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An outbreak of aseptic meningitis associated with a previously unrecognized virus

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INTRODUCTION

With the discovery of the ECHO viruses in tissue culture and of Coxsackie viruses by suckling mouse inoculation, has come the recognition that the syndrome of non-bacterial meningitis without paralysis is not the same as non-paralytic poliomyelitis. Aseptic meningitis, the name generally used for the syndrome, is indeed frequently caused by polioviruses during poliomyelitis epidemics, but at other times outbreaks are mainly due to ECHO and Coxsackie viruses. ECHO viruses Types 4, 6 and 9 have all caused epidemics in Europe and America, but in Britain only ECHO Type 9 has been associated with any large outbreaks (see review by Macrae, 1959). In Scotland the epidemic of 1956 was due to ECHO Type 9. In 1958 paralytic poliomyelitis was widely prevalent, and the epidemic of aseptic meningitis that year was caused by poliovirus Type 1. In 1959, in the almost complete absence of poliovirus from the community (Duncan & Sommerville, 1960), a considerable epidemic of aseptic meningitis took place. Virological investigations showed that this epidemic in fact was made up of two main outbreaks partly superimposed upon one another. The outbreak in the early summer was caused by Coxsackie A7 virus (Grist, 1960) and that in the late summer and autumn was due to a previously unrecognized virus which has been provisionally named Frater virus and which appears to belong to the ECHO group. A brief preliminary account of Frater virus and the epidemic associated with it has already been published (Duncan, 1960*a*), and the present paper gives a more detailed description of the outbreak and of the epidemiology of the virus. The outbreak was of considerable size. Only patients admitted to hospital with clinically proven aseptic meningitis were studied, and of these 69 were found to show evidence of infection with Frater virus. The virus was isolated from 61 patients and in the other eight the evidence was purely serological.

MATERIALS AND METHODS

Stool specimens

During the 6 months from July to December 1959, the period of the Frater virus epidemic, stool specimens were examined from 165 patients with aseptic meningitis. Over the same period stools were also tested from a control group of 215 patients admitted to hospital suffering from other diseases. These were mainly non-

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infectious neurological conditions, diarrhoea, and respiratory diseases. With few exceptions the stools were collected shortly after the patient's admission to hospital.

Isolation of virus

Each stool was homogenized to give an approximately 20% suspension in phosphate-buffered saline containing penicillin, streptomycin and mycostatin. The suspension was centrifuged at 3000 r.p.m. for 30 min. and the clear supernatant stored at $-40°$ C. Every stool extract was inoculated into two tubes of rhesus monkey kidney and two tubes of human thyroid tissue culture, and another aliquot of any specimen yielding a virus in either type of tissue was later inoculated to human amnion tissue cultures. These methods are described more fully elsewhere (Duncan, 1960*b*). Tubes were examined daily for at least 14 days and if cytopathic changes were observed, the virus was passed on an average four times in human amnion tissue cultures to give high-titred seed. This was used in a neutralization test with an antiserum made by inoculating a guinea-pig with the Frater type strain of virus. 100TCD50 doses of seed were incubated for 1 hr. at room temperature with dilutions of the antiserum and 0·2 ml. vol. of the mixtures then transferred to tubes of human amnion tissue. The virus was identified as a Frater virus if it was neutralized by approximately the same dilution of serum as that neutralizing the type strain.

Sera from aseptic meningitis patients

A serum sample during the acute phase of illness and another 10–14 days later was obtained from as many patients as possible suffering from aseptic meningitis during 1959. Such paired sera were obtained from 37 of the 61 patients from whose stools Frater virus was isolated, and a single serum only was received from another 16 of them. Paired sera were also obtained from 57 patients with aseptic meningitis from whose stools either no virus was isolated or a virus other than Frater virus.

Other sera examined

In order to determine the level of antibodies to Frater virus in the general community, sera were examined from blood donors in the Glasgow area. Sera were obtained from 117 donors bled in the latter part of 1958, at least 6 months before the outbreak of aseptic meningitis, and from 100 donors bled early in 1960, a few months after it.

Paired sera from 55 patients who had aseptic meningitis or clinical poliomyelitis in 1958 were examined in an attempt to find if Frater virus had been endemic in the population before the 1959 epidemic.

Sera from 10 of the patients who suffered from aseptic meningitis due to Frater virus in 1959 were taken 1 year later to find if raised levels of antibody to the virus persisted for that time.

γ-Globulin samples

Eight samples of pooled γ-globulin prepared from blood from donors bled in Edinburgh and the east of Scotland at intervals over the years from 1951 to 1959 were tested for antibodies to Frater virus, ECHO virus Type 9, and poliovirus Type 1. This was done to obtain an estimate of the prevalence of Frater virus in the community as compared with that of other enteroviruses. γ-Globulin for use throughout Scotland is prepared in Edinburgh and therefore γ-globulin prepared from the blood of donors in other parts of the country was not available for testing.

Titration of antibodies

Antibody levels in patients' sera were determined by mixing a series of dilutions of the serum, previously inactivated for 30 min. at 56° C., with equal volumes of Frater type strain virus in a dose of 100TCD50. After 1 hr. at room temperature 0·2 ml. volumes of the mixtures were transferred to tubes of human amnion tissue culture which were incubated at 37° C. The presence or absence of cytopathic changes in the tubes was noted after control tubes containing virus unmixed with serum showed complete degeneration. The antibody titre of the serum was taken as the reciprocal of the greatest dilution neutralizing the virus. Dilutions are expressed as final dilutions of serum in serum–virus mixtures.

γ-Globulin samples were titrated in exactly the same way. The sera from aseptic meningitis patients from whose stools Frater virus was isolated, were also titrated for antibodies to Coxsackie A7 virus. A similar method was used employing a tissue-culture adapted strain of this virus (Habel & Loomis, 1957) and rhesus monkey kidney tissue cultures. Antibodies to ECHO virus Type 9 and poliovirus Type 1 in gamma globulin samples were titrated using a locally isolated strain of ECHO 9 and the Brunenders strain of poliovirus Type 1, and the tests were carried out in human amnion tissue cultures.

RESULTS

Features of the epidemic

This summary of the features of the epidemic is based on the records of the 69 patients who showed both clinical evidence of aseptic meningitis and virological evidence of infection with Frater virus. The clinical criteria of aseptic meningitis were febrile illness, meningismus, and increase in cells in the C.S.F. in the absence of a bacterial cause of meningitis. A detailed clinical description of the patients will be included in a joint clinical and laboratory account of aseptic meningitis in Scotland in 1959 which is being published elsewhere. In brief, cases associated with Frater virus most often had a sudden onset with headache, vomiting and fever. Photophobia was a common complaint, and muscle pain was present in a number of cases. Nuchal rigidity and Kernigism were detectable in most cases. The patients usually were moderately ill on admission to hospital, made a rapid recovery, and were discharged after a hospital stay of about 2 weeks. The cells in the C.S.F. were mainly lymphocytes and numbered between 18 and 500 per $mm.^3$.

The age and sex distribution are shown in Table 1. The entire range of ages was from 4 months to 39 years. Most of the patients were between 5 and 14, the age group of children attending school, but a fair number of adults were involved. There was a definite preponderance of males in the series. Most of the cases occurred in the Glasgow region, 32 in the city itself, 14 in towns in the Clyde valley to the west of the city, and nine to the east in Lanarkshire. Of the remaining 14 cases 10 were in Dundee, two in Stirlingshire, one each in Perth and Ayrshire. There were several examples of infections with Frater virus affecting different children in the same family. The epidemic began with nine cases in July 1959, rose to a peak with 23 cases in August and 25 in September, and gradually diminished through October and November with six and three cases to December with only one. There were two sporadic cases, diagnosed by serological methods only, in March and June.

Table 1. *Age and sex distribution*

	Age (years)								
Sex	0–2	3–4	5–9	10–14	15–19	20–24	25–29	30+	All ages
Male	0	8	14	15	6	3	2	0	48
Female	1	1	7	3	1	2	3	3	21
Total	1	9	21	18	7	5	5	3	69

Isolations of Frater virus

Frater virus was isolated from 61 of the 165 patients with aseptic meningitis whose stools were examined during the second half of 1959. By contrast, the virus was isolated from only 12 of the 215 patients in the control group whose stools were examined during the same period. Three of the 12 suffered from otherwise unexplained febrile illnesses, three had diarrhoea without a demonstrable bacterial cause, and the remaining six had illnesses of known cause unlikely to be associated with the virus. Frater virus was thus isolated from 37% of aseptic meningitis cases and from 6% of the control group during the same period. The greater frequency of isolation of Frater virus from aseptic meningitis patients than from control patients was found at all ages. For the age groups 0–4 years, 5–9 years, 10–19 years, and over 20 years in that order the percentage isolations were 22, 42, 48 and 33 among aseptic meningitis cases and 6, 7, 9 and 0 in control groups.

In addition to these isolations of the virus from patients with uncomplicated aseptic meningitis and from control patients, Frater virus was isolated from the stools of one patient aged 1 year suffering from clinically typical paralytic poliomyelitis from whom poliovirus was not obtained.

Frater virus was isolated from a total of 74 patients and, as it was obtained on two occasions from one patient, the virus was isolated in all from 75 stool specimens. Each of these stools was inoculated into human thyroid, human amnion, and monkey kidney tissue cultures. In human thyroid 65 isolations were made, in human amnion 58, and in monkey kidney 26.

Serological investigation of aseptic meningitis patients

Antibody titres to Frater virus were estimated in the paired acute and convalescent sera obtained from 37 of the 61 patients with aseptic meningitis from whose stools Frater virus was isolated. These titres are given in Table 2. In 23 patients there was a fourfold or greater rise in titre between the specimens, in 11, titres of 64 or more were found in both but no fourfold rise, and in three, titres

Table 2. *Antibody titres to Frater virus in acute and convalescent-phase sera of 37 aseptic meningitis patients from whom Frater virus was isolated*

Case	Day of illness acute/convalescent	Frater antibody titre acute/convalescent	Case	Day of illness acute/convalescent	Frater antibody titre acute/convalescent
1	2/13	< 16/128	20	4/13	< 16/256
2	3/12	64/256	21	3/14	< 8/16
3	6/16	256/256	22	7/20	128/128
4	5/18	64/512	23	3/13	< 16/64
5	2/13	< 16/256	24	5/28	64/128
6	4/15	64/128	25	8/19	16/128
7	2/13	< 16/32	26	10/20	256/1024
8	2/13	< 16/32	27	13/24	16/1024
9	6/19	64/64	28	3/15	32/128
10	3/17	256/64	29	2/14	128/256
11	3/10	512/2048	30	1/12	< 16/128
12	4/11	< 8/< 8	31	2/14	< 16/128
13	5/11	256/4096	32	11/22	64/256
14	3/11	128/512	33	3/14	1024/1024
15	3/13	8/512	34	4/14	64/64
16	3/13	16/1024	35	4/16	< 16/64
17	...	32/256	36	3/18	128/128
18	3/14	128/128	37	6/15	< 8/< 8
19	1/10	< 16/< 16			

..., Not accurately known.

of 16 or less were found in both. Single sera were tested from 16 others of the 61 with Frater virus in the stool. Five which were taken early in the illness had titres of 32 or less, nine had titres ranging from 64 to 4096, and two had titres of 32 or less though taken in the phase of convalescence. Antibody titres to Coxsackie A7 virus were also determined in 31 of the 37 paired sera which were tested for antibodies to Frater virus. The other six pairs could not be tested because they were completely used up in previous tests. In all except two of the pairs the titre of Coxsackie A7 antibodies in the convalescent sample was less than eight. The titres in the other two pairs were 16/32 (patient no. 15 in Table 2) and 8/16 (patient no. 18).

The titres of antibody to Frater virus were tested in paired sera from 57 patients who had aseptic meningitis during 1959 and whose stools did not yield Frater virus. Four showed fourfold rises in titre and four had high but not rising titres of 64 or more. These eight patients are included with the 61 from whom Frater virus was isolated, as cases showing definite evidence of infection with Frater virus.

Serological investigation of the patient with paralytic disease

Sera taken on the 16th and 29th days of illness from the patient with clinically typical paralytic poliomyelitis, were examined for antibodies to Frater virus, polioviruses, and Coxsackie A7 virus. The titres to each of the five viruses were the same in both specimens—Frater, 256; poliovirus Type 1, < 8; Type 2, 32; Type 3, 32; and Coxsackie A7, < 8.

Antibodies to Frater virus in the general community

The levels of antibody to Frater virus in the general population before and after the 1959 epidemic were estimated by testing sera from blood donors bled in 1958 and 1960. These results are given in Table 3. Only 9% of donors in 1958 had a detectable level of antibodies to the virus and in these the titres were low. In 1960, 25% of donors showed some level of antibodies to Frater virus, 16% showing titres higher than any found among the 1958 donors.

Table 3. *Levels of antibody to Frater virus in the sera of west of Scotland blood donors in* 1958 *and* 1960

Antibody titre ...	< 8	8	16	32	64
Percentages of 1958 donors	91	7	2	0	0
Percentages of 1960 donors	75	5	4	9	7

The interpretation of these antibody levels in donors' sera is much easier if it is known how long antibodies persist after infection by Frater virus. To obtain some estimate of this, antibody levels were measured in sera taken from 10 patients a year after their meningeal infection with the virus. At the time of convalescence the antibody titres in these patients' sera were in the range 64 to 512. One year later the titre was exactly the same in three, was twice the original level in two, and was half the original level in five. These closely similar levels a year after infection show that antibodies remain in the sera for some considerable time after infection, and the very low levels in the 1958 donors' sera would therefore suggest that the community had little experience of the virus before 1959.

Some indication of the possible prevalence of Frater virus in the Scottish community in earlier years was obtained from the examination of samples of pooled γ-globulin prepared from donors' blood. The levels of antibodies to Frater virus, ECHO virus Type 9, and poliovirus Type 1 in eight samples of γ-globulin are shown in Table 4. A fairly constant level of antibodies to each of the three viruses was found in all the samples, around 32 for Frater, 64 for ECHO 9, and 2000 for poliovirus Type 1. The titre of 128 for Frater virus antibodies in the sample of γ-globulin prepared from blood taken in the middle of the 1959 epidemic is high and, as the antibody levels of the other two viruses are no higher in this than in other samples, is probably a true reflexion of a high level of Frater antibodies in the community at this time. This along with the levels of Frater anti-

bodies in Glasgow donors in 1960 suggests that the 1959 epidemic of Frater virus meningitis was accompanied by a widespread symptomless or mild infection of the community with the virus, which apparently had previously been present in Scotland only to a limited but fairly constant extent.

Table 4. *Titres of antibodies to Frater virus, ECHO virus Type* 9, *and poliovirus Type* 1 *in γ-globulin prepared from blood of east of Scotland donors*

Year donors were bled	Titres of antibodies to Frater virus	ECHO 9	Poliovirus Type 1
1951	32	64	4000
1952	32	64	1000
1953	32	64	1000
1954	16	128	4000
1956*	16	64	2000
1957	32	128	2000
1958*	32	128	1000
1959	128	128	2000

* Sample contains γ-globulin from members of the armed forces temporarily stationed in the area as well as that from donors permanently resident in the east of Scotland.

The results of testing paired sera from patients who had aseptic meningitis or clinical poliomyelitis in 1958 for Frater antibodies did not suggest that the virus had been epidemic in that year. Only two of the 55 had raised titres, 64 without a fourfold rise in both cases. The other 53 had titres of less than 16 and most had titres under 8.

DISCUSSION

The Committee on the Enteroviruses (1957) have stated that in order to establish an aetiological connexion between an enterovirus and a particular disease the virus must be isolated from a much higher proportion of patients with the disease than from other people of the same age group in the same area at the same time. This condition has been fulfilled for Frater virus and aseptic meningitis in Scotland during 1959. A fourfold or greater rise in titre of antibodies to the virus in the sera of 23 of the 37 patients from whom the virus was isolated and from whom acute and convalescent sera were tested, affords further evidence that infection with the virus coincided with the clinical manifestations of aseptic meningitis. The very low titres of antibody to the virus in the community before the epidemic would reasonably permit the acceptance of high titres of antibody of 64 or more in convalescent sera alone as suggestive evidence of recent infection with the virus. On these grounds a further 20 patients from whom the virus was isolated may be considered to have serological evidence of recent infection with the virus. Thus of 48 patients from whom suitable serum specimens were obtained 43 showed evidence of recent infection with Frater virus. Together with the finding of very different virus isolation rates in cases and in controls this strongly suggests that Frater virus is a cause of the aseptic meningitis syndrome.

The single case of paralytic disease from whom Frater virus was isolated may have been caused by this virus. Neither polioviruses nor Coxsackie viruses were isolated from the patient's stools. Because no acute-phase serum was available the evidence cannot be complete, as it was impossible to show that a rise in poliovirus antibodies during the illness did not occur. However, titres, unchanged at the 16th and 29th days of illness, of < 8, 32 and 32 to the three types of poliovirus are all low. None is as high as might have been expected if the child, who had previously had two injections of Salk vaccine, had received an antigenic boost from one or other type of virus in the course of a recent attack of poliomyelitis. The titre of 256 suggests recent Frater virus infection. The absence of antibodies to Coxsackie A7 is strong evidence against this virus having been the cause of the illness. Moreover, the virtual absence of polioviruses from the community at the time makes the possibility all the greater that Frater virus was the cause of this child's disease. A further eight paralytic cases in Scotland in 1959 probably due to viruses other than polioviruses have been reported (Grist, 1960; Grist & Duncan, 1960).

The outbreak was probably of considerable size because this series of 69 cases includes only those who were admitted to hospital, had their C.S.F. examined, and had sera or faeces sent for virological examination. Almost certainly there were others in hospital from whom specimens were not taken for virology and many milder cases who were treated by their own doctors at home. The low level of antibodies in the population before the epidemic and the number of adult patients involved suggest that the virus was a relative newcomer to the community. The two patients in 1958 with raised levels of antibody to the virus and the constant low levels of antibody in the γ-globulin samples further suggest that the virus may previously have been endemic in the community. This interpretation can, however, be only tentative because so little is known about the extent of antigenic crossing between different enteroviruses that it is quite conceivable that these antibodies in γ-globulin could have been formed in response, not to Frater virus, but to some other member of the enterovirus group. The appearance in human sera of antibodies which react in a complement-fixation test with several ECHO viruses other than the infecting type has been described (Halonen, Rosen & Huebner, 1959). This is in general more likely to happen with complement-fixing than with neutralizing antibodies but it is quite possible that some cross-reacting antibodies may also be formed in ECHO virus infections. In hyperimmune monkey sera cross-reactions have been described between ECHO Types 1, 8 and 12. (Committee on the Enteroviruses, 1957.)

A brief description of the properties of Frater virus has already been published (Duncan, 1960*a*) and a full account of its biological and physical properties will be published later. These properties point to its belonging to the ECHO group, and serologically it has not been shown to be one of the presently recognized members of the group. It is considered therefore to be a new member of the group or possibly an antigenic variant of one of the present members.

SUMMARY

In 1959, 69 cases of aseptic meningitis were admitted to various hospitals in Scotland—all apparently due to a hitherto unrecognized virus. This agent had the characteristics of an ECHO virus but differed from the 28 ECHO viruses at present recognized. Seventy-five strains of the virus were isolated, and human thyroid and human amnion tissue cultures proved much superior to monkey kidney tissue cultures for its isolation.

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