

CRYPTOCOCCOSIS—A REPORT OF 3 CASES WITH SPECIAL EMPHASIS ON MANAGEMENT AND A REVIEW OF THE LOCAL LITERATURE

By *J. M. J. Supramaniam, M.B. (Mal.), M.R.C.P. (Glas.), F.R.C.P. (Edin.), T.D.D. (Wales),
F.C.C.P., A.M. (S'pore)

† Ng Kwok Choy, M.B. (Mal.), M.R.C.P. (Edin.), D.T.C.D. (Wales)

** J. Tambyah, M.B. (S'pore), M.R.A.C.P. ** Goh Choo Woon, M.B.B.S. (S'pore)
(Unit III, Tan Tock Seng Hospital, Singapore)

Cryptococcus neoformans is widespread in soil, and infection in domestic animals has been reported. The organism is present in large numbers in pigeon excreta and inhalation of cryptococcus-laden dust by susceptible persons may cause primary pulmonary cryptococcosis (Littman 1959), Meningitis is the commonest mode of presentation of disseminated cryptococcosis and is usually fatal if untreated; but occasional cases of spontaneous remission for up to sixteen years have been reported. However, this type of infection with spread to the meninges tends to occur in persons with low resistance or in association with reticulo-endothelial diseases, such as leukaemia, malignant lymphoma and Hodgkin's disease, or with prolonged treatment with corticosteroids and other immunosuppressive agents, or in chronic renal failure (MacGillivray 1966). Though the portal of entry is via the respiratory tract, the presentation of the pulmonary form alone is not common.

SURVEY OF LOCAL LITERATURE

A survey of the local literature revealed only 11 cases of cryptococcosis, 4 in Singapore and 7 in Malaysia. All were the meningitic form and every case succumbed except three. One case (Goh, 1961) was well at the time of report. One (Poopalasingam, 1967) absconded after an interrupted treatment with amphotericin B. The C.S.F. was still abnormal though the patient was clinically well. The third case discharged himself against advice soon after treatment was started and was not traced (Lim and Chan, 1962).

Though sporadic cases may have been seen and treated, the first reported case was described by Pallis (1949) in Singapore. A Chinese sailor presented with meningitis and died 3 weeks after

admission. The diagnosis was made at post-mortem. The chest X-ray showed a rounded opacity in the right infraclavicular region but this was found to be tuberculous in aetiology at necropsy. Loh (1951) reported the second cryptococcus meningitis in Singapore. The diagnosis was made in the very late stage when the cryptococcus was isolated from the C.S.F. The patient died 3 weeks after admission. The third case came from the I.M.R. Kuala Lumpur and the fourth was reported by Ross-Russell and Dean (1957) from the B.M.H. at Taiping in a Chinese sailor from Hong Kong. The fifth reported cryptococcus meningitis was a boy of 10 who was diagnosed at post-mortem, (Muir and Ransome, 1959). Cryptococci were seen lying free in the alveolar spaces at necropsy, but the chest X-ray was normal. Goh (1961) described another case of cryptococcus meningitis in Singapore. This was a boy of 8 who was treated with amphotericin B and was one of the 3 cases who survived at the time of report. In this case the fungus could still be seen on direct smear though the culture became negative. Lim and Chan (1967) mentioned 4 cases, 3 of whom died and one case was a Malay boy who discharged himself against medical advice soon after starting treatment with amphotericin B. He was not traced and presumably died of the cryptococcus meningitis. The eleventh case was that of Poopalasingam (1967) described above. He absconded after 2,125 mg. of amphotericin B. As his C.S.F. was still abnormal, it is justifiable to presume that he was not cured of the cryptococcus meningitis when he absconded.

In this paper we report another three cases of cryptococcosis, all successfully treated with amphotericin B. One case had both pulmonary and meningeal involvement. The second had

* Ag. Sr. Consultant Chest Physician, Tan Tock Seng Hospital, Singapore.

† Senior Medical Registrar, Tan Tock Seng Hospital, Singapore.

** Medical Officers, Tan Tock Seng Hospital, Singapore.

meningitis and presumably pulmonary involvement though not proven but suggestive in the chest X-ray. The third case had only the pulmonary form. We believe that these are the first three reported cases of pulmonary cryptococcosis in Singapore and Malaysia who are alive and well at the time of report.

CASE REPORT

The following is a report of the 3 successfully treated cases with emphasis on the method of treatment and an account of some problems encountered in their management.

Case 1: C.S.H. (Adm. No. 015877) a school-girl, Chinese, aged 10 was admitted to the medical ward in Tan Tock Seng Hospital on 8-7-65 with a history of fever and bitemporal headache for two weeks prior to admission. She was referred by a general practitioner who had treated her with antibiotics with no relief of her symptoms. There was no history of contact with tuberculosis nor with birds.

PHYSICAL EXAMINATION

She was febrile, T. 100⁴F, irritable and photophobic. The tonsils were slightly enlarged and the pharynx inflamed. There was no neck rigidity and Kernig's sign was negative. The reflexes were not exaggerated and the plantar response was flexor. The fundi were normal. The liver was palpable one cm. below the right costal margin. Other systems were normal.

INVESTIGATIONS

Hb. 9.6 gm.%, T.W. 8,700 N 84, L 12, M 1, E 3, E.S.R. 30mm. 1st hour. Urine: few rbc. per high power field, albumin+, sugar nil. Lumbar puncture revealed clear fluid under 50 mm. pressure, cell count 84 per c. mm. mainly lymphocytes, globulin trace, total proteins 65 mg. per cent, chlorides 740 mg. per cent, sugar 52 mg. per cent. Smears showed no organisms but an Indian ink preparation was not done on this occasion. X-ray chest showed increased markings in the right upper zone and an opacity in the right mid zone and the possibility of tuberculosis was considered. Tuberculin test using RT 23 1TU and 3 TU was however, negative.

CLINICAL COURSE

The general condition of the patient continued to deteriorate and in view of the possibility of a tuberculous meningitis, streptomycin and isoniazid therapy was commenced. The second lumbar puncture a few days later showed an increase in pressure—250 mm.—but

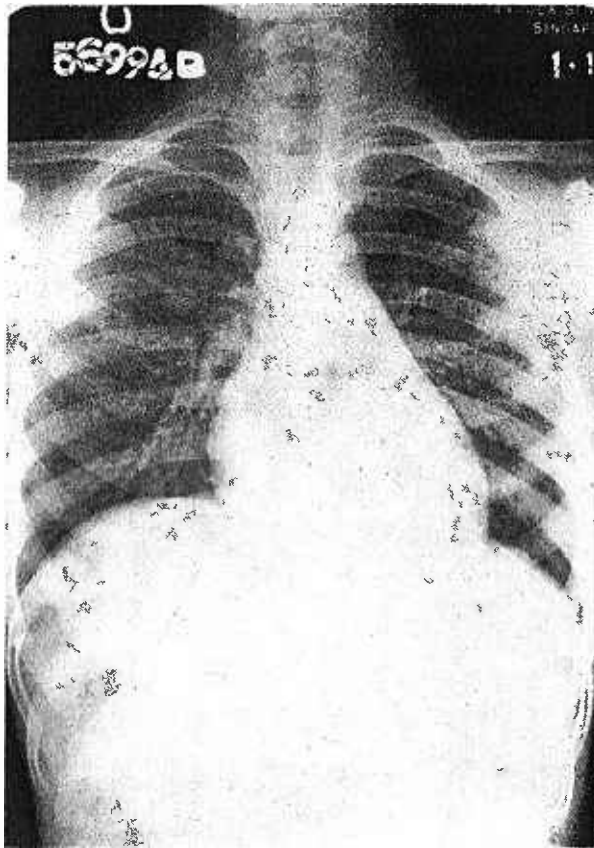
with essentially similar findings as the previous examination. No Cryptococci were demonstrated. The fourth lumbar puncture four weeks after admission, however, showed Cryptococci on smear and culture. Anti-tuberculous chemotherapy was stopped and treatment with intrathecal and intravenous Amphotericin B was commenced, using Winn's Intravenous Method. Soon after commencement of therapy with Amphotericin B the patient began to improve symptomatically, headache was relieved and bacteriologically fewer Cryptococci were demonstrated. Within a month of therapy the fungus could not be grown on culture media but degenerate forms could be demonstrated on smears. Culture of sputum was negative for *Cryptococcus neoformans*.

Radiologically, about 4 months after admission the opacity in the right mid zone was seen to become rounded (Fig. 1), and a tomogram demonstrates the lesions well (Fig. 2).

After a total of 1030 mg. Amphotericin B had been administered thoracotomy was performed by Mr. J.J. Murugesu, and he removed the anterior segment of the right upper lobe (Figs. 3a and 3b).

The patient was discharged in March 1966 after having had another 1222.5 mg. Amphotericin B and she remains well under follow-up two years later (Fig. 4). Her CSF was normal in subsequent lumbar punctures. Her performance at school has remained satisfactory. Side effects encountered in this patient were minimal and consisted of minor gastrointestinal disturbance namely nausea and vomiting, and nephrotoxicity manifested by transient elevation of the blood urea. Temporary cessation of therapy was required when the blood urea was raised.

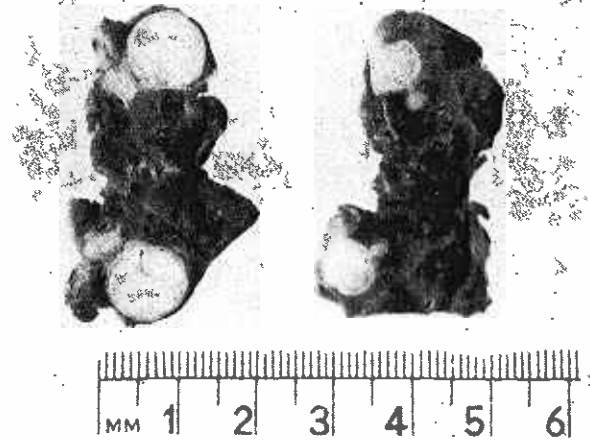
Case 2: L.A.H. (Adm. No. 015981), a schoolgirl, Chinese, aged 17 was first seen in July 1965, complaining of drooping of both upper eyelids for one week, worse in the left eye. She had had headache over the left temporal region but did not complain of diplopia, dysphagia, dysphonia or weakness of the limbs. There was no history of contact with tuberculosis. Clinical examination showed bilateral ptosis more marked on the left. The fundi were normal. No other abnormality was detected. The blood picture, urinalysis, Kahn Test and V.D.R.L. were negative. Tuberculin Test using RT 23 1TU was weakly positive (8 mm.). Intravenous Edrophonium (Tensilon) produced an immediate favourable response. X-ray chest



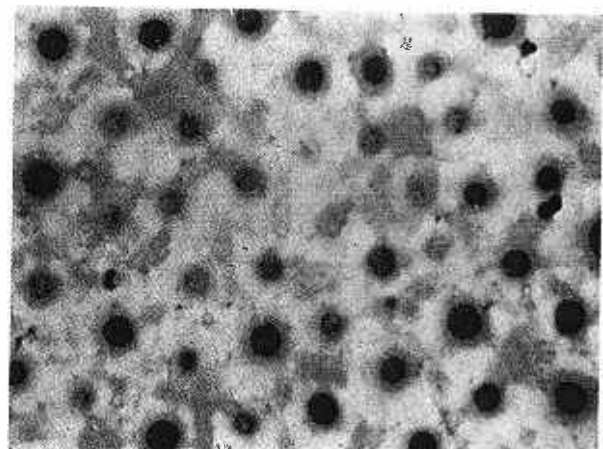
Case 1. Fig. 1. C.S.H. Showing rounded opacities in RUZ and RMZ.



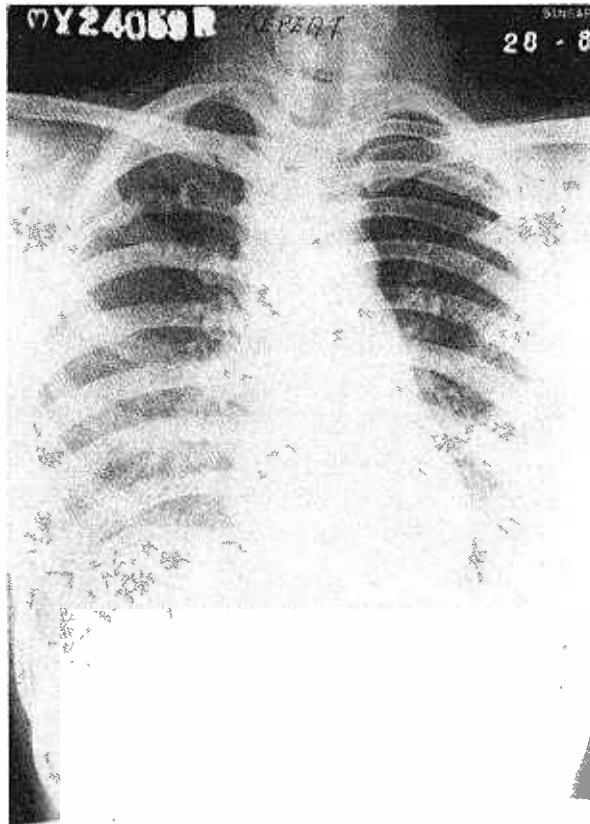
Case 1. Fig. 2. C.S.H. Tomos showing rounded opacities in RUZ and RMZ.



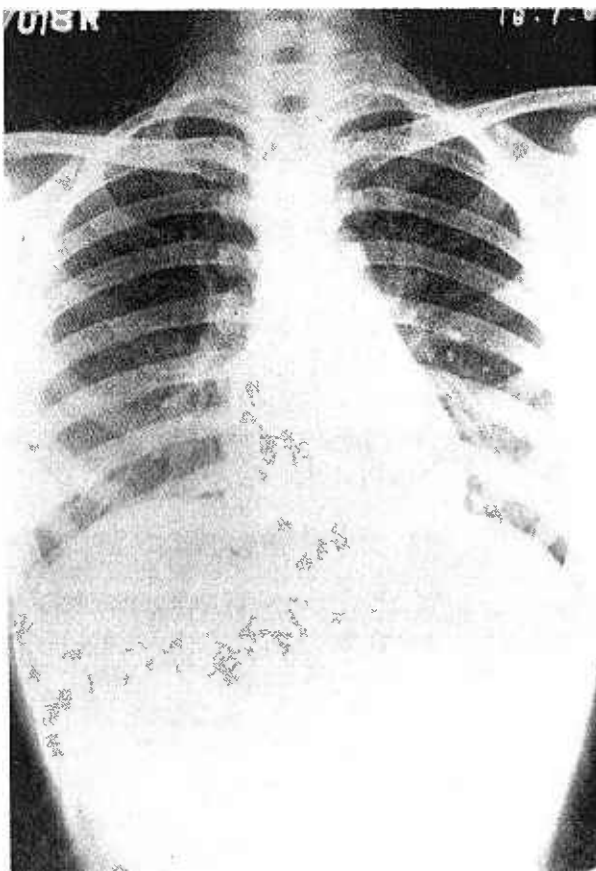
Case 1. Fig. 3(a). C.S.H. Gross appearance of anterior segment of upper lobe of right lung removed at operation showing Toruloma.



Case 1. Fig. 3(b). C.S.H. Section of upper lobe of right lung removed at operation showing numerous Torula.



Case 1. Fig. 4. C.S.H. 28 months after surgery.



Case 2. Fig. 5.



Case 2. Fig. 6.

Figs. 5 & 6. L.A.H. Showing opacities in LUZ.

showed a nodular opacity over the left upper zone (Figs. 5 and 6), and the possibility of a tuberculous lesion was considered. A clinical diagnosis of minimal pulmonary tuberculosis and myasthenia gravis was made and the patient was discharged with antituberculous drugs and pyridostigmine.

2ND ADMISSION

She was readmitted in January 1966 complaining of frontal and nuchal headache which had become progressively worse over the past six months. The ptosis had improved since she had been taking pyridostigmine. Clinical examination revealed normal fundi, no neck rigidity and Kernig's sign was negative. Other systems were normal. Lumbar puncture showed clear fluid under 135 mm. pressure and microscopy revealed 306 cells per c. mm., mainly lymphocytes, globulin present, proteins 100 mg., chlorides 700 mg. and sugar 35 mg. per cent. Smear showed *Cryptococcus neoformans* and culture produced growth of the organism. The blood picture and the blood urea were normal.

CLINICAL COURSE

Treatment with Amphotericin B, 1 mg. per kilogram, was commenced using Winn's intravenous technique. Intrathecal Amphotericin B, 0.5 mg. dissolved in 10 ml. cerebro-spinal fluid previously withdrawn, was instilled every other day. This had to be stopped after five weeks when she developed weakness of legs. One week later, while still on intravenous Amphotericin B, she developed weakness of all four limbs. Reflexes were present and equal, plantar responses flexor, Kernig's negative and no neck rigidity was detected. Intravenous Edrophonium (Tensilon) produced no improvement. Investigations showed marked hypokalaemia — Potassium 1.7 meq. Sodium 140 meq. and chlorides 91 meq. per litre. The blood urea was 60 mg. per cent. Electro-cardiogram confirmed hypokalaemia and there was evidence of bigeminy. Cessation of therapy with Amphotericin B and potassium supplements produced remission of symptoms and signs within two weeks. Blood urea, however, only returned to normal one month later. She also had a microcytic hypochromic anaemia which responded to transfusion of packed cells and iron therapy. The patient completed a course of 2006 mgm. Amphotericin B in September 1966. She is being followed up as an out-patient and remains well. Radiologically the chest lesions appear to have cleared up considerably (Fig. 7).

Case 3: A.T. (Adm. No. 024987) a 35 year old Chinese housewife was admitted on 19-7-67 to Surgical 'B' Unit of the Outram Road General Hospital, Singapore, for complaints of a productive cough for 6 months with occasional staining of sputum. There was no fever and there was no history of contact with tuberculosis nor with birds.

PHYSICAL EXAMINATION

Her general condition was good. There was no neurological deficit and no abnormality was detected in the respiratory system or other systems.

INVESTIGATIONS

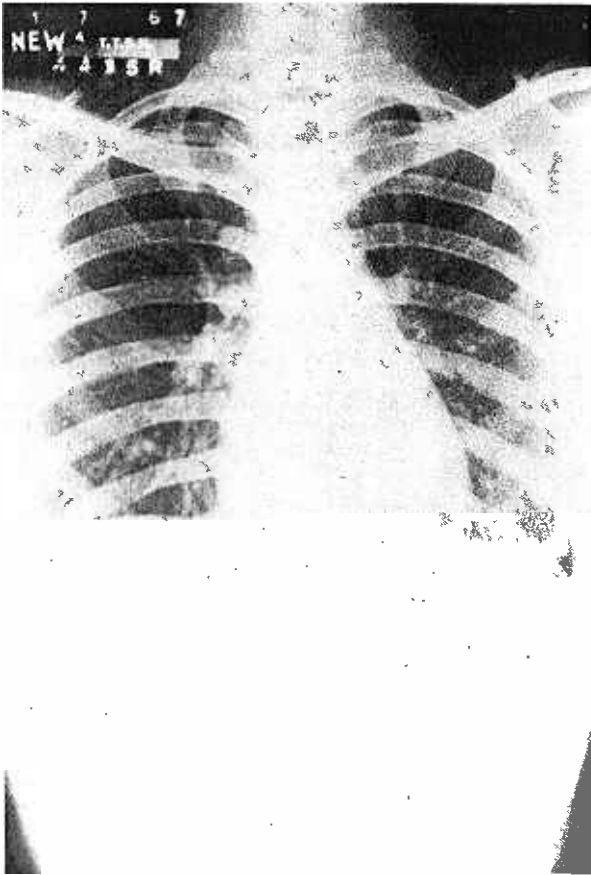
A chest X-ray showed an opacity in the left lower lobe (Figs. 8 and 9). Tomography revealed a well defined solitary lesion with cavitation (Fig. 10).

Hb. 14.7 gm. %, TW 9200/cm. P 80 %, L 14 %, M 4 %, E 2 %. ESR 7 mm./hr. L.P: Normal. Mantoux Test was negative 1/1000 and 1/100. Sputum was repeatedly negative for AFB. Bronchoscopy revealed no growth.

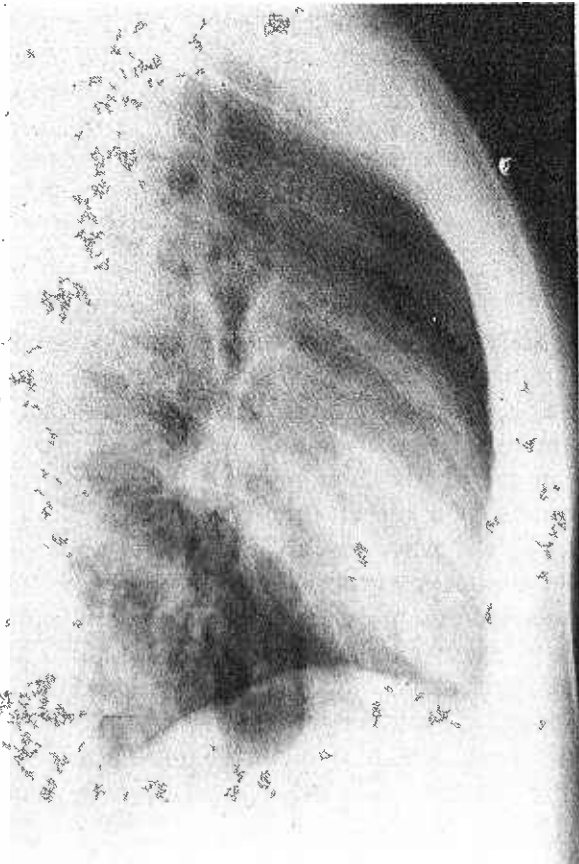
CLINICAL COURSE AND TREATMENT

In view of the staining of sputum and a solitary opacity in the chest X-ray and negative results for pulmonary tuberculosis, an exploratory thoracotomy was done on 18-8-67 by Mr. J.J. Murugesu and a soft friable necrotic greyish tumour, measuring 3-4 cm. in diameter was found in the apex of the left lower lobe. The periphery of the mass felt hard. The hilar nodes were enlarged. A left lower lobectomy was done and the histology (Fig. 11), reported was "section shows lung with an area of granulation tissue. Section of hilar lymph nodes shows granulomatous nodules and giant cells with *Cryptococcus neoformans* in the giant cells. On culture of the tissue *Cryptococcus neoformans* was grown." Subsequently, a blood culture was negative for *Cryptococcus*.

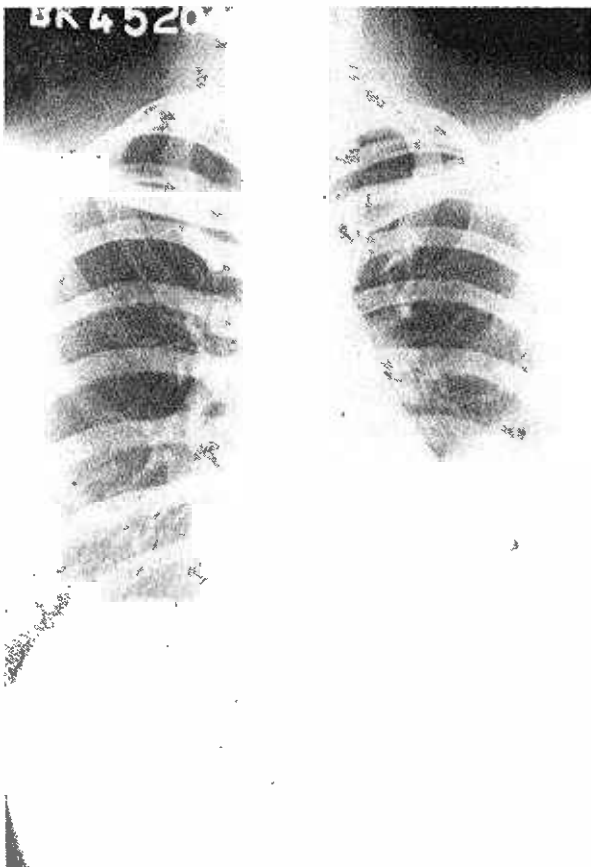
The patient was initially covered with anti-tuberculous drugs, but these were stopped when the histology was known. She was transferred to Tan Tock Seng Hospital, Singapore on 25-8-67 when Amphotericin B was started. This patient had slight fever (99°F-100°F) in the afternoons when Amphotericin was given. Occasionally, she experienced chills. However, treatment was persisted. In the first week of treatment, the SGPT was raised to more than 400 King Units and the serum K⁺ was lowered to 2.9 meq./L. When therapy was interrupted for a few days these figures returned to normal.



Case 2. Fig. 7. L.A.H. After treatment.



Case 3. Fig. 9. A.T. Lt. lateral pre-operative film.



Case 3. Fig. 8. A.T. Pre-operative film showing opacity in LLZ.



Case 3. Fig. 10. A.T. Tomo showing opacity in LLZ with suggestion of cavitation.

A total of 1991 mg. of Amphotericin B was administered to this patient. She was discharged well on 17-12-67 with good radiological progress. Her sputum was always negative for *Cryptococcus*. On follow up, her chest X-ray was clear (Fig. 11).



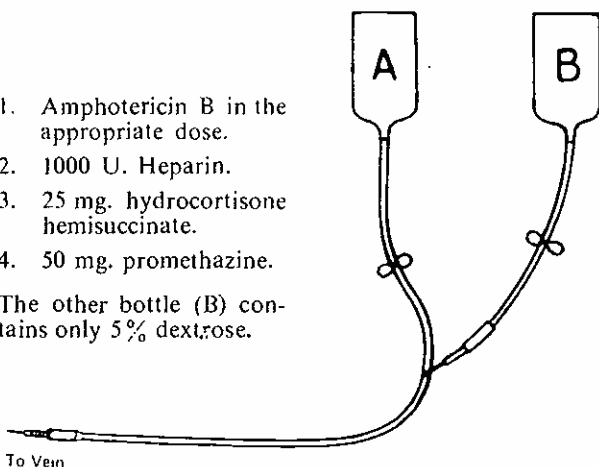
Case 3. Fig. 11. A.T. 9 months after surgery.

Method of Administration of Intravenous Amphotericin B

The cut-off technique for administration of intravenous Amphotericin B as described by Winn (1959) consists of 2 solutions running through a Y—connection (see diagram). One bottle (A) of one pint 5% dextrose, contains:-

1. Amphotericin B in the appropriate dose.
2. 1000 U. Heparin.
3. 25 mg. hydrocortisone hemisuccinate.
4. 50 mg. promethazine.

The other bottle (B) contains only 5% dextrose.



The Amphotericin powder which must be stored away from light and heat is dissolved in

10 ml. of dextrose. After complete solution it is transferred to the bottle A. Separate needles and syringes are used to deliver the various drugs into bottle A in order to prevent precipitation. Each addition is followed by thorough mixing of the solution. Bottle A is covered with a dark cloth as the administration of the drip runs over several hours.

The drips are run one at a time at 30 drops per minute. Bottle A is run for 15 minutes after which its tube is clamped and bottle B is then run for 5 minutes and this alternating procedure is continued until drip A is finished.

Venesection is done with an ordinary injection needle (gauge 21 or 19) and it is important that venesection is commenced at the periphery of the limbs in order to conserve veins. With a little care it is possible to use finger veins. It is advisable to use veins of alternate limbs at successive infusions. When the needle is removed from the vein, extravasation of blood is prevented by firm pressure on the vein for 5 minutes.

The first dose of Amphotericin B is 1 mg. and it is then increased by 5-10 mg. daily till the desired dose is reached, namely 1 mg./Kg. body weight—Littman (1959). This dose is infused daily or on alternate days if there is untoward side effects. Generally, we prefer to give it on alternate days. When the interruption of therapy is longer than a week, Amphotericin B is re-started again with an initial dose of 1 mg. and then worked up to the desired dose as described above. Generally a full course of treatment requires between 1.5 gm.-3.0 gm. of Amphotericin B.

Promethazine 50 mg. and dispirin 300 mg. are given orally half an hour before starting the infusion in an attempt to minimize reactions. Potassium chloride 1 gm. b.d. is given as a routine when the patient is on Amphotericin B in order to replace loss due to nephrotoxic effects of the drug. If the patient develops fever during infusion, the drip is slowed and a further dose of promethazine and dispirin is given.

During the course of Amphotericin B therapy the following investigations are carried out:-

- | | |
|----------------------------|-------------------|
| 1. Hb., TW, Platelet count | } Every other day |
| 2. Clotting time | |
| 3. Serum Electrolytes | |
| 4. Urine analysis | |
| 5. Blood urea | } 2—3 × /week |
| 6. S.G.P.T. | |

7. Lumbar puncture and CSF for culture—initially E.O.D. and then weekly
8. Sputum culture for *Cryptococcus*
9. E.C.G.—as indicated
10. Chest X-ray—as indicated

INTRATHECAL AMPHOTERICIN

When meningitis is present, intrathecal Amphotericin B is also administered but this is generally not well tolerated by the patient. 0.5 mg. of Amphotericin B is dissolved in 10 ml. of C.S.F. previously withdrawn and this is then instilled into the cerebrospinal space. The procedure is repeated every other day till the C.S.F. is clear of the fungus or till the side effects become troublesome.

DISCUSSION

Disseminated Cryptococcosis is a relatively uncommon infection in Singapore. In ninety per cent of the cases reported in the world literature the central nervous system was affected, the remaining ten per cent involved the lungs or other systems like the skin and bone (Houk, 1965). It is generally believed that the causative organism gains access to the body through the respiratory tract. The initial pulmonary lesion may be subclinical and, if clinically apparent, may heal with or without dissemination of the disease. Spontaneous remission with or without scar formation has also been reported. A case has been made by Houk *et al* (1965) to treat pulmonary Cryptococcosis with resection alone without the use of Amphotericin B because of the toxicity of the drug but Chan (1960) at Tan Tock Seng Hospital is treating a case of pulmonary Cryptococcosis, with Amphotericin B only, as the patient refused surgery. The pulmonary lesion has regressed satisfactorily. We are of the view that it is safer to perform surgery only after a course of Amphotericin B. The presence of meningitis makes the use of Amphotericin B mandatory.

Campbell (1966) in a review of the British literature noted that sixty two cases out of a total of one hundred and one reported cases of pulmonary Cryptococcosis were diagnosed following thoracotomy, emphasizing the importance of biopsy in the diagnosis of these cases. Sixty of these patients were treated by surgical excision only without the use of Amphotericin B. Seven subsequently developed meningitis while none of those treated with Amphotericin B developed this complication. Thus one of our

cases, A.T., was given Amphotericin B after resection. Viable organisms have been cultured from resected specimens (Hickie, 1964) and it seems reasonable to assume that resection removes an endogenous source of viable *Cryptococci* thus reducing the incidence of relapse.

Side effects encountered with Amphotericin B are common and have been extensively reported. The commonest side effects are fever, with or without chills and rigors, backache, occasionally joint pains, hypokalaemia with or without symptoms and transient rise in blood urea and S.G.P.T. More serious complications of treatment are convulsions, renal failure and liver failure. In all 3 patients described an adequate dose of chemotherapy was administered, in spite of some side effects which were severe only in one patient (L.A.H.). Phlebotrombosis was not encountered and only a mild transient phlebitis was observed in two of our cases. This was made possible by the cut-off technique for intravenous administration of Amphotericin B described by Winn (1959). It is important that venesection is commenced at the periphery of the limbs in order to conserve veins and in our experience with a little care it is possible to use finger veins and often repeated infusions may be given into the same vein. The incidence of thrombo-phlebitis was very rarely encountered with the use of this technique.

In the first patient described, only transient gastro-intestinal disturbances like nausea and vomiting, and mild impairment of renal function was encountered. On suspension of the drug the blood urea fell to normal and therapy could be recommenced. In the second case there was rather severe renal functional impairment. Hypokalaemia has been reported by Sternberg *et al* (1961) and by Andriole and Kravetz (1962) who suggest that this may be due to renal or intestinal losses. Treatment of the hypokalaemia with supplementary potassium as suggested by Spickard *et al* (1963) produced improvement. The unusual feature of the hypokalaemia was the flaccid paralysis of the limbs so that the patient was unable to get out of bed. The coexistence of myaesthesia gravis with disseminated cryptococcosis has been reported by Rowland *et al* (1965) and in this patient the possibility of myaesthesia accounting for the weakness was initially considered. The failure to respond to intravenous Edrophonium suggests that the hypokalaemia was most probably the cause of the weakness.

The third case had only mild fever during the period of therapy but in spite of this we continued

with the treatment. It is important that therapy should not be interrupted too long otherwise the whole process of initiating treatment has to be repeated with smaller doses and the danger of resistance to the drug might well develop.

The absence of a skin test antigen or a reliable serologic technique makes examination of the cerebro-spinal fluid obligatory in diagnosis (Salvin, 1959). The cerebro-spinal fluid findings are not diagnostic, but in advanced cases a high pressure, turbid fluid, moderate high cell count, high protein content and a low sugar content are usual (Littman, 1959). Demonstration of the organism in the cerebro-spinal fluid is the only absolute diagnostic measure and in the investigation of a problem of meningitis where the causative organism is not obvious, an Indian ink preparation should be routine. This procedure has proved rewarding although, occasionally, repeated examinations have to be made. Recently Gordon and Vedder (1966) have suggested that an agglutination test using latex particles coated with Cryptococcal antigen has proved to be a useful test for screening, diagnosis and prognosis. They indicate that occasionally false negative results may be obtained with an Indian ink preparation when the serology is positive.

SUMMARY

1. A review of the reported cases of Cryptococcus neoformans infection in local literature is made.
2. Three new cases of disseminated Cryptococcosis are described, two with chest involvement and the third case with probably chest lesions. These are the first three cases of pulmonary Cryptococcosis locally reported who are alive and well.
3. The value of Amphotericin B and the importance of the cut-off technique and careful venesection in its administration are stressed.
4. Some problems of diagnosis and management are discussed.

ACKNOWLEDGEMENT

Our thanks are due to Mr. J.J. Murugesu, F.R.A.C.S., Surgeon 'B' Surgical Unit, Outram

Road General Hospital who performed resection in two of the cases.

REFERENCES

1. Andriole, V.T. and Kravetz, H.M. (1962): "The use of Amphotericin B in Man." J. Amer. Med. Ass. 180, 269.
2. Campbell, G.D. (1966): "Primary Pulmonary Cryptococcosis." Amer. Rev. Resp. Dis. 94, 236.
3. Chan, William (1968): "Personal communication."
4. Goh, K.T. (1961): "Cryptococcus Neoformans meningitis." J. of Singapore Paed. Soc. Vol. 3, No. 2: 72.
5. Gordon, M.A. and Danica K. Vedder (1966): "Serologic tests in diagnosis and prognosis of Cryptococcosis J.A.M.A. 197." 961.
6. Hickie, J.B. and Thomas Walker (1964): "Cryptococcosis (Torulosis): Some problems in diagnosis and management." Aust. Ann. Med. 13, 229.
7. Houk, V.N., and Moser, K.M. (1965): "Pulmonary Cryptococcosis." Must all receive Amphotericin B? Ann. Int. Med. 63, 583.
8. I.M.R. Kuala Lumpur (1953): "Annual Report." Pg. 20.
9. Lim, T.W. & Chan, K.E. (1962): "Observations on the laboratory diagnosis of cerebral torulosis." Med. J. of Mal. Vol. 16, No. 3:193.
10. Littman, M.L. (1959): "Cryptococcosis (Torulosis) Current concepts and therapy." Amer. J. Med. 27, 976.
11. Loh, S.W. (1951): "Torula Meningitis." Proc. Alum. Ass. K.E. VII Coll. of Med. 4:143.
12. MacGillivray, J.B. (1966): "Two cases of Cryptococcosis." J. Clin. Path. 19, 424.
13. Muir, C.S. and Ransome, G.A. (1959): "Cryptococcus Neoformans Meningitis." Med. J. of Mal. Vol. 14, No. 2: 125.
14. Pallis, C. (1949): "Torulosis." Proc. of Alum. Ass. of K.E. VII Coll. of Med. 2:452.
15. Poopalasingham, T. (1967): "Cryptococcus Meningitis." Med. J. of Mal. Vol. 21, No. 4:337.
16. Ross-Russell, R.W. and Dean D. (1957): "Torula Meningitis in Malaya." B.M.J. 2: 627.
17. Rowland, L.P. Griffiths, C.O., Kabat, E.A. (1965): "Myaesthesia gravis, thymoma and Cryptococcal meningitis." New. Eng. J. Med. 273, 620.
18. Salvin, S.B. (1959): "Current concepts of diagnostic serology and skin hypersensitivity in the mycoses." Amer. J. Med. 27, 96.
19. Spickard, A., Butler, W.T., Andriole, V., and Utz, J.P. (1963): "The improved prognosis of Cryptococcal meningitis with Amphotericin B therapy." Ann. Int. Med. 58, 66.
20. Sternberg, T.H., Newcomer, V.D. and Reisner, R.N. (1961): "Current status of Griseofulvin and Amphotericin B." Med. Clin. N. Amer. 45, 781.
21. Winn, W.A. (1959): "The use of Amphotericin B in the treatment of Coccidiodal disease." Amer. J. Med. 27, 617.