Original articles

Evaluation of adenosine deaminase activity and antibody to *Mycobacterium tuberculosis* antigen 5 in cerebrospinal fluid and the radioactive bromide partition test for the early diagnosis of tuberculosis meningitis

Y M COOVADIA, A DAWOOD, M E ELLIS, H M COOVADIA, AND T M DANIEL

Departments of Microbiology and Paediatrics and Child Health, University of Natal, Durban, South Africa, and Department of Medicine, Case Western Reserve University and University Hospitals, Cleveland, Ohio, United States of America

SUMMARY   A number of different biochemical and serological tests have been described recently for the early and accurate diagnosis of tuberculous meningitis. None of these tests has yet gained widespread acceptance in clinical medicine or in microbiology laboratories. To investigate this problem we evaluated adenosine deaminase activity (ADA), an enzyme linked immunosorbent assay (ELISA) that detects antibody to antigen 5 of *Mycobacterium tuberculosis*, and the radioactive bromide partition test (BPT) in the cerebrospinal fluid (CSF).

Cerebrospinal fluid specimens from children with tuberculous, pyogenic, and viral meningitis as well as from patients with pulmonary tuberculosis without meningitis and from controls with normal CSFs were included in the study. In addition, we estimated ADAs in serum samples from selected children in these groups. The sensitivity and specificity of the three tests evaluated in the CSF were: ADA assay 73% and 71%; BPT 92% and 92%; and ELISA for antibody to antigen 5, 53% and 90%, 40% and 94%, and 27% and 100%, respectively, at titres of more than or equal to 1:20, 1:40, and 1:80. The serum ADA was lower ($11 \cdot 0 \pm 6 \cdot 15$ IU/l) in children with tuberculous meningitis when compared with those with pulmonary tuberculosis alone ($25 \cdot 8 \pm 20 \cdot 9$ IU/l). The BPT was found to be the most reliable test in the early differentiation of tuberculous from other causes of meningitis and remained abnormal for a period of up to five months after the beginning of treatment. Accordingly, we believe that the BPT should be used in conjunction with bacterial and fungal antigen detection systems for the initial differentiation of clinically suspicious tuberculous meningitis from Gram or culture negative cases, or both, of bacterial and fungal meningitis.

The diagnosis of tuberculous meningitis is usually based on a history of contact, clinical findings, a positive tuberculin skin test, chest roentgenogram, characteristic cerebrospinal fluid (CSF), and demonstration of acid fast bacilli on direct microscopy or culture.[1–3] The CSF findings, however, are often ambiguous, especially in children,[2] and interpretation of the tuberculin skin test is difficult as it may be reactive because of immunisation or falsely negative

because of malnutrition or severe infection. Furthermore, in our own experience, acid fast bacilli are shown rarely in direct Ziehl-Neelsen smears of CSF specimens and are cultured in only 42–75% of patients.[4 5] Therefore, the diagnosis of tuberculous meningitis is often delayed, and this can adversely affect the outcome.

In recent years various biochemical and serological tests have been evaluated for the early diagnosis

of tuberculous meningitis, including detection of tuberculostearic acid and 3-(2[1]-ketohexyl) indoline by gas liquid chromatography,[6 7] measurement of CSF adenosine deaminase activity (ADA),[8 10] the bromide partition test (BPT),[11 13] and the detection of mycobacterial antigens and antibodies by enzyme linked immunosorbent assay (ELISA).[14–16] To date, however, none of these tests has gained widespread acceptance for the rapid diagnosis of tuberculous meningitis in routine microbiology laboratories. These methods often gave disappointing results when tested in a clinical setting or required sophisticated, expensive equipment not readily available in most laboratories.[17]

In this paper we report on the use of the ADA assay, the radioactive BPT, and an ELISA antibody to antigen 5 of *Mycobacterium tuberculosis* in the diagnosis of tuberculous meningitis. The latter has already proved to be useful in the diagnosis of pulmonary tuberculosis.[18]

Materials and methods

Patients. CSF samples were obtained from three groups of African patients, aged 1 month to 12 years, who were admitted to the paediatric wards of the King Edward VIII Hospital or King George V Hospital, which serves as a referral hospital for patients with confirmed tuberculosis in the Durban area.

(1) Group A consisted of 38 children with tuberculous meningitis and was divided into two further groups. Group A1 consisted of 13 children admitted to the King Edward VIII Hospital with a strong clinical suspicion of tuberculous meningitis—that is, with typical CSF findings of tuberculous meningitis plus at least two of the following: positive results of Mantoux test; chest roentgenogram suggestive of pulmonary tuberculosis; or CSF positive for acid fast bacilli on direct Ziehl-Neelsen stain or culture, or both. These children were assessed within 72 hours of admission or before the beginning of antituberculous chemotherapy, and thereafter serially at regular intervals until transfer or discharge. Group A2 comprised 25 children at King George V Hospital who were already on treatment for tuberculous meningitis for varying periods. These children were assessed on a single occasion only.

(2) Group B was made up of 49 children presenting with clinical findings suggestive of meningitis from whom CSF was obtained for diagnostic purposes. This group included 16 patients with non-tuberculous bacterial meningitis (designated as group B2), 13 with aseptic meningitis (group B3), and 20 with normal CSF (group B1).

(3) Group C included 14 children with pulmonary tuberculosis with no evidence of meningitis. Tests had been undertaken in these children on an initial suspicion (which subsequently proved to be unfounded) of meningitis.

Not all tests were performed in every patient; the exact number of patients investigated by a particular test is indicated in the text. Clinically, severity of meningitis was based on the following:[19]

Stage 1: patients were fully conscious and rational with signs of meningeal irritation but with no focal neurological signs or signs of hydrocephalus.

Stage 2: patients were mentally confused and/or had such neurological signs as squints or hemiparesis.

Stage 3: patients were mentally inaccessible, owing to the depth of stupor or delirium and/or had a complete hemiplegia or paraplegia.

Methods. Routine microscopy and biochemical investigations were performed on all CSF specimens received, and they were all cultured on chocolate agar plates. CSF specimens received from patients with suspected tuberculosis were cultured in addition onto Lowenstein-Jensen media and examined by Ziehl-Neelsen stain for acid fast bacilli. If not tested for bacterial antigens or ADA immediately the specimens were stored at −20°C and tested within 24 hours. CSF samples for ELISA testing were stored at −70°C until the end of the study, when they were shipped by air to Cleveland frozen on dry ice.

Phadebact coagglutination test
The Phadebact coagglutination test (Pharmacia Diagnostics, Sweden) consists of specific antibodies against *Haemophilus influenzae* type B, *Streptococcus pneumoniae* 83 serotypes, *Neisseria meningitidis* groups A, B, C, Y, and W135, and *Streptococcus agalactiae* bound to protein A rich staphylococci. The tests were performed on CSF specimens according to instructions supplied with each kit.

Adenosine deaminase activity (ADA) assay
Adenosine deaminase activity (ADA) was assayed at 37°C by colorimetric method described by Giusti[20] using commercially available reagents (Boehringer Mannheim, W Germany). The optical density was measured at 628 nm with a Beckman Model 42 clinical analyser. Enzyme activity was expressed in IU/l (37°C). Control specimens of known values were included with each run. For statistical analysis specimens not showing any ADA

were assigned a value of 0·1 IU/l, which is the lower limit of sensitivity of this particular assay.

Enzyme linked immunosorbent assay (ELISA) for antibody to Mycobacterium tuberculosis *antigen 5*[18]
The ELISA microtitre assay for IgG antibody to antigen 5 was performed in Cleveland using methods described previously,[18] with modifications to allow the use of an automated ELISA plate reader (MR 580 Micro ELISA Auto Reader, Dynatech Laboratories, Arlington, Virginia). Controls on each plate included a 1:500 dilution of a known standard positive serum and a colour standard with an optical absorbancy of 0·24 when read on the automated plate reader set to read the ratio of 405/630 nm. Plates were read when the positive standard gave a colour intensity equal to that of the colour standard, and all wells with readings equal to or greater than that of the positive standard were considered positive. CSF samples were stored at −70°C for periods of up to six months and analysed at the end of the study. For the calculation of geometric means titres less than 1:20 were arbitrarily assigned a value of 1.

Radioactive bromide partition test (BPT)
This test was performed as previously described by Wiggelinkhuizen and Mann.[13] Briefly, 0·6–0·8 uCl^{82}Br/kg body weight in isotonic sodium chloride was administered orally, and specimens of serum and CSF were obtained simultaneously 48 hours later for measurement of ^{82}Br content. The results were expressed as serum to CSF^{82}BR ratio.

Blood
ADA was determined on venous blood from selected patients.

Statistical analysis
Statistical analysis was performed using Student's *t* test. For the ELISA results the test was performed after logarithmic transformation of the values obtained. In the patients with tuberculous meningitis only results obtained in the first month after admission were included for statistical comparison with the other groups. For the bromide partition test the results were analysed using the χ^2 test. Significance was taken at the 0·01% level.

Results

Cerebrospinal fluid results.

Adenosine deaminase activity
The results of CSF ADA in the different groups of patients studied are shown in Figure 1 and Table 1. Of the 38 children in group A, only 73% (11/15) of patients investigated in the first month of admission had ADA values higher than 10 IU/l, and thereafter only 21% (8/38) of specimens tested had ADA values of more than 10 IU/l. Twelve children in group A1 had serial ADA assays performed during the course of their stay in hospital. Most of these children showed a decrease in CSF ADA to values
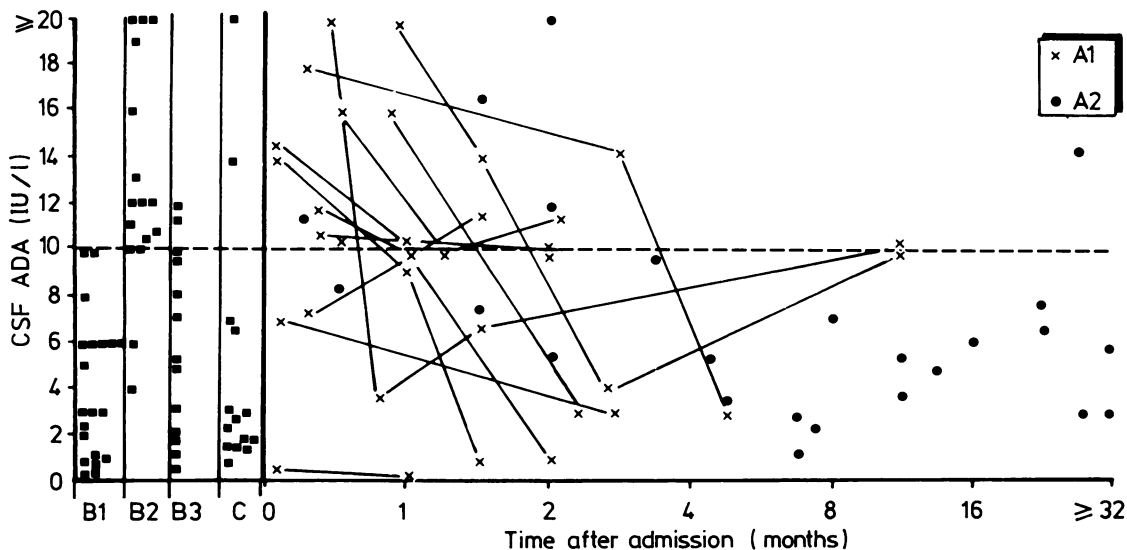

Fig. 1   *Distribution of adenosine deaminase activity (ADA) in cerebrospinal fluid (CSF) in different groups of patients.*
Key to groups: A1: Tuberculous meningitis followed up serially; A2: Tuberculous meningitis on treatment; B1: Normal cerebrospinal fluid; B2: Bacterial meningitis; B3: Aseptic meningitis; C: Pulmonary tuberculosis.

Table 1 *Mean ADA and geometric mean titre of ELISA antibody to antigen 5 in CSF and mean serum ADA in the different groups of patients*

Patient groups	No tested	CSF			Serum	
		ADA (IU/l)		Antigen 5 antibody	ADA (IU/l)	
		Mean	Range	Geometric mean	Mean	Range
Group A1 (tuberculous meningitis)	13* ⎱	11·7	7·1–32·5	1:6	11	2·1–23·6
A2 (tuberculous meningitis)	2* ⎰					
	23†	8·1	1·1–4·7	1:3·6	18·7	7·8–45
Group B1 (normal)	20	4·2	0·1–9·8	1:1	0·12	0·1–0·4
B2 (bacterial meningitis)	16	13·1	4·2–20	1:2	1·12	0·2–3·1
B3 (aseptic meningitis)	13	5·8	0·5–11·9	ND	ND	
Group C (pulmonary tuberculosis)	14	5·4	0·8–24·3	1:1	25·8	3·5–76·5

*Tested within one month of admission.
†Tested more than one month after admission.
ND=not done.

below 10 IU/l within one to five months after treatment had begun (Fig. 1). We did not show any correlation, however, between CSF ADA and either severity of disease or presence of complications (data not shown).

In group B all the normal children and 85% (11/13) of the children with aseptic meningitis had ADA values less than 10 IU/l, whereas the vast majority (14/16) of the patients with non-tuberculous bacterial meningitis had ADA values more than 10 IU/l. Eighty six per cent (12/14) of the children with pulmonary tuberculosis alone had ADA values less than 10 IU/l. The mean ADAs in CSF (Table 1) in the patients with bacterial and tuberculous meningitis (investigated in the first month of admission) were significantly higher (p<0·01) than in the other groups studied (B1, B3, and C). There was no significant difference in ADA, however, between the patients with tuberculous and bacterial meningitis. If a value of more than 10 is identified as the cut off point then CSF ADA showed a sensitivity of 73% (11/15) for diagnosis of tuberculous meningitis in the first month after admission. In control groups B and C a value of less than 10 was only 71% (45/63) specific for absence of the disease.

ELISA antibody to antigen 5 of M. tuberculosis
Figure 2 shows the distribution of CSF antigen 5 antibody concentrations in the different groups of patients studied. Of the children in group A who were tested within the first month of admission, only 53% (8/15) had ELISA titres of 1:20 or more. Similarly, of all the specimens tested on group A, only 42% (23/55) were found to be positive at this titre, and 21 (91%) of these positive results were detected in the first five months after admission. Twelve of the children in group A had serial antibody assays performed during the course of their

illness. In four of these children the titres remained below 1:20 over a period of one to 10 months, while an equal number showed a rise (four) or fall (four) in antibody titres. Peak concentrations of CSF antibody were detected as early as one week or as late as two and a half months after presentation. We did not observe any correlation, however, between antibody concentrations and severity of disease or the presence of complications. With the exception of five children who had titres of more than or equal to 1:20, the remaining 31 children tested in group B had titres of less than 1:20. All the children in group C had values of less than 1:20.

CSF specimens from children in group A, which were tested in the first month of admission, gave a geometric mean titre of 1:6 (Table 1), which was significantly higher than that obtained in normal children or in patients with pulmonary tuberculosis alone. No significant difference was evident, however, between antigen 5 antibody in the CSF of the patients with either tuberculous or bacterial meningitis. At CSF dilutions of more than or equal to 1:20, 1:40, and 1:80, respectively, the ELISA test had sensitivities of 53% (8/15), 40% (6/15), and 27% (4/15) when performed within the first month of admission. In the control groups B and C a titre of less than 1:20 was 90% (45/50) specific for the absence of tuberculous meningitis, whereas titres of less than 1:40 and 1:80, respectively, were 94% (47/50) and 100% (50/50) specific.

Radioactive bromide partition test (BPT)
Results of the radioactive bromide partition test are shown in Figure 3 and Table 2. In group A1 the BPT was performed within 72 hours after admission, in groups B and C within a week after admission, and in group A2 the time of the test varied from a week to four years after admission. If a BPT ratio of less than 1·5 is taken as indicative of tuberculous
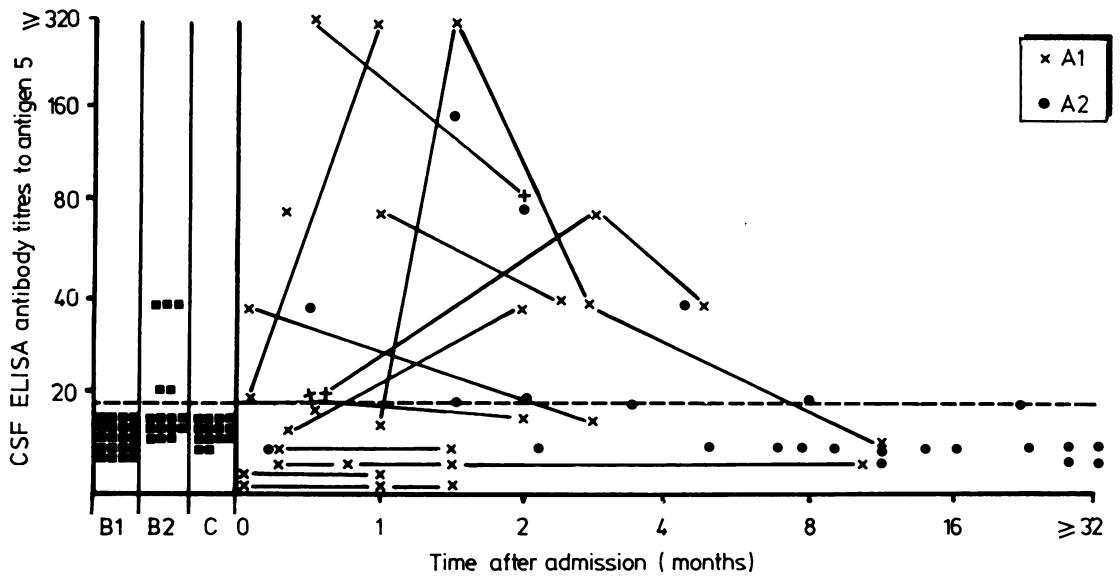
Fig. 2   *Distribution of ELISA antibody titres to antigen 5 in cerebrospinal fluid (CSF) in different groups of patients.*
Key to groups: A1: Tuberculous meningitis followed up serially; A2: Tuberculous meningitis on treatment; B1: Normal cerebrospinal fluid; B2: Bacterial meningitis; B3: Aseptic meningitis; C: Pulmonary tuberculosis.
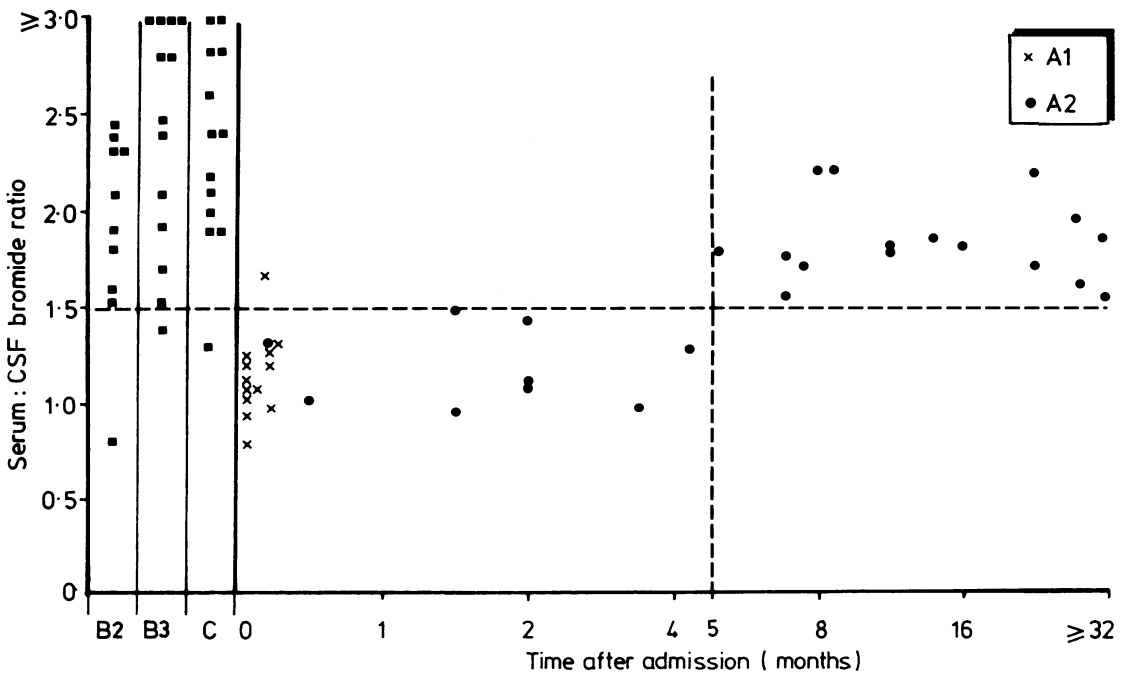

Fig. 3   *Results of radioactive bromide partition test (BPT) in different groups of patients.*
Key to groups: A1: Tuberculous meningitis followed up serially; A2: Tuberculous meningitis on treatment; B1: Normal cerebrospinal fluid; B2: Bacterial meningitis; B3: Aseptic meningitis; C: Pulmonary tuberculosis.

Table 2 *Mean radioactive bromide partition ratio in the different groups of patients*

Patient groups	No tested	Mean	Range
Group A1 (tuberculous meningitis)	13*	1·15 ⎫	0·96–1·68
A2 (tuberculous meningitis)	2*	1·15 ⎬ 1·15	1·01–1·3
Group B2 (bacterial meningitis)	10‡	1·93	0·78–2·4
B3 (aseptic meningitis)	13	2·52	1·38–3·8
Group C (pulmonary tuberculosis)	13	2·3	1·2–3·6

*Within one month of admission.
†A mean of 1:18 was obtained for the period five months after admission.
‡*Haemophilus influenzae* four, *Streptococcus pneumoniae* five, *Neisseria meningitidis* one.

meningitis, and a value of >1·5 as against the diagnosis, then the BPT was positive in 92% (12/13) of patients in group A studied in the first month after admission and in 95% (21/22) within five months after admission. In group B only one of the 13 patients with viral meningitis had a BPT ratio of less than 1·5, and this was a child with mumps who also had positive results for the Mantoux test; with the exception of one child with pneumococcal meningitis, all the others with bacterial meningitis in whom the test was performed were found to have values of more than 1·5. Of the 13 children tested in group C, only one had a ratio of less than 1·5. Children in group A had BPT ratios that were significantly different from those obtained in the control groups, but a significant difference was not shown between patients in the control groups themselves (Table 2).

Of the 13 CSF specimens in group A1 examined for acid fast bacilli by the Ziehl-Neelsen stain, none were found to be positive, and only six (46%) were positive on culture.

Serum tests

In both the groups with tuberculous meningitis and with pulmonary tuberculosis the mean ADA in serum was significantly higher than in the non-tuberculous control group (Table 1). The serum from patients with pulmonary tuberculosis alone had significantly higher ADA titres than those with tuberculous meningitis (p<0·01).

Discussion

This study was undertaken to evaluate appropriate tests that could reinforce a clinical diagnosis of tuberculous meningitis. We evaluated the usefulness of three tests that, in our view, have been inadequately assessed (BPT and ADA) or untried (ELISA antigen 5 antibody assay). Unexpectedly,

we found the BPT, which was first used for this purpose as far back as 1954, to be the most reliable.

The CSF ADA was found to have relatively low sensitivity (73%) and specificity (71%) for diagnosis of tuberculous meningitis. Most importantly, for this is a major clinical problem, we did not show a significant difference between ADA in the CSF of patients with acute bacterial and tuberculous meningitis. These findings compare favourably with those reported by Mann *et al*[9] and Mallan *et al*[10] but differ significantly from those reported by Piras and Gakis[8] and Blake and Berman.[21] CSF ADA has also been reported to be of value in distinguishing tuberculous and viral causes of meningitis.[8] [10] We observed, however, that CSF values in patients with viral meningitis overlapped with those found in patients with acute bacterial and tuberculous meningitis. As a result of these findings, we believe that ADA in CSF has very limited application in routine microbiology laboratories for the early and accurate diagnosis of tuberculous meningitis. Serum ADAs were lower in children with tuberculous meningitis when compared with those with pulmonary tuberculosis alone. This requires further investigation.

The ELISA test was found to be highly specific but relatively less sensitive for the diagnosis of tuberculous meningitis. Hernandez *et al*, using a different antigen antibody system, reported on an ELISA test for detection of antibodies to bacille Calmette-Guérin (BCG) in CSF, which they found to be very sensitive and highly specific for diagnosis of tuberculous meningitis.[15] Similarly, Kalish *et al* detected IgG antibodies to purified protein derivative in the CSF of all three patients with tuberculous meningitis and in none of the 33 control specimens.[16] Sada *et al*, using ELISA to detect mycobacterial (BCG) antigens in CSF, found the test to be highly specific (95%) and 81% sensitive for diagnosis of tuberculous meningitis.[14] A possible explanation for the relatively low sensitivity (27%–53%) shown by the ELISA antigen 5 antibody assay in this study is the presence of excess antigen or antigen-antibody complexes in CSF samples, which would not be detected by this assay. Further studies need to be undertaken to answer this question. The findings of this study also suggest that CSF antigen 5 antibody concentrations do not reflect on the severity of the disease process, nor were they useful for monitoring response to treatment.

The results of this study confirm that the radioactive bromide partition test (BPT) is of value in distinguishing tuberculous meningitis from viral and pyogenic meningitis and that the diagnostic value of the test is not affected by antituberculous treatment for a period of up to five months after treatment is

begun. Using a bromide partition ratio of 1·5 as the upper limit for the diagnosis of tuberculous meningitis, we obtained a sensitivity of 92% and a specificity of 92%. Wiggelinkhuizen and Mann, using a ratio of 1·6, reported an incidence of 5·9% false negative results and 12% (4/32) false positive results in children with suspected tuberculous meningitis.[13] As most of the false positive results have been reported in children with purulent bacterial meningitis the value of the BPT in distinguishing such patients from those with tuberculous meningitis has been far less clear.[9 13]

In the present series the BPT was performed in 10 children with pyogenic meningitis with only one false positive result, which was seen in a child with pneumococcal meningitis. False positive results have also been reported in patients with neurosyphilis, multiple sclerosis, spinal block, congenital hypothyroidism, and mumps meningoencephalitis;[13] the last mentioned also posed difficulties in this study with a ratio of 1·38.

The BPT has previously been shown to be of particular value in the differential diagnosis of lymphocytic meningitis, including that due to neoplastic and other non-infective conditions.[12] This is of particular importance in adults in whom these latter conditions may often need to be differentiated from tuberculous meningitis. The role of this test in differentiating cryptococcal meningitis from tuberculous meningitis still needs to be determined, but this is now far less of a problem with the ready availability of the highly sensitive and specific cryptococcal latex agglutination test. We should point out that a possible disadvantage of the BPT is the need for a gammacounter to read the results.

Despite an exhaustive and careful search for acid fast bacilli, we did not obtain any positive results in the CSF specimens examined, and the organism was cultured in only 46% of patients. Similar observations have been reported by others.[22]

The detection of antigens in CSF and other body fluids by ELISA or indirect agglutination tests is now routinely performed in most microbiology laboratories for the rapid and accurate diagnosis of acute bacterial and also cryptococcal meningitis. These techniques have now been described for the rapid diagnosis of tuberculous meningitis and have potential for application at clinic or peripheral hospital level.[14 23] Both these tests, however, will need to fulfil very strict criteria of sensitivity and specificity before they can be accepted for the rapid diagnosis of tuberculous meningitis in routine laboratories.

Of the three tests evaluated in this study, the BPT was found to be the most reliable for the early differentiation of tuberculosis from other common causes of meningitis. Accordingly, we believe that for the present, where facilities are available, the BPT should be performed in conjunction with bacterial and fungal antigen detection systems for the initial differentiation of clinically suspicious tuberculous meningitis from Gram or culture negative cases, or both, of bacterial and cryptococcal meningitis.

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Correspondence to Dr Y M Coovadia, Department of Microbiology, Faculty of Medicine, University of Natal, P O Box 17039, Congella 4013, Durban, South Africa.

Fifty years ago

Dwarfism with retinal atrophy and deafness

E A Cockayne (London)—Arch Dis Child 1936;**11**:1–8

'The two dwarfs (brother and sister) have small heads and faces with sunken eyes and prominent superior maxillae. They are slightly built with short, slender trunks and unduly long legs and their feet and hands are too large in proportion. Both are active and their movements quick and bird-like. They frequently make noises but actual words are seldom recognised. Although not totally deaf their hearing is greatly impaired and it is difficult to tell how much their backwardness is due to deafness and how much to mental deficiency. Both have a scaly, erythematous dermatitis. The optic discs show considerable atrophy with narrowing of the retinal arteries and scattered over the fundus are a number of blackish dots. In some respects this syndrome resembles juvenile amaurotic idiocy but pigmentation of the retina is more uniform and widespread, the mentality is different and dwarfism has not been found in association with amaurotic idiocy.'

(Cockayne's syndrome is now well recognised. There are 14 references in McKusick's book, and now the syndrome has been broadened to include dwarfism, precocious senility, mental retardation, pigmentary retinal degeneration, optic atrophy, deafness, marble epiphyses in some digits, sensitivity to sunlight, intracranial calcification, microcephaly, hyperlipoproteinaemia, fasting hyperinsulinaemia, renal insufficiency with acidosis, and peripheral neuropathy. No definite cause has yet been found for the condition. NEIL GORDON.)