November 14, 1957

Dr. Albert Sabin
Children's Hospital Research Foundation
Elland Avenue and Bethesda
Cincinnati, Ohio

Dear Dr. Sabin:

I am enclosing a copy of our "Diarrhea and Echo 18" manuscript, which I thought might be of interest to you. Because we planned to send it to the Journal of the American Medical Association, I left out most of the technical material.

I greatly appreciated the help you gave me in our attempts to identify the agent. Any comments and suggestions you might care to make would be gratefully received.

Copies of the figures will be available in a week or so, and I will send these to you then.

With best regards,

Sincerely,

Heinz F. Eichenwald, M. D.

hfe.ek
enc.
EPIDEMIC DIARRHEA IN PREMATURE AND OLDER INFANTS CAUSED BY ECHO VIRUS TYPE 18*

by

Heinz F. Eichenwald, M. D., Alexander Abadio, B. A., Albert M. Arky, M. D., & Alan P. Hartman, M. D.

from

Department of Pediatrics
The New York Hospital-Cornell Medical Center

* This study was supported by Research Grant E-998 of the Institute of Allergy and Infectious Diseases, National Institutes of Health.
Despite the ubiquity of diarrheal disease in clinical practice, failure to identify a specific bacterial or parasitic cause is the usual happening. Even in those underdeveloped areas in the world where bacterial diarrheas are highly endemic, no etiologic agents can be found in approximately 65% of the cases. (1) It has therefore been generally assumed that a number of viruses may be responsible for a majority of these illnesses.

Impressive, although indirect, evidence does exist that various different, poorly defined viral agents may be a cause of a number of clinical syndromes characterized by diarrhea (2). With some of these agents, diarrhea has been produced in human volunteers by inhalation (3) or on oral inoculation (4) and some have been passed serially in humans (5), cats (5) calves (6) and guinea pig cornea (7). Nevertheless, according to Higgins (8), no complete evidence has yet been adducted in proof of a viral agent in diarrheal disease, largely because such an agent has not been propagated in suitable laboratory animals or in tissue culture.

The present report deals with 2 related outbreaks of diarrhea among infants from whom it was possible to isolate a virus clearly responsible for the illness.

During the summer of 1956, an outbreak of diarrhea occurred among infants in the premature nursery of The New York Hospital. The inmates of this particular nursery had been studied closely for the preceding six months as part of a general investigation
into the effect of viral infection on premature infants. In the four week period preceding the outbreak, no viruses had been detected in this population by weekly throat and rectal swabs.

**Epidemiologic Investigation**

As shown in Figure I, the first case of diarrhea occurred on July 29; two days later, a second infant became ill. The outbreak then built up gradually and ended abruptly within one week of its onset. Twelve of 21 infants in the nursery were affected.

The epidemiologic features of the outbreak are of interest. The premature nursery consists of 4 separate units, opening into a common corridor. The nursery and medical personnel is the same for all 4 units. Standards of cleanliness and of nursing and medical care are very high.

Although all infants are individually isolated, cases of diarrhea occurred in all 4 rooms and appeared distributed at random. Infants in incubators were affected as frequently as those in bassinets. As demonstrated in figures 2 and 3, age and weight did not affect the attack rate. The ages of the affected infants ranged from 6 to 46 days and the weights from 1000 to 2200 gm.

A detailed epidemiologic survey failed to suggest any non-infectious causes for the diarrhea. There was no evidence of bacteriologic or chemical contamination of formula or water, nor could antibiotics or vitamins be shown to be responsible.

**Description of Illness**

Clinically, the disease was not severe. None of the sick infants showed significant temperature elevations or hypothermia.
Two babies developed moderate abdominal distension, six others appeared lethargic or listless. Physical examinations were otherwise unremarkable; no mucous membrane lesions were noted.

Generally, the diarrhea persisted from one to five days, with a mean duration of 3 days. Most of the infants passed five or six fairly large, watery, greenish stools each day; on occasion these were expelled explosively. Mucus or pus cells were not present, but in two of the infants small flecks of bright blood were noted on a single occasion.

The illness was treated by a reduction in caloric content of feedings, supplemented by the parenteral administration of water and electrolytes whenever indicated. Following subsidence of the diarrhea, the babies were gradually returned to their normal formulas, without recurrence of disease.

**Microbiologic Investigations**

Because of the obscure origin of the diarrhea, intensive microbiologic investigations were conducted. Rectal, and nose and throat swabs were collected on at least two separate occasions from all infants in the nursery, irrespective of whether they were sick or well. Additional specimens were collected from sick infants as soon as the onset of diarrhea was noted. Acute and convalescent blood samples were obtained 3 weeks apart from sick infants, and a single blood specimen was collected 2 to 4 weeks after termination of the outbreak from those who had remained well. Throat and stool cultures and blood samples were obtained from the nursing and medical personnel, all of whom were in good health at the time.
The rectal swabs from infants were examined for the presence of bacterial agents using the methods described by Edwards and Ewing (9). No salmonella or shigella were found. E. coli were isolated from swabs of 7 of the 12 sick infants, and from a similar proportion of those who remained well. These strains were tested with typing sera* against the potentially pathogenic sero-types (0-26, 0-55, 0-86, 0-111, 0-119, 0-125, 0-126, 0-127), but no agglutination was obtained.

Throat swabs were plated using standard bacteriologic methods; again no differences between the flora of the sick and well infants were observed.

Stool and throat cultures from the staff were unrevealing, no salmonella or shigella were found in the stools, and the respiratory tract flora were unremarkable.

Investigations aimed at detecting the presence of viral agents were carried out concurrently.

Each specimen from nose, throat and rectum was suspended in a balanced salt solution containing penicillin, streptomycin, and mycostatin. After further clarification of stool suspensions by centrifugation, aliquots of all materials were inoculated directly into monolayer stationery cultures of both human amnion and monkey kidney tissue. The cultures were observed for possible

* Kindly supplied by Dr. W. H. Ewing of the Communicable Disease Center, U. S. Public Health Service.
cytopathogenic effect for 7 to 14 days; those which failed to show
definite tissue changes were sub-cultured through 3 successive
passages before being considered negative.

No cytopathogenic effects appeared in any human amnion cultures
inoculated with stool specimens from infants, but in monkey kidney
tissue, stools from 10 of 12 sick infants showed an agent by the sixth
day of the first passage. The cytopathogenic effect produced was
similar to that observed with poliomyelitis viruses. No agent was
isolated from the rectal swabs of well infants.

On the other hand, stool cultures from 5 members of the nurs-
ing staff revealed agents with a poliomyelitis-like tissue action;
2 of these produced cytopathogenic changes in kidney tissue only,
while 3 others grew out simultaneously in the first amnion passage.

Throat cultures from infants and staff failed to reveal any
agents in these tissues. These results are summarized in Table 1.

In order to determine the relationship of the 15 newly iso-
lated gastrointestinal agents to each other, antiserum was prepared
in rabbits against the virus isolated from one infant, designated
the B1 strain. All agents were then tested against the B1 antiserum
by standard tissue-culture neutralization methods. The results are
shown on Table 2. The B1 antiserum neutralized in approximately equal
dilutions not only the homologous virus, but all other strains iso-
lated from infants and 2 of the 5 obtained from the nursing staff.
Cross-neutralization tests subsequently confirmed the fact that these
strains are immunologically identical, except for S2, S4, S5. The
12 identical strains will be referred to collectively as the N5 virus.
S₂ was subsequently identified as poliomyelitis virus type I, the identity of S₄ and S₅ has not been determined.

The blood specimens obtained were tested for the presence of neutralizing antibodies to the N₅ virus. Because of the small amounts of blood obtainable from premature infants, only serum dilution of 1 to 2 and 1 to 16 were tested against 50 tissue culture doses of virus. All twelve infants with diarrhea showed a rise in antibody to 1:16 or above, none of the well infants had antibodies of 1:2 or higher. Both nurses from whom the N₅ agent was recovered showed antibody titers of 1:8 in their acute phase specimen, which had risen to 1:64 two weeks later.

Table 3 summarizes the virological and serological data obtained. It is apparent that all infants with diarrhea showed evidence of infection with N₅ virus, while none of the well infants did. A Chi-square test showed that this difference is highly significant (p > 0.001).

Two weeks following the end of this outbreak, tissue cultures of stool specimens collected from all infants on the premature nursery once again failed to show the presence of a virus.

**Outbreak of Diarrhea Among Fullterm and Older Infants**

Four days after the termination of the premature infant outbreak, diarrhea was noted among patients on a sick infant ward in the same hospital. Within a four day period, five babies became ill with signs and symptoms similar to but somewhat more severe than those shown by the prematures. The age of these patients ranged from one week to two months and their weight from 3300 gm. to 6200 gm.
Bacteriological studies on stools and respiratory tract secretions were unremarkable, but using monkey kidney tissue cultures, cytopathogenic agents subsequently shown to be identical to the N<sub>5</sub> virus were recovered from the stools of all five infants with diarrhea. No virus was found in ten well infants on the same ward. Paired acute and convalescent serum specimens from the sick patients showed four-fold rises in antibody titer to N<sub>5</sub> virus.

An investigation into the source of this second outbreak revealed that one of the two nurses from whom N<sub>5</sub> virus had been recovered in the survey of the Premature Nursery Staff had supervised the sick infants floor three days prior to the outbreak of diarrhea in the first baby. She recalled having handled at least two of the infants who subsequently became ill.

**Identification of N<sub>5</sub> Virus**

Identification of the N<sub>5</sub> virus was attempted. The tissue affinity and the type of cytopathogenic effect obtained suggested that this agent belongs to the enteric cytopathogenic human orphan (ECHO) group. This impression is supported by the findings that it is non-pathogenic to suckling mice, and therefore probably not a Coxsackie agent; antisera to the three types of poliomyelitis virus do not neutralize it, and it does not possess the complement-fixing antigen common to the adenoviruses.
The virus was tested against antisera prepared with the 14 prototyped ECHO agents recognized at the time, and against antisera** to five similar enteric viruses more recently isolated by Ramos-Alvarez in A. B. Sabin's laboratory (9). Comprehensive neutralization and cross-neutralization tests revealed the immunologic identity of the N₅ virus and the D-3 prototype of Ramos-Alvarez and Sabin. The D-3 virus has subsequently been accepted as ECHO Type 18 by the Committee on the ECHO viruses.

Discussion

Following the advent of tissue culture methods, virology entered an era similar to that faced by bacteriologists more than 60 years ago. The application of new tools to the search for microbes led to the discovery of innumerable new agents. At the present time, the chief problem in virology probably is not so much the isolation of new agents, but rather the determination of the role that already known agents play in the causation of disease.

The simple, temporal association of commonly occurring viruses with some disease entity cannot be accepted as proof that this particular agent is responsible for the observed illness. Before a virus can be called the "cause" of any disease, much additional evidence must be obtained, most important of which is perhaps that available through epidemiologic methods.

**Antisera and virus prototypes were kindly supplied by Dr. Albert B. Sabin, whose generous advice on carrying out some of the identification procedures is also gratefully acknowledged.
The evidence that ECHO 18 is etiologically related to diarrhea in infants may be summarized as follows:

1. Evidence of infection with the virus was found in every infant with diarrhea, but not in those who remained well. These differences showed a high order of significance by statistical test, indicating that chance association was unlikely.

2. A significant rise in antibodies to ECHO 18 occurred in all infants with the disease and was temporally related to the course of the illness.

3. ECHO 18 was found among infants in the premature nursery only during the course of the outbreak; the virus was not present before or after the epidemic had run its course.

4. A second outbreak occurred among other infants in a different part of the same hospital following exposure to a nurse known to be excreting ECHO 18. Again, a definite association between the presence of the virus and the appearance of diarrhea was demonstrated.

5. ECHO 18, in our experience, is not a commonly occurring virus. We have been unable to isolate it from nearly 100 stool specimens obtained from young children with various types of illnesses.

The conclusion that ECHO 18 caused the two separate but related outbreaks of diarrhea is thus strongly supported by epidemiologic, immunologic and statistical analysis. It is perhaps fortunate that in this instance ECHO 18 had an "all or none" effect on its hosts; when the virus was present, diarrhea
invariably occurred. Had the agent caused any appreciable amount of subclinical illness, then the statistical approach might not have provided an answer.

It is obvious that absolutely incontrovertible evidence in favor of the association between the virus and diarrhea might be obtained by suitably designed observations with human volunteers. The possibility must then be considered that this agent may on occasion produce an entirely different clinical syndrome in older children and adults, thus studies of this type might not be worth the risk.

Of some clinical interest are the observations concerning the course of the illness in premature infants. Considering their relatively poor response to bacterial infection, these infants withstood their illness surprisingly well. Not only was the disease relatively mild, but a prompt antibody response occurred, sufficient to control the infection in a short time.

Summary

Evidence is presented linking ECHO Virus Type 18 to 2 separate but closely related outbreaks of diarrhea in premature and older full-term infants. This represents the first instance in which a virus isolatable by laboratory methods has been shown to be a cause of diarrhea.
REFERENCES


### RESULTS OF VIRUS ISOLATION PROCEDURES

#### INFANTS AND STAFF

<table>
<thead>
<tr>
<th></th>
<th>NUMBER</th>
<th>NUMBER WITH VIRUS IN STOOL</th>
<th>NUMBER WITH VIRUS IN NOSE OR THROAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>INFANTS WITH DIARRHEA</td>
<td>12</td>
<td>10*</td>
<td>0</td>
</tr>
<tr>
<td>INFANTS WITHOUT DIARRHEA</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>STAFF (NURSES, DOCTORS, ETC.)</td>
<td>26</td>
<td>5**</td>
<td>0</td>
</tr>
</tbody>
</table>

* ALL ISOLATIONS IN MONKEY KIDNEY TISSUE

** TWO STRAINS RECOVERED IN MONKEY KIDNEY TISSUE ONLY, THREE IN BOTH MONKEY KIDNEY AND HUMAN AMNION
<table>
<thead>
<tr>
<th>VIRUS STRAIN</th>
<th>CYTOPATHOGENIC ACTION IN MONKEY KIDNEY CULTURE</th>
<th>HUMAN AMNION CULTURE</th>
<th>NEUTRALIZED BY RABBIT ANTISERUM TO B-1 STRAIN</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FROM INFANTS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-1</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>B-2</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>B-3</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>B-4</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>B-5</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>B-6</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>B-7</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>B-8</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>B-9</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>B-10</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td><strong>FROM STAFF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-1</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>S-2</td>
<td>YES</td>
<td>YES</td>
<td>NO**</td>
</tr>
<tr>
<td>S-3</td>
<td>YES</td>
<td>YES</td>
<td>NO**</td>
</tr>
<tr>
<td>S-4</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>S-5</td>
<td>YES</td>
<td>YES</td>
<td>NO**</td>
</tr>
</tbody>
</table>

* NEUTRALIZED BY POLIOMYELITIS TYPE 1 ANTISERUM

** UNIDENTIFIED AGENTS
### Table 3

**SUMMARY OF VIRUS ISOLATION AND SEROLOGIC STUDIES IN PREMATURE INFANTS**

<table>
<thead>
<tr>
<th></th>
<th>Total Number</th>
<th>Number with Virus Isolated from Rectal Swabs</th>
<th>Number with Fourfold Rise in Antibody to N-5 Virus</th>
<th>Percent with Either Antibody Rise or Virus Isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants with Diarrhea</td>
<td>12</td>
<td>10</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>Infants without Diarrhea</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>