THE INCIDENCE OF INFECTION WITH POLIOVIRUS AND OTHER VIRUSES IN CASES OF ASEPTIC MENINGITIS ('NONPARALYTIC POLIOMYELITIS') IN SHEFFIELD IN 1954

BY D. A. J. TYRRELL, D. BALDUCCI, B. SNELL AND T. ELISABETH ZAIMAN

From the Virus Research Laboratory of the University of Sheffield, Lodge Moor Hospital, Sheffield, 10

When poliomyelitis is prevalent there is a natural tendency to make the diagnosis of 'non-paralytic poliomyelitis' on patients who are found to have an aseptic meningitis. Sometimes this is correct, but often it is not. American and other workers (Godenne & Riordan, 1955; Francis, Korns, Woight, Boisen, Hemphill, Napier & Tolchinsky, 1955; McLeod, Beale, McNaughton & Rhodes, 1956) failed to recover poliovirus from about 60% of patients with an aseptic meningitis. Other agents which have sometimes been found to cause aseptic meningitis are the viruses of mumps, herpes simplex, lymphocytic chorio-meningitis, arthropod-borne encephalitis (in certain areas) and leptospira. (Adair, Gauld & Smadel, 1953; Report, Virus Reference Laboratory, 1953). But in about half the cases of aseptic meningitis no evidence can be demonstrated that any of these agents, or the polioviruses, are responsible and it seems likely that there are other viral causes. Strains of the Coxsackie B group of viruses have been recovered from patients in some outbreaks of aseptic meningitis (Melnick, Shaw & Curnen, 1949; Galpine & Macrae, 1953; Kirby & Evans, 1955; Johnsson 1955; McLeod *et al.* 1956; Wilkins, Kotze, Melvin, Gear, Prinsloo & Kirsch, 1955), while, of the Cox-sackie A group, Type 9 has been found in a number of patients. Since some members of the group of 'enteric cytopathogenic human orphan' (ECHO) viruses have been found in the faeces or cerebrospinal fluid of patients suffering from aseptic meningitis it seems likely that they also may be causal agents particularly Types 6 and 4. (Committee on ECHO viruses, 1955; Melnick, 1955; Beale, Duncan, Stackiw, Davis, Dempster & Rhodes, 1956; Karzon, Baron, Winkelstein & Cohen, 1956; Davis & Melnick, 1956; Krech, 1957.) Viruses which are neutralized by ECHO virus Type 9 serum and are also pathogenic for suckling mice apparently caused widespread epidemics of an aseptic meningitis associated in some places with a rash (see review in Tyrrell, Lane & Snell, 1957) . Other workers believe that viruses of the Columbia SK-M.M.-Encephalomyocarditis-Mengo Meningo encepha-litis group may cause this disease (see Bieling & Koch, 1956).

The purpose of the present investigation was to discover what viruses caused aseptic meningitis in an average unselected group. Patients with paralytic polio-myelitis were examined as controls.

MATERIALS AND METHODS

Patients studied

The group comprised forty patients admitted to hospital from Sheffield and the area around during the period June to October in 1954 when the prevalence of poliomyelitis was low. Their ages varied from $1\frac{1}{2}$ to 48 years; thirty were under 14 years.

Clinical examination

Nearly all the patients were examined personally by one of us (B.S.) who recorded on an appropriate form a detailed account of the clinical history and physical examination, together with an abstract of the hospital notes. When the patients were discharged from hospital, and before the results of the laboratory tests were known, each illness was classified on clinical grounds as paralytic poliomyelitis, aseptic meningitis or some other disease. Paralytic poliomyelitis was diagnosed by the presence of the typical lower motor neurone type of paralysis. Aseptic meningitis was diagnosed on the basis of neck stiffness or positive Kernig's sign and the absence of any other condition likely to produce meningism.

Virus isolation

Two specimens of faeces, or faecal swabs, were collected from each patient within a few days of admission. Blood was collected on admission and 2 and 4 weeks later. All specimens were stored at $-20°$ C. Faecal material was inoculated into monkey kidney tissue cultures (Melnick, 1955). If poliomyelitis virus was not isolated from patients with aseptic meningitis reserve material was inoculated into rabbit kidney cultures, human kidney cultures and HeLa cell cultures. In addition to these procedures faecal specimens from twelve patients were inoculated into 1-day-old suckling mice. The cerebrospinal fluids (C.S.F.) from eighteen patients were also cultured—thirteen in monkey kidney tissue and five in human kidney tissue.

Virus neutralization tests

A convalescent serum specimen from each patient was titrated for neutralizing antibody against poliovirus Types 1–3, using monkey kidney-cultures in roller tubes (Tyrrell, Keeble & Wood, 1956).

Complement fixation and haemagglutination tests

Serum samples from the patients were tested for antibodies to poliovirus, Coxsackie virus, herpes simplex virus, Adenoviruses, mumps virus and the virus of lymphocytic choriomeningitis by the complement fixation methods and against Columbia SK virus by the haemagglutination inhibition technique of Horvath & Jungeblut (1952). Poliovirus antigens were made from tissue culture fluids heated to 60° C. for $\frac{1}{2}$ hr. and the CFT used was that described by Balducci, Zaiman & Tyrrell (1956). Coxsackie virus antigens were prepared from the ground-up carcases of infected suckling mice and purified by six cycles of freezing and thawing and then allowing them to stand for 3 days at 4° C. before centrifuging at 1000–1500 g.

Coxsackie B Types 1–5 and Coxsackie A Type 9 antigens were further concentrated in the ultra-centrifuge. Mouse immune sera were used to standardize these antigens (Melnick & Ledinko, 1950). The herpes virus antigen was made in suckling mice (Balducci, Tyrrell & Stuart-Harris (1956) and those for the Adenoviruses in tissue culture (Balducci *et al.* 1956). Mumps and lymphocytic choriomeningitis CFT's were performed by Dr J. E. M. Whitehead of the Public Health Laboratory, Sheffield with Public Health Laboratory Service antigens.

RESULTS

Table 1 shows the virus isolations in monkey kidney or HeLa cell cultures from faeces or faecal swabs; there were no isolations of any virus from the cerebrospinal fluids. Rectal swabs are known to yield virus less frequently than faeces (Nolan, Wilmer & Melnick, 1955) and from seventeen of the twenty-four cases of aseptic meningitis rectal swabs only were obtained. It is, however, unlikely that the small number of virus isolations from these cases was entirely due to this.

Table 1. *Virus isolation from faeces and rectal swabs from cases of paralytic poliomyelitis and of aseptic meningitis*

	Paralytic disease	Aseptic meningitis
No. of cases tested	16	24
Virus isolation:		
Poliovirus Type 1	9	2
Poliovirus Type 2	3	1
Poliovirus Type 3	1	0
Unidentified virus	0	1
No virus isolated	3	20

Table 2. *Results of neutralizing antibody titrations on sera from cases from which poliovirus was not isolated*

	Paralytic disease	Aseptic meningitis
No. of cases tested	3	21
Cases with antibody against one or more Types of poliovirus	2	20
No. of paired sera tested	1	12
Fourfold or greater rise versus:		
Poliovirus Type 1	1	0
Poliovirus Type 2	0	0
Poliovirus Type 3	0	1

The results of neutralizing antibody tests on sera of patients from whom poliovirus was not recovered are shown in Table 2. It was not possible to complete tests on paired sera in every case owing to lack of serum. Whenever complement-fixing antibody against a particular poliovirus was found the corresponding neutralizing antibody was also found. Neutralizing antibody was often found, however, in the absence of complement-fixing antibody. Of the twenty-two sera

reacting positively to the complement-fixation test, fifteen reacted with only one Type of poliovirus, the remaining seven with more than one Type. Fourteen of the fifteen and five of the seven mentioned above were from children under the age of 10 years. Using the same complement-fixing antigens—culture fluids heated to 60° C. for 30 min.—we found no antibody to the polioviruses in the sera of nineteen

Table 3. *Results of complement-fixation tests*

Virus antigens used		Sera from: Paralytic poliomyelitis	Sera from: Aseptic meningitis
Poliovirus 1–3	No. of cases tested	14	19
	Total no. positive (titres 1–4 and over)	13 (11)*	9 (2)*
	No. positive isolations with CF antibody to homologous Type virus only	9	0
	No. of cases with fourfold or more antibody rise	6 (6)*	0
	No. of cases with twofold or more antibody rise	9 (8)†	3
Coxsackie	No. of cases tested	16	24
A. Types 1–10	Total no. positive (titres 1–16 and over)	5	5
B. Types 1–5	Antigen A_2	1	0
	Antigen A_3	3	0
	Antigen A_4	0	1
	Antigen A_6	1	1
	Antigen B_3	0	1 (1)*
	Antigen B_5	0	2‡†
	(All sera negative to other Types)		
Herpes simplex	No. of cases tested	15	20
	Total no. positive (titres 1–4 and over)	7	7
	No. showing fourfold or more antibody rise	1†	0
Adenoviruses	No. of cases tested	15	20
	Total no. positive (titres 1–4 and over)	3	7
	No. showing fourfold or more antibody rise	0	2†

* Figures in parentheses indicate the number of cases in which the poliovirus was isolated.

† One case in each group so marked showed rising antibody titre only in the third specimen of serum.

‡ Fourfold or greater rise in antibody titre.

medical staff or children suffering from diseases other than poliomyelitis or aseptic meningitis. Paul & Melnick (1956) have recently reviewed the diagnostic significance of tests with this type of antigen. No positive results were found in the complement-fixation tests for lymphocytic chorio-meningitis virus. Nor was it considered that evidence of infection with Columbia SK virus was obtained. The haemagglutination inhibition test for this was indeed positive in sera from three of sixteen paralytic patients and from eight of twenty-four patients with aseptic meningitis. The titres ranged from 1:8 to 1:1024 and in two cases they rose, while in six they fell. However, even the serum with a haemagglutination inhibition titre of 1:1024 failed to protect mice against as little as 10 LD_{50} of Columbia SK virus inoculated intraperitoneally.

Clinical studies

The clinical condition of the patient is related to the results of virus studies in Table 4.

It was hoped to find clinical signs or symptoms which would distinguish aseptic meningitis caused by different viruses. In general this was not possible. However, among the aseptic meningitis patients in whom evidence of poliovirus infection was found, one had loss of tendon reflexes without any motor or sensory change and

Table 4. *Diagnosis of virus infection in forty cases*

Clinical type of illness	Infection concurrent with acute phase of illness (virus isolation or rising antibody level)		Recent infections (elevated or falling antibody level)		No infection detected
Paralytic poliomyelitis	Poliovirus only	9	Poliomyelitis and mumps	1	1
	Poliovirus and Coxsackie A	5			
Aseptic meningitis	Poliovirus only	2	Poliovirus	6	9*
	Poliovirus and Coxsackie B	2	Coxsackie A	2	
	Adenovirus	1	Mumps	1	
	Unidentified	1			
		20		10	10

* Three cases in this group were not tested for poliovirus complement-fixing antibody owing to anticomplementary sera.

two others had transient urinary retention. It is of interest that in one patient with paralytic poliomyelitis and in eight with aseptic meningitis the cerebrospinal fluid was normal on admission; four of the latter had a poliovirus infection.

DISCUSSION

Whereas evidence of poliovirus infection was obtained, either by virus isolation or by the demonstration of a rising antibody titre, in fifteen of sixteen patients with paralytic poliomyelitis, it was found in only ten of twenty-four patients with aseptic meningitis. A diagnosis of non-paralytic poliomyelitis is, therefore, justified in only about 50 % of the cases of aseptic meningitis. High-titre antibodies for Coxsackie A viruses which often fell in titre during the illness, suggesting a recent infection, were found in four of twenty-four patients with aseptic meningitis, but they were also found in five of sixteen patients with paralytic poliomyelitis. Moreover, of the two patients with aseptic meningitis whose sera showed a rising titre for Coxsackie B Type 5 antibodies, one had clear-cut evidence of infection with poliovirus Type 3, and in the other the antibody rise took place between the taking of the second and third specimens of serum, suggesting that his infection with Coxsackie B Type 5 virus took place after admission to hospital. There is, therefore, little evidence that known Coxsackie viruses caused any of these cases of aseptic meningitis. One patient with aseptic meningitis had a very marked rise of Adenovirus antibody during his illness which may have been caused by a member of this group. None of the cases appears to have been due to the Columbia SK or related viruses.

From one patient a virus was apparently isolated in monkey kidney cultures and died out on passage. Although it was re-isolated and was shown not to be poliovirus, it was not further identified. The patient showed no evidence of recent or concurrent infection with any of the viruses used in the serological tests. This points to the desirability of carrying out further intensive studies using improved methods, in the effort to find new viruses causing aseptic meningitis.

SUMMARY

1. Sixteen cases of paralytic poliomyelitis and twenty-four cases of aseptic meningitis ('non-paralytic poliomyelitis') occurring in the Sheffield area were studied clinically and by virological methods.

2. Poliovirus was isolated from thirteen of the sixteen cases of paralytic poliomyelitis and from three of the twenty-four cases of aseptic meningitis. In another two of the cases of paralytic poliomyelitis and another six of the cases of aseptic meningitis serological evidence of poliovirus infection was obtained.

3. Serological evidence of Coxsackie virus infection was found in four of the cases of aseptic meningitis; but in one there was also clear-cut evidence of poliovirus Type 3 infection, and in another it seemed that the infection was contracted in hospital. Evidence of Coxsackie virus infection was also obtained in five cases of paralytic poliomyelitis.

4. Serological evidence of Adenovirus infection was found in one case of aseptic meningitis.

5. No acceptable evidence was found of infection with Columbia SK or related viruses, or with the virus of lymphocytic choriomeningitis.

6. From one patient with aseptic meningitis an unidentified virus was isolated.

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