

Association of Echo Virus Type 6 with Aseptic Meningitis.* (22630)

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A number of viruses shown to be outside of the poliomyelitis, Coxsackie, and adenovirus groups, have been isolated from stool specimens of cases diagnosed as nonparalytic poliomyelitis, as well as from specimens obtained from normal children. Labeled *echo* (*enteric cytopathogenic human orphan*) *viruses*, they have been found to fall into at least 14 distinct antigenic types(1). The etiological relationships of the echo viruses to disease remain to be established. Evidence is presented here to show that one virus of the group, Type 6, seems capable of producing the aseptic meningitis syndrome. Suspicion of an etiological relationship was aroused when the virus was first recognized in 1954, for the first 3 strains isolated came from 3 cases of aseptic meningitis in Providence(2). We have found it only once among 212 enteric viruses isolated during a longitudinal study of *healthy* children(3,4).

Methods. Viruses were isolated and identified in monkey kidney cultures, and antibody determinations made, by methods already described(4). Specimens were collected as widely as possible in 1955 from patients in Connecticut hospitalized with the diagnosis of poliomyelitis, as a part of a study on the evaluation of poliomyelitis vaccine. They included a stool and serum sample obtained from each patient as soon after onset as possible and a second serum a few weeks later. Complement-fixing (CF) and neutralizing

(Nt) antibodies against the polioviruses were determined on all sera collected. Nt antibody titrations were carried out using 4-fold dilutions of serum against 100 TCD₅₀ of virus.

Results. Virus isolations. Fecal specimens were collected and tested from 58 paralytic and 137 nonparalytic patients. All but 4 of the specimens considered here were collected within 3 weeks of onset. In the nonvaccinated group of 144 cases, the ratio of paralytic to nonparalytic cases was 1 to 1.8, similar to that of previous years in this area, but markedly different from the ratio of 1 to 7.5 among the 51 vaccinated cases.

The results of the tests (Table I), may be summarized as follows: Poliovirus, chiefly type 1, was isolated from 39 of 52 nonvaccinated paralytic cases (75% positive) and from 3 of 6 vaccinated paralytic cases. In contrast, poliovirus was isolated in only 23 of 92 nonvaccinated nonparalytic cases (25% positive), and in only 5 of 45 vaccinated, nonparalytic cases (11% positive). There was no significant difference in the isolation of echo viruses or Coxsackie viruses in the vaccinated and nonvaccinated groups.

Of 45 viruses isolated from paralytic patients, 42 were poliovirus (39 type 1). Type 6 echo virus was isolated from a paralyzed patient, who probably had a mixed polio-echo infection. Even though poliovirus was not isolated, the Nt and CF antibody responses were those characteristic of poliomyelitis infection(5). Another paralyzed patient also was considered to have had a mixed infection, polio and Coxsackie A9. Although poliovirus was not isolated from this patient, it was isolated from his brother, and in addition, the patient gave Nt and CF antibody responses of poliomyelitis. A third case yielded only Coxsackie B4 virus, but as only a single acute phase specimen was obtained, the serum studies are incomplete.

Of 69 enteric viruses isolated from the nonparalytic cases, 28 (41%) were polioviruses

* Aided by a grant from the National Foundation for Infantile Paralysis, Inc. and by a contract with the Communicable Disease Center, U. S. Public Health Service. We wish to acknowledge the participation of the Connecticut State Department of Health and particularly of Dr. Mila Rindge, who not only informed us of many of these cases but also assisted in the collection of specimens. Material was also made available from Providence, R. I., through the kindness of Dr. Joseph Smith, Supt. of Health.

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TABLE I. Viral Isolations Obtained in Specimens Submitted from Cases of "Poliomyelitis" in Conn. during 1955.

	Nonvaccinated		Vaccinated		Total	
	Paralytic	Non-paralytic	Paralytic	Non-paralytic	Paralytic	Non-paralytic
Total cases studied (specimens collected within 3 wk of onset)*	52	92	6	45	58	137
No. yielding virus	42	45	3	24	45	69
Poliovirus Type 1	36	22	3	5	39	27
2	2	0	0	0	2	0
3	1	1	0	0	1	1
Echo virus Types 1, 2, 3, 4, 5	0	0	0	0	0	0
6	1†	9	0	12	1†	21
7, 8, 9, 10, 11, 12, 13, 14	0	0	0	0	0	0
Not typed	0	1	0	3	0	4
Coxsackie virus Type A9	1‡	1	0	0	1‡	1
B1	0	0	0	0	0	0
B2	0	5	0	1	0	6
B3	0	2	0	0	0	2
B4	1§	4	0	3	1§	7
B5	0	0	0	0	0	0

* Stool specimens from 10 paralytic cases collected between 22 and 115 days yielded 3 positives: one, a type 1 poliovirus isolated from a nonvaccinated case 50 days after onset; one, a type 2 poliovirus isolated from a nonvaccinated case 35 days after onset; and one, a type 1 poliovirus isolated from a vaccinated case 29 days after onset. Of 8 stools from nonparalytic cases collected between 22 and 45 days, one nonvaccinated case yielded type 1 poliovirus 22 days after onset. Of the late specimens, only these 4 are included in the results above.

† Probably mixed polio-echo type 6 infection. Although poliovirus was not isolated from the patient, CF and Nt antibody responses were characteristic of a polio-infection.

‡ Probably mixed polio-Coxsackie A9 infection. Although poliovirus was not isolated from the patient, it was isolated from his brother. Furthermore, the patient gave a CF and Nt antibody response characteristic of polio-infection.

§ Only Coxsackie B4 virus was isolated. An acute serum, the only one collected, failed to show polio-CF antibodies.

(27 type 1), 25 (36%) were echo viruses, and 16 (23%) were Coxsackie viruses. Of the Coxsackie viruses, 6 were B2, 2 were B3, 7 were B4, and only 1 was A9.‡ Of the echo viruses, 21 were type 6, and 4 were unrelated to any of the 14 recognized types. Thus with the exception of poliovirus type 1, echo type 6 was encountered in this series more than any other virus.

When the nonparalytic cases are separated by age groups (Table II), the results may be summarized as follows: In the 5-10 age group, the only group vaccinated in 1955 in Connecticut, poliovirus was isolated from 5 of 45 vaccinated cases (11% positive), all type 1,

and 6 of 22 nonvaccinated cases (27% positive). In the same age group, type 6 echo virus was isolated from 12 of 45 vaccinated cases (27% positive) and 6 of 22 nonvaccinated cases (27% positive). Coxsackie viruses were isolated from 4 of the vaccinated cases (9% positive) and 2 of the nonvaccinated cases (9% positive). Poliovirus was isolated from all of the age groups studied (with the exception of the 5 patients over 30 years). Similarly, Coxsackie viruses were found in all the age groups. However, the type 6 echo viruses were all isolated from children aged 5-13 years. The paralytic cases are not considered by age groups because most of the cases were positive and yielded poliovirus.

Serological findings. Multiple collections of serum were taken from the echo type 6 excretors, and Nt antibody titers were determined against the patient's own virus as well as against the D'Amori strain, the prototype

‡ In early spring 1956, however, when only 3 viruses were isolated in Conn. from the first 9 cases of aseptic meningitis seen, all 3 proved to be Coxsackie A9. This virus was isolated again in August from another patient with aseptic meningitis, Type A9 being obtained both from the stools and from the cerebrospinal fluid.

TABLE II. Viral Isolations from Nonparalytic Cases in Relation to Age.

Age groups	1-4	5-10 Non- vaccinated	5-10 Vaccinated	11-20	21-30	>30	Total
Cases studied	11	22	45	25	22	5	130
Poliovirus isolations	5	6	5	8	4	0	28
Echo-6 "	0	6*	12	3	0	0	21*
Coxsackie "	2	2	4	4	2	2	16

* Not included in this table are 3 additional cases from Providence, R.I., studied in 1954, which yielded echo-6 virus.

strain of echo type 6 virus. As similar homologous and homotypic titers were found, hereafter only the titers against the prototype strain are considered. The results of the tests, as shown in Table III, may be summarized as follows: Echo-6 excretors produced homotypic antibodies more slowly, and at lower titer than the poliovirus excretors produced homotypic polio-antibodies. Polio-antibody titers were essentially unaffected in excretors of echo-6 virus.

Specific examples giving vaccination status, poliovirus Nt and CF, and echo-6 Nt antibody titers are listed in Table IV. All of these cases were excreting echo-6, the stool being collected at the time the first serum was drawn. The first two patients listed were not vaccinated. Each demonstrated poliovirus Nt antibodies to a single poliovirus type, with-

out a change in titer being noted during the course of the disease. As the CF tests were negative, the Nt antibodies are assumed to represent a past subclinical polio-infection, for no history of poliomyelitis was obtained. Other patients failed to have any detectable antibodies against any of the poliovirus types. Two patients listed had been vaccinated, each having received 2 doses of formalinized vaccine, about 2 months prior to infection with the echo virus, which probably accounts for their elevated levels of poliomyelitis Nt antibodies. Again the polio-antibodies remained constant during the course of the illness. In contrast all 4 patients demonstrated a rise in antibodies to echo-6 virus, as did all of the echo-6 excretors from whom adequately spaced serum specimens were collected.

Signs and symptoms. Clinical data were obtained from the patients studied. Only the results of the study of aseptic meningitis cases associated with echo-6 virus, the Coxsackie group B viruses, and poliovirus-1 are shown in Table V, where the cell counts of the cerebrospinal fluids are also noted. It may be seen that the symptomatology among the 3 groups was not significantly different. Fever, malaise, headache and stiff neck were most commonly encountered. Stiff back, back pains, muscle pain, vomiting and sore throat were occasionally encountered, and abdominal pains and constipation were infrequently found. An increased number of cells in the spinal fluid was noted in all excretors of echo-6 virus in which a lumbar puncture was performed. The number of cells ranged from 12 to over 300 with about 75 cells per c. mm. being the average count. Eight of 11 Coxsackie excretors showed increased cells in the spinal fluid. Twenty of 23 poliovirus-1 excretors showed increased cells in the spinal

TABLE III. Neutralizing Antibody Titers* in Aseptic Meningitis Patients (Age 5-13 Years) Infected with Echo Virus Type 6 or Poliovirus Type 1.

	Acute, 1-7 days†	Convales- cent, over 7 days†
Echo-6 donors		
Nonvaccinated sera tested	9 pairs	
Echo-6 serum titers	<10	100
Polio-1 " "	50	50
Vaccinated sera tested	11 pairs	
Echo-6 serum titers	<10	50
Polio-1 " "	256	256
Polio-1 donors		
Nonvaccinated sera tested	4 pairs	
Polio-1 serum titers	150	200
Vaccinated sera tested	3 pairs	
Polio-1 serum titers	100	200

* Geometric mean.

† Interval in days between onset of illness and collection of serum. Only paired sera in which the acute specimen was drawn during first week are included.

TABLE IV. Titers of Polio-Types I, II and III and Echo-6 Antibodies in Paired Sera of Aseptic Meningitis Patients Excreting Echo-6 Virus.

Age	Days after onset	Poliovirus antibody titer						Echo-6 neutralizing antibody titer
		Neutralization I	Neutralization II	Neutralization III	Complement fixation I	Complement fixation II	Complement fixation III	
6 (nonvaccinated)	3	<4	<4	64	0	0	0	<10
	45	<4	<4	64	0	0	0	100
6 "	1	256	<4	<4	0	0	0	<10
	31	256	<4	<4	0	0	0	100
8 (vaccinated)	5	256	1024	1024	0	0	0	<10
	12	256	1024	1024	0	0	0	50
6 "	4	64	1024	16	0	4	0	10
	18	64	1024	16	0	4	0	100

fluid. Here again, by use of the cell count, no significant differences were present between the 3 groups.

Discussion. Even though it has long been known that aseptic meningitis may be caused by a variety of agents(6,7), a large proportion of cases are still of unknown etiology. With the increasing use of tissue culture techniques, a number of new agents have been isolated from patients manifesting this syndrome. This might well be merely a reflection of the type of case studied. Before a newly recognized agent can be said to be the etiological agent of a disease, it must be shown to have a high correlation with the oc-

currence of the illness. The situation is not simple in the case of a syndrome having a diverse etiology and a virus which does not produce disease in laboratory animals. Nevertheless, the present report of echo virus type 6 as the only agent associated with 24 cases of the disease lends strong support to the view that this virus is one of the etiological agents of aseptic meningitis. Infections produced with the virus in this series of cases were of the classical type, in that antibody rises were readily demonstrated in paired sera. At the recent Conference on Viruses in Search of Disease, 3 different laboratories(8,9,10) also commented on finding a high degree of association of echo-6 virus with aseptic meningitis cases, including the isolation of the agent from the cerebrospinal fluid in some of the patients studied(9).

TABLE V. Clinical Features of Aseptic Meningitis Patients Associated with Echo-6, Coxsackie B and Poliovirus Excretion.

Signs and symptoms	Echo-6	Coxsackie B	Polio-virus
Fever and malaise	21/21	11/13	19/20
Headache	17/21	11/13	17/20
Stiff neck	18/21	12/13	17/20
" back	8/21	3/13	10/20
Back pain	4/21	3/13	8/20
Muscle "	4/21	3/13	7/20
Vomiting	15/21	5/13	10/20
Abdominal pain	3/21	0/13	0/20
Sore throat	5/21	4/13	10/20
Diarrhea	0/21	0/13	0/20
Constipation	0/21	1/13	3/20
CSF pleocytosis (LP performed 1-10 days after onset with the majority being done on 2nd day)			
Less than 10 cells	0/18	3/11	3/23
10- 24 cells	1/18	0/11	1/23
25- 99 "	9/18	4/11	8/23
100-300 "	5/18	1/11	8/23
Over 300 "	3/18	3/11	3/23
More than 10 cells	18/18	8/11	20/23

Summary. In a study of vaccinated and non-vaccinated "poliomyelitis" patients in Connecticut during the summer and fall of 1955, 78% of the paralytic, but only 50% of the nonparalytic, cases yielded viruses cytopathogenic for monkey kidney cultures. Of the 45 agents isolated from paralytic patients, 93% were polioviruses, chiefly type 1. Two patients had mixed infections: echo-6 and polio-1, and Coxsackie A9 and polio-1. Of the 69 enteric agents isolated from the nonparalytic cases, 41% were polioviruses (type 1, with a single exception), 23% were Coxsackie viruses (B2, B3, B4, A9), and 36% were echo viruses. Of the 25 echo viruses, 21 belonged to echo-6 and 4 were unrelated to any of the 14 recognized echo types.

In addition, echo-6 was recovered from 3 cases of aseptic meningitis in Rhode Island during the summer of 1954. Together with evidence being obtained in other laboratories, these data strongly support the view that echo virus type 6 is one of the etiological agents of the aseptic meningitis syndrome.

1. Committee on the Echo Viruses. *Science*, 1955, v122, 1187. Only 13 types are listed in the original communication of the Committee. Type 14, recently recognized in this laboratory, has now been recovered from 3 patients with aseptic meningitis and from 2 healthy children.

2. Melnick, J. L., and Emmons, J., unpublished

work.

3. Honig, E. I., Melnick, J. L., Isacson, P., Parr, R., Myers, I. L., and Walton, M., *J. Exp. Med.*, 1956, v103, 247.

4. Melnick, J. L., *Ann. N. Y. Acad. Sci.*, 1955, v61, 754; *ibid.*, 1956, in press.

5. Melnick, J. L., *ibid.*, 1955, v61, 1005.

6. Adair, C. V., Ross, L. G., and Smadel, J. R., *Ann. Int. Med.*, 1953, v4, 675.

7. Rhodes, A. J., *Ann. N. Y. Acad. Sci.*, 1956, in press.

8. Enders, J. F., and Kibrick, S., *ibid.*

9. Karzon, D., *ibid.*

10. Svedmyr, A., *ibid.*

Received July 13, 1956.

P.S.E.B.M., 1956, v92.

Epinephrine-Chlortetracycline Induced Arrhythmia in the Isolated Rat Heart. (22631)

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(Introduced by C. H. Hine.)

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The effect of antibiotics on the host has received considerable attention. Their possible effect on cardiac action has been emphasized. One of the authors, with another worker(2) found that epinephrine-chlortetracycline induced arrhythmia can be demonstrated in isolated frog and turtle hearts. This led to the experiments reported here on the effect of epinephrine-chlortetracycline on the isolated mammalian heart.*

Methods. A white rat was killed by a blow at the base of the skull. The heart was excised and the coronary arteries were perfused through a modified Straub cannula in the aorta. The heart was allowed a 10-15 minute stabilization period and its contractions were recorded kymographically. After an additional 10 minutes, the drug was administered by injection into the perfusate and in approximately 20-25 minutes following this a challenge dose of epinephrine (1 μ g) was added to the perfusate.

The temperature for each experiment was

kept to within $\pm 0.5^{\circ}\text{C}$ and ranged between $34-37^{\circ}\text{C}$ in different experiments. The perfusate was well-oxygenated Lockes' solution; a glucose concentration of 0.2% strength rather than the lower limit of 0.1% aided in reducing irritability and in stabilizing the heart (Fig. 1). The elapsed time per experiment averaged 1.9 hours although a normal record without irregularity of beat for up to 3.4 hours was made.

Results. An isolated rat heart treated with 100 mcg of chlortetracycline-HCl[†] followed by 1 μ g of epinephrine developed both inotropic and chronotropic irregularities (Fig. 2) in which the normal beat was interrupted for from 5-30 minutes, usually 15-20 minutes, by one or more of several different kinds of arrhythmia as follows: 1. Cyclic variation in amplitude; 2. Periods of increased amplitude followed by 2-20 dropped beats or by contractions of smaller amplitude; 3. Series (2-5) of beats irregular in rate and amplitude; 4. Complete cessation of beat (Fig. 5).

* With the aid of a grant from the San Francisco Heart Assn.

[†] Through the courtesy of the Lederle Laboratories Division, American Cyanamid Co.