MEMORANDUM

TO: Members of the Enterovirus Committee

FROM: Executive Secretary

In the event you may not have this article in your files, I am enclosing, for your information, a copy of the article in The Lancet - August 27, 1960, entitled "Aseptic Meningitis Associated with A Previously Unrecognized Virus."

Marvin M. Harris, Ph.D.
During 1959 a virus which could not be identified as any recognised type was isolated from the faeces of 61 patients admitted to hospital with aseptic meningitis. A further 8 patients with aseptic meningitis showed serological evidence of infection with the same virus though it was not isolated from their stools, making a total of 69 cases apparently due to the same agent. At present the virus is referred to as Frater virus after the type strain.

The cases occurred in an outbreak which reached its peak in August and September, 1959, and were distributed widely over the whole of Central Scotland, with over half in the cities of Glasgow and Dundee. Almost 60% of the patients were children of school age, a considerable number were adults, and the full range of ages was from 4 months to 39 years.

Investigations

The virological investigation of these patients consisted in the examination of stools for virus, and of sera taken at the acute and convalescent stages of illness for antibodies. Technical methods are described elsewhere. Stool extracts were tested in human-thyroid, human-amnion, and monkey-kidney tissue cultures, and virus isolates were identified as Frater virus by neutralization tests with an antiserum made by immunising a guinea-pig with the type strain of Frater virus.

Stool specimens were received from 68 of the 69 patients in the series and Frater virus was isolated from 61. Paired acute-phase and convalescent-phase sera were obtained from 46 of the 69 patients. There was a 4-fold or greater rise in titre of Frater antibodies between the two specimens in 26; in 14 other pairs titres (expressed as reciprocals of final serum dilutions) of 64 or more were found in both sera but without a 4-fold rise; and in 6 pairs titres of 16 or less were found in both. A single convalescent-phase serum only was obtained from 10 further patients in the series. Eight had titres ranging from 64 to 4096 and 2 had titres of 32 or less.

The interpretation of raised titres in single convalescent sera, and in pairs with the same level in both, is possible only if the level of antibodies to the virus in the general population is known. To determine this, 117 sera taken from blood-donors six months before the outbreak of aseptic meningitis were titrated for antibodies to Frater virus. The levels were: under 8, 91%; 8, 7%; 16, 2%. These results strongly suggest that a titre of 64 or more may be taken as evidence of recent infection with Frater virus. Thus sera from 56 of the 69 patients in the series were tested, and definite evidence obtained in 26 cases and suggestive evidence in 22 that infection with Frater virus coincided with the clinical manifestations of aseptic meningitis.

Further evidence of the connection between Frater virus and aseptic meningitis is afforded by the much greater frequency of isolation of the virus from
the stools of patients with aseptic meningitis than of control patients. During
the six months from July to December, 1959, stool specimens from 165 patients
with aseptic meningitis were examined and Frater virus isolated from 61, an
isolation-rate of 37%. During the same period Frater virus was isolated from
only 12 of 215 patients without symptoms of aseptic meningitis, an isolation-
rate of 6%. These control patients were admitted to hospital with non-infectious
neurological diseases, diarrhea, and respiratory diseases. The table (attached)
shows that the greater frequency of isolation of Frater virus from aseptic mening-
itis patients held true at all age-groups. These findings appear to fulfill the
requirements of the Committee on the Enteroviruses for the establishment of an
etiological connection between an enterovirus and a particular disease.

In addition to the 69 cases of aseptic meningitis, 1 case of paralytic
disease clinically indistinguishable from poliomyelitis was found to be associated
with Frater virus. Poliovirus was not isolated from this patient—a baby of 1
year—and there was no clear serological evidence of recent poliomyelitis infection.

The Virus

Frater virus is not yet fully identified. The characters of the virus so
far determined suggest that it belongs to the enterovirus group.

It is cytopathogenic—with changes like those of enteroviruses—in tissue
cultures of human thyroid, human amnion, and rhesus-monkey kidney, and is not
cytopathogenic in HeLa cells. From 10- to 100-fold higher titres in thyroid than
in amnion, and in amnion than in kidney, are found when the same seed is titrated
in all three tissues. When 75 stool extracts known to contain Frater virus were
tested in parallel in the three tissues, 65 isolations were made in thyroid, 58
in amnion, and 26 in kidney. The virus is non-pathogenic for suckling mice, adult
mice, and guineapigs, and for chick embryos by various routes of inoculation.

In this laboratory the virus was not neutralized by antisera, made by
immunising rabbits, to polioviruses types 1, 2 and 3, Coxsackie viruses types A7
and 9 and B1 to 5, and ECHO viruses types 1 to 9, 11 to 15, and 17 to 20. Anti-
sera to Frater virus, made by immunising guineapigs, failed to neutralize prototype
strains of polioviruses types 1, 2 and 3, Coxsackie viruses types A7 and 9
and B1 to 6, and ECHO viruses types 1 to 27. Dr. A. D. Macrae of the Central
Public Health Laboratory, Colindale, also examined the virus and reported that
it was not neutralized by his antisera to Coxsackie A9 or B1 to 5, or ECHO 1 to
27. It has now been sent to Dr. J. Melnick for further examination. Complement-
fixation tests of the virus were negative with adenovirus and herpes simplex
antisera and positive with sera known to contain neutralizing antibodies to
Frater virus. Pooled human gamma-globulin neutralized the virus to a titre of
32. It is a relatively stable virus and is ether-resistant.

Frater virus has the properties of an ECHO virus as laid down by the
Committee on the ECHO Viruses. Serologically it differs from ECHO types 1 to
27, and its growth in tissue culture is very different from that of 2060 virus
of Mogabgab and Pelon which is now designated as ECHO type 28. Frater virus
appears, therefore, to be a new ECHO virus or an antigenic variant of an existing
one.
Summary

In 1959 69 cases of aseptic meningitis in Central Scotland were found to be associated with a previously unrecognised virus, apparently of the ECHO group but differing from ECHO types 1 to 28. Human thyroid and amnion tissue cultures were considerably more efficient than monkey kidney in isolating the virus.

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ISOLATIONS OF FRATER VIRUS FROM STOOLS OF ASEPTIC MENINGITIS CASES AND CONTROL PATIENTS, JULY-DECEMBER, 1959

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>0-4</th>
<th>5-9</th>
<th>10-19</th>
<th>20+</th>
<th>All Ages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aseptic meningitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of patients</td>
<td>41</td>
<td>45</td>
<td>46</td>
<td>33</td>
<td>165</td>
</tr>
<tr>
<td>Number from whom virus isolated</td>
<td>9 (22%)</td>
<td>19 (42%)</td>
<td>22 (48%)</td>
<td>11 (33%)</td>
<td>61 (37%)</td>
</tr>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of patients</td>
<td>94</td>
<td>27</td>
<td>42</td>
<td>52</td>
<td>215</td>
</tr>
<tr>
<td>Number from whom virus isolated</td>
<td>6 (6%)</td>
<td>2 (7%)</td>
<td>4 (9%)</td>
<td>0 (0%)</td>
<td>12 (6%)</td>
</tr>
</tbody>
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