Cincinnati, Ohio
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Foundation and Dept. of
Pediatrics, Univ. of Cincinnati

Elland Ave. and Bethesda, Cincinnati, Ohio

12,400.00

A. The Natural History of Experimental Poliomyelitis Infection

III. Pattern of virus distribution in Cynomolgus monkeys infected by the cutaneous route.

IV. Role of virus and host in infection by oral route.

V. Is there inapparent or abortive poliomyelitis infection in Cynomolgus monkeys receiving the virus by the oral route?

B. The Natural History of Human Poliomyelitis.

IV. Study of a case of paralytic poliomyelitis in a laboratory worker exposed to the virus.

V. Is there winter poliomyelitis?

C. Can fish act as intermediate hosts for poliomyelitis virus?

(See attached sheets for details)
A (III). Pattern of virus distribution in Cynomolgus monkeys infected by the cutaneous route. - Our studies on human poliomyelitis revealed a pattern of virus distribution in which the alimentary tract and certain parts of the nervous system were predominantly affected, with no indication that the peripheral tissues were involved as a result of generalized dissemination or centrifugal spread of the virus. In order to be certain, however, that the human pattern is the result of infection by the alimentary tract it is necessary to know whether or not under experimental conditions in which the portal of entry is known and limited to a certain site a similar pattern is obtained only with infection by the oral route. We have already found [A(I) in press] that in rhesus monkeys infected by the sciatic route with the M.V. strain no virus is found in the alimentary tract, and it is already known from the work of a number of investigators (including our own) that the human pattern is totally different from that found in rhesus monkeys infected by the nasal route. Our work in 1941 on Cynomolgus monkeys infected by the oral route [A(II)] not only revealed that the human pattern of virus distribution is reproduced in these animals but also disclosed additional facts whose significance is still to be interpreted. It becomes essential, therefore, to determine in the same host whether or not infection by a totally different route such as the cutaneous (a route of infection occasionally considered in human beings) can in any way simulate the pattern found in human beings or infected Cynomolgus monkeys. We have already found in preliminary tests that virus does not occur in the stools of Cynomolgus infected with the Per. strain (early human strain used in our feeding experiments) by the intracerebral route, and the
results of the proposed investigation will go far in establishing the
presence of virus in the stools as an index to primary infection of the
tissues of the alimentary tract.

The plan of the investigation is to inject our Per. virus intra-
cutaneously into about 20 Cynomolgus monkeys, and to search for the virus
in the following specimens obtained from 5 paralyzed animals:

<table>
<thead>
<tr>
<th><strong>Alimentary tract.</strong></th>
<th>1. Oropharyngeal washings</th>
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<tbody>
<tr>
<td></td>
<td>2. Buccal tissue</td>
</tr>
<tr>
<td></td>
<td>3. Tongue</td>
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<tr>
<td></td>
<td>4. Tonsils</td>
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<td>5. Pharyngeal wall</td>
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<td></td>
<td>6. Small intestine - washed wall</td>
</tr>
<tr>
<td></td>
<td>7. &quot; &quot; &quot; contents</td>
</tr>
<tr>
<td></td>
<td>8. Large &quot; &quot; washed wall</td>
</tr>
<tr>
<td></td>
<td>9. &quot; &quot; &quot; contents</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Blood and viscera.</strong></th>
<th>10. Whole blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11. Lungs</td>
</tr>
<tr>
<td></td>
<td>12. Liver, spleen, and kidneys</td>
</tr>
<tr>
<td></td>
<td>13. Adrenals</td>
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<td></td>
<td>14. Urinary bladder</td>
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<table>
<thead>
<tr>
<th><strong>Lymph nodes.</strong></th>
<th>15. Axillary</th>
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<tbody>
<tr>
<td></td>
<td>16. Inguinal</td>
</tr>
<tr>
<td></td>
<td>17. Mesenteric</td>
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<td>18. Cervical</td>
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</tbody>
</table>
Collateral sympathetic ganglia. - 

19. Abdominal (celiac)
20. Superior cervical sympathetic

Central nervous system. -

21. Cerebrospinal fluid
22. Cord and medulla
23. Mesencephalon
24. Diencephalon
25. Motor cortex
26. Anterior frontal and occipital cortex
27. Anterior perforated substance
28. Olfactory bulbs

Other tissues. -

29. Nasal mucosa
30. Salivary glands
31. Site of inoculation (skin)

Minimum number of Cynomolgus monkeys required for this study ....... 185

A(IV). Role of virus and host in infection by oral route. - It is well known that all attempts to produce poliomyelitis in Rhesus monkeys by feeding them large amounts of virus (practically all of the reported work was done with the M.V. strain) have been unsuccessful, while Cynomolgus monkeys are now believed to differ in this respect from the Rhesus in some unknown way. In our work this year 6 (and possibly 7) out of 15 Cynomolgus which were fed our Per. virus developed poliomyelitis. When 5 Cynomolgus were fed similar quantities of "M.V." virus all remained
well. It is to be recalled that in the earlier negative feeding experi-
ments on Cynomolgi reported by Clark et al., and Flexner, M.V. virus was
used. The question which naturally arises is this: to what extent is
successful infection by the oral route determined by the host, the strain
of virus, or both?

Plan of investigation: a) 20 Cynomolgi + M.V. virus

20 " + Per. "

20 Rhesus + Per. "

b) Determine number of minimal infective cerebral
doses in M.V. and Per. strains of virus used in
these tests.

Minimum number of monkeys required for this study (including those needed
to supply virus for feeding). = ..................... 100

A(V). Is there inapparent or abortive poliomyelitis infection in Cynomolgus mon-
keys receiving the virus by the oral route? = 50 per cent or more of the
Cynomolgus monkeys which are fed a suitable strain of virus exhibit no
signs of disease, and our examination of the central nervous system of 8 such
animals has revealed no lesions. The purpose of this investigation is to
determine whether they might have an infection which is limited to the ali-
mentary tract. Cynomolgus used in part A(IV) will supply material for this
study.

Plan of investigation: a) Test stools of 12 monkeys (without apparent signs
of disease) obtained at a time when their mates
are succumbing with paralysis

b) Same for osopharyngeal washings

c) Sacrifice 12 monkeys about 6 weeks after feeding,
test colon contents for virus and examine
central nervous system histologically.

Minimum number of monkeys required for this study............. 36

B(IV). Study of a case of paralytic poliomyelitis in a laboratory worker exposed to the virus. - The circumstances of this unusual case of poliomyelitis have already been reported and some of the proposed studies have already been carried out. Poliomyelitis virus has been isolated 3 times from the enema return obtained from this patient on the 2nd, 4th, and 6th days of paralysis. The specimen obtained 1 month after onset has been tested and found negative; two specimens obtained 2 months after onset still have to be tested. A good strain of virus has been established in Cynomolgus monkeys and the following questions are still to be answered.

1) Is the virus isolated from this patient immunologically identical or different from the Per. virus to which she was exposed?

2) Did this patient’s serum at the onset of paralysis contain neutralizing antibodies against a standard strain of poliomyelitis virus (M.V.), the Per. strain to which she was exposed, and the B.J. strain which was isolated from her. Serum obtained 2 months after onset also to be tested.

Minimum number of monkeys for this study ..................... 40

B(V). Is there winter poliomyelitis? - The purpose of this investigation is to obtain data which would throw some light on the following questions which are of great importance to the ultimate understanding of the epidemiology of poliomyelitis.
1) Is total poliomyelitis (inapparent + abortive + nonparalytic + paralytic) predominantly a summer disease, or does it also occur frequently in the winter but more often in the nonparalytic form?

2) Are cases diagnosed as poliomyelitis in the winter actually some other disease [e.g., infectious polyneuritis (neuronitis, Guillain-Barré syndrome, etc.)]?

To the best of my knowledge no virus has been isolated from cases diagnosed as poliomyelitis in the winter. Trask, Paul, and Vignec (1940) reported tests on stools from one Winter case and 6 Spring cases—all with negative results. Our plan is to establish contact with a number of contagious disease and Children's hospitals (as well as in cooperation with Dr. Don W. Gudakunst who receives reports from health officers) and have them send us stools or enema returns on the following types of cases, occurring in Northern States between December, 22 and March, 22.

   a) those diagnosed as paralytic poliomyelitis
   b) those presenting the syndrome of fever, vomiting, headache, stiff neck or spine, with pleocytosis—i.e. those which would have been diagnosed as nonparalytic poliomyelitis during an epidemic of the disease.

We propose to test a total of 50 specimens, 25 from patients in each category. The incidence of positive isolation of virus from patients with poliomyelitis is high enough with present techniques to make this a satisfactory sample. This study will undoubtedly require more than one winter for completion.

Minimum number of monkeys ...................... 60
G. Can fish act as intermediate hosts for poliomyelitis virus? - While we were carrying out certain studies on the Cleveland epidemic (1941) my associate Dr. Robert Ward pointed out the desirability of searching for the virus in fish caught in Lake Erie near the outlet of the sewage plant and adjacent to a beach which was significantly connected with a number of cases of poliomyelitis. With the agreement and aid of the Health Department, 37 fish were caught and their viscera and intestines preserved in the frozen state (solid CO₂). These specimens will be tested as soon as Cynomolgus monkeys are again available.

Regardless of the outcome of these tests, however, we propose to determine under experimental conditions whether or not fish may act as intermediate hosts for the virus. Scavenger fish are known to exist in polluted streams and on many occasions epidemics have been believed to occur in relation to rivers or streams, the spread occurring upstream as well as downstream.

Plan of investigation. - About 30 carp will be kept in a tank contaminated with sufficient virus (human and recent human origin) to be just detectable by subinoculation. The fish will be left in the tank for one week and then removed to clean, flowing water. The intestines and viscera of 3 fish will be pooled and tested for virus (by inoculation and feeding in 2 Cynomolgus) during the following periods.

1) After 2 days' sojourn in contaminated tank
2) " 7 " " " " "
3) One week after removal from " "
4) 2 weeks " " " " "
5) 4 weeks after removal from contaminated tank
6) 3 months
7) 6

Minimum number of monkeys (including those required for supplying virus to contaminate tank) ................... 30
ADDENDUM TO APPLICATION FOR GRANT

In view of the unexpected shortage in cynomolgus monkeys (the animals boarded for us by the dealer since the spring of 1941 were sold by the dealer to others without notifying us), the work covered by the 1941 grant will not be completed in 1941. It is impossible at this date (October 1, 1941) to estimate how much of it will be finished by December 31, 1941.

At the present time we still have the following to complete:

A. (A-2) about 40 to 50 monkeys required to complete proposed studies on orally infected cynomolgus.

B. (B-3) the required human autopsy material has been collected and about 170 monkeys will be required for testing.

C. (C-1) flies and fish have been collected in urban outbreaks and about 20 to 25 monkeys will be required for tests.

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