeon droppings. Symmers (1967) has described cryptococcal infections occurring under similar conditions in five hospital patients being treated for Hodgkin's disease and sarcoidosis. However, despite these highly suspicious circum-

stances, we failed to recover cryptococci from this attic or from any adjacent sites. Later, we did isolate *C. neoformans* from a pigeon loft about one mile away from the hospital, which suggests that the epidemiological pattern here is likely to resemble that elsewhere, though the possibility of some other source of cryptococci cannot be entirely ruled out.

The strains of *C. neoformans* isolated from the two patients differed in their degree of capsulation and in their pathogenicity for mice, strain 1 being less well capsulated and less virulent than strain 2. Similarly the insidious onset, chronic course, and small numbers of cryptococci in the first patient were in marked contrast to the rapidly progressive infection with many organisms in the second. There are conflicting opinions on the relationship between capsulation of *C. neoformans* and virulence (Bodenhoff, 1969); our clinical and experimental observations with these two strains are consistent with the view that the degree of capsulation is a factor in virulence.

EFFECTS OF STEROIDS

Primary pulmonary cryptococcosis is often asymptomatic and self-limiting, and may be discovered incidentally in otherwise healthy people. By contrast, disseminated cryptococcosis is associated in 30 to 50% of cases with serious underlying diseases, such as Hodgkin's disease or other reticuloses (Zimmerman and Rappaport, 1954; Symmers, 1967), or diabetes mellitus (Butler, Alling, Spickard, and Utz, 1964). Zimmerman and Rappaport (1954) studied 60 cases of cryptococcosis and concluded that there was no real evidence that steroids, used in the treatment of Hodgkin's disease or lymphomas, had any bearing on the frequency or dissemination of cryptococcal infections.

However, recent studies strongly suggest that corticosteroid therapy does predispose to cryptococcal infection, and that it may cause localized infection to become generalized. Goldstein and Rambo (1962) reported on a 49-year-old housewife who developed cryptococcal meningitis after nine years of steroid therapy for rheumatoid arthritis. They also reviewed the literature in English between 1950 and 1960 and found that, of those cases with sufficient details available, eight out of 147 (5.4%) were steroid-associated. In three of these, a dormant lesion had apparently been activated by the steroid therapy. Several more recent reports (Spickard, Butler, Andriole, and Utz, 1963; Bennington, Haber, and Morgenstern, 1964; Gordon and Vedder, 1966; Randall, Stacy, Toone, Prout, Madge, Shadomy, Shadomy, and Utz, 1968) give even higher proportions of steroid-associated cases, ranging from 25 to 75%, with an overall average of 40% (25 cases out of 62). While some of these patients had Hodgkin's disease and other illnesses which themselves may predispose to cryptococcosis, many were re-

ceiving steroids for conditions such as asthma and rheumatoid arthritis which are not usually considered to increase susceptibility to infections. These clinical observations, which indicate that steroids do predispose to cryptococcal infections, are supported by experimental studies in mice in which cortisone promoted the dissemination of the infection from localized cryptococcal lesions (Levine, Zimmermann, and Scorza, 1957); the extent of this steroid-induced enhancement of the disease may vary in different strains of mice (Louria, Fallon, and Brown, 1960).

It seems probable that the occurrence of cryptococcal meningitis in these two patients was related to their steroid treatment. Cryptococcal meningitis has not previously been reported in Northern Ireland, and it is unlikely to be coincidental that the first two cases were both receiving steroid therapy. There have been several reports associating cryptococcosis and sarcoidosis, but the majority of these patients were also on steroids, and it is difficult to assess the relative importance of the two factors. Cryptococcal meningitis has also been described previously in asthmatic patients who were receiving steroids.

DIAGNOSIS

The recognition of two cases of cryptococcal meningitis in Northern Ireland within a few months suggests that the disease may not be as uncommon in Britain as is generally supposed. Laboratory diagnosis of the condition may be easy in some instances and difficult in others, depending on the numbers of cryptococci which are present in the cerebrospinal fluid; this is well illustrated in the two patients reported here. If the possibility of cryptococcosis is not considered then there may be delay, or even failure, in establishing the diagnosis, as the necessary laboratory procedures may not be employed. Physicians should be aware of the increased risk of this infection in certain susceptible groups of patients, while laboratory workers need to remember cryptococcosis when investigating specimens of cerebrospinal fluid from patients with obscure forms of meningitis.

The findings in cerebrospinal fluid may resemble those in tuberculous or viral meningitis. The protein and cell levels are raised in over 90% of cases, but the sugar is reduced in only about 50% (Butler *et al*, 1964). Microscopic examination for cryptococci is positive in only 50 to 60% of cases (Butler *et al*, 1964; Gordon and Vedder, 1966) and it should be emphasized that the spherical cryptococci may not be recognized in a counting chamber, as they are easily mistaken for either erythrocytes or leucocytes. Budding of the cells may indicate their true nature, but usually it is necessary to make Indian ink preparations and demonstrate the capsulated yeast-like cells.

If the organisms are only present in small numbers direct microscopy may be negative, but

cryptococci can be cultured from the spinal fluid in most cases. They will grow in two to four days on blood agar or Sabouraud's medium, both at 37°C and at room temperature, and yeast colonies appearing in these circumstances should not be dismissed as contaminants.

Recently serological methods have been introduced which are an additional aid in the diagnosis of cryptococcosis (Gordon and Vedder, 1966). These may be particularly helpful in those instances where there is difficulty in obtaining positive cultures, and it has been suggested that they should be used in any case of obscure meningitis (Watkins, Campbell, Gardner-Medwin, Ingham, and Murray, 1969).

TREATMENT

Before 1956 cryptococcal meningitis was nearly always fatal. In recent years amphotericin B treatment has been used successfully, with improvement in 70 to 80% of patients, but relapse may occur in about 30% (Butler *et al*, 1964). The average dosage is 1 mg/kg/day, although this can cause a variety of side effects and toxic reactions. In our first patient a rising blood urea level resulted in the dose being reduced to 25 mg on alternate days, and her first course of 1 gram took 63 days to complete. The cerebrospinal fluid cell count and protein level remained abnormal, and, though the culture was negative, the patient relapsed within four months.

Her second course of amphotericin B was given over a period of only 20 days, despite a maintained rise in blood urea. This returned rapidly to normal following the cessation of amphotericin B therapy. Clinical improvement was more rapid on this occasion, though cerebrospinal fluid protein was still raised on completion of the course. When reviewed seven months later the patient was well and findings for cerebrospinal fluid were normal, except for an unexpected positive culture. It seems reasonable to assume that higher tissue levels of the drug were achieved during the second course, accounting for the better response. The stopping of steroids may have been an additional favourable factor on this occasion, as Gordon and Vedder (1966) considered that they had an adverse effect on clinical response to amphotericin B.

The toxicity of amphotericin B prevents its effective use in some cases. In the absence of any other satisfactory form of therapy, it may be necessary to accept some temporary impairment of renal function in order to maintain an adequate dosage of the drug. Recent reports of the successful use of 5-fluorocytosine (Tassel and Madoff, 1968; Watkins *et al*, 1969) raise the hope that this may prove to be a suitable alternative to amphotericin B in the treatment of cryptococcal meningitis and other systemic mycoses.

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Addendum

While this paper was being prepared, a third patient with a disseminated cryptococcal infection has been seen here. This was a 77-year-old man who was admitted to the Belfast City Hospital in cardiac failure, with dyspnoea, ascites, and some lymphadenopathy. Gland biopsy showed a malignant reticulosis of Hodgkin's type.

Before any specific therapy was started, the patient developed a fluctuant subcutaneous swelling on the right side of the chest. Thick, purulent material was aspirated, a few yeasts were seen in a stained smear, and on culture *C. neoformans* was isolated. The patient's condition deteriorated rapidly, with increasing drowsiness and weakness, and he died eleven days later. Permission for a necropsy was not obtained.

This third case of disseminated cryptococcosis in Northern Ireland in less than two years tends to support our supposition that these infections may be commoner in Britain than is usually realized. Both physicians and bacteriologists need to be alert regarding the possibility of cryptococcosis, and indeed of other generalized fungal infections, in those groups of patients who are particularly at risk.

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References

- Bennington, J. L., Haber, S. L., and Morgenstern, N. L. (1964). Increased susceptibility to cryptococcosis following steroid therapy. *Dis. Chest*, **45**, 262-263.
- Bodenhoff, J. (1969). Alteration in virulence in strains of *Cryptococcus neoformans* resistant to amphotericin B and polymyxin B. *Acta path. microbiol. scand.*, **75**, 153-168.
- Butler, W. T., Alling, D. W., Spickard, A., and Utz, J. P. (1964). Diagnostic and prognostic value of clinical and laboratory findings in cryptococcal meningitis. *New Engl. J. Med.*, **270**, 59-67.
- Emmons, C. W. (1955). Saprophytic sources of *Cryptococcus neoformans* associated with the pigeon (*Columba livia*). *Amer. J. Hyg.*, **62**, 227-232.
- Evans, E. G. V. (1968). Personal communication.
- Goldstein, E., and Rambo, O. N. (1962). Cryptococcal infection following steroid therapy. *Ann. intern. Med.*, **56**, 114-120.
- Gordon, M. A., and Vedder, D. K. (1966). Serologic tests in diagnosis and prognosis of cryptococcosis. *J. Amer. med. Ass.*, **197**, 961-967.
- Levine, S., Zimmermann, H. M., and Scorza, A. (1957). Experimental cryptococcosis (torulosis). *Amer. J. Path.*, **33**, 385-409.
- Louria, D. B., Fallon, N., and Brown, H. G. (1960). The influence of cortisone on experimental fungus infections in mice. *J. clin. Invest.*, **39**, 1435-1449.
- Mackenzie, D. W. R. (1968). Personal communication.
- Partridge, B. M., and Winner, H. I. (1966). *Cryptococcus neoformans* in bird droppings. *Lancet*, **2**, 1251.
- Randall, R. E., Stacy, W. K., Toone, E. C., Prout, G. R., Madge-

- G. E., Shadomy, H. J., Shadomy, S., and Utz, J. P. (1968). Cryptococcal pyelonephritis. *New Engl. J. Med.*, 279, 60-65.
- Randhawa, H. S., Clayton, Y. M., and Riddell, R. W. (1965). Isolation of *Cryptococcus neoformans* from pigeon habitats in London. *Nature (Lond.)*, 208, 801.
- Smith, F. B., and Crawford, J. S. (1930). Fatal granulomatosis of the central nervous system due to a yeast (torula). *J. Path. Bact.*, 33, 291-296.
- Spickard, A., Butler, W. T., Andriole, V., and Utz, J. P. (1963). The improved prognosis of cryptococcal meningitis with amphotericin B therapy. *Ann. intern. Med.*, 58, 66-83.
- Symmers, W. St. C. (1967). *Cryptococcus neoformans* in bird droppings. *Lancet*, 1, 159-160.
- Tassel, D., and Madoff, M. A. (1968). Treatment of *Candida* sepsis and *Cryptococcus* meningitis with 5-fluorocytosine. *J. Amer. med. Ass.*, 206, 830-832.
- Watkins, J. S., Campbell, M. J., Gardner-Medwin, D., Ingham, H. R., and Murray, I. G. (1969). Two cases of cryptococcal meningitis, one treated with 5-fluorocytosine. *Brit. med. J.*, 3, 29-31.
- Zimmerman, L. E., and Rappaport, H. (1954). Occurrence of cryptococcosis in patients with malignant disease of reticuloendothelial system. *Amer. J. clin. Path.*, 24, 1050-1072.