

be drawn from Series II metabolism (Table IV) and retention (Table III) values.

While these conclusions seem to be an accurate evaluation of the data obtained, the long term effects of mutual feeding of both Sr^{89} and fluoride under a variety of different conditions should be evaluated before making definite conclusions. It does appear, however, that presence of fluoride, at 3 different levels, is not associated with increased retention of Sr^{89} in the body, nor, conversely, is the presence of Sr^{89} associated with increased retention of fluoride in the body.

Conclusions. Administration of Sr^{89} and

fluoride together does not increase retention of either Sr^{89} activity or fluoride in the whole carcass or femur of the rat. Preliminary evidence suggests, on the contrary, that presence of fluoride may decrease the amount of Sr^{89} retained by the rat.

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ECHO 11 Virus Associated with Aseptic Meningitis.* (25347)

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Several enteroviruses have clearly been associated with aseptic meningitis (1-3). Most prominent among this group are ECHO virus types 4, 6, 9, and 16; Coxsackie types A7 and A9, B1-5; and poliovirus types 1-3. Other types have been isolated from sporadic cases. Until the time of this study, ECHO 11 had been isolated only from normal children (4,5) and from sporadic cases of diarrhea (6). The present report is concerned with an outbreak of aseptic meningitis connected with this virus.

History of outbreak. During latter part of June and July, 1958, at least 7 children were admitted to Jefferson Davis Hospital with a febrile illness characterized by pleocytosis of the cerebrospinal fluid. In addition a mother of one of these children was admitted to the hospital as a "suspected case of poliomyelitis." The most common symptoms at time of admission were: frontal headache, vomiting, spiking fevers to 103°F , stiff neck and joints, acute abdominal pains, and occasional diarrhea and swollen cervical glands. Further physical examination did not show skin rash or mouth lesions. Duration of acute illness was 3 to 7 days. Hematological examination

usually yielded a few immature blood cell forms. White blood cells, generally lymphocytes, in the cerebrospinal fluid ranged from 35 to $440/\text{mm}^3$. The diagnosis was aseptic meningitis, etiological agent unknown. Five of the 7 children lived in a low rental housing project of 1000 dwelling units for low income families. The majority of units are rows of 2-story apartments. The buildings are constructed of concrete with adequate bathroom and cooking facilities in each family unit. Occasional inspection by the administration keeps the living quarters in good repair. Insect control is part of this service. There are ample playground facilities for children. Visiting among neighbors in adjacent homes is very common, especially among children. It is not unusual for the Public Health Nurse to visit each family periodically to check on communicable diseases. A vaccination program is carried out through the hospital clinic and most children visited had received vaccines against diphtheria, whooping-cough, poliomyelitis, typhoid, and tetanus. *Field study.* Because of ease of collection and handling, it was decided to obtain only rectal swabs. Specimens were collected in 3 ml of M-H medium (7) containing a 10-fold concentration of

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antibiotics; swabs were stored on dry ice, and kept frozen in laboratory until tested. Thirty-six families, contributing 157 single specimens, were studied for symptoms and for virus. The criteria for choosing families were as follows. Specimens were obtained from 18 families who either reported a child with a recent history of admission to the hospital with presumptive diagnosis of aseptic meningitis, or had close contact with such a child. Samples were taken from affected child, from each available member of his family, and from members of contact families. As cases were limited to one side of the housing project, a control population of 18 families was selected from the opposite side of the courts. Although it was possible for older children to have mingled through community projects, the vast majority of children in this group were too young for such social admixture and their associations were generally limited to adjacent homes. Families in the control group were randomly selected from among larger families.

Laboratory methods. Monkey kidney tissue cultures were used throughout the isolation and identification process(7). Swab suspensions were thawed at room temperature, centrifuged at 2000 rpm, and 0.1 ml of the supernate inoculated into each of 3 culture tubes. Inoculated cultures were incubated at 37°C and examined for cytopathic effects (CPE) until fifteenth day. If CPE occurred the tissue culture was harvested by freezing and thawing to liberate the cell-associated virus. If culture was harvested after sixth day of incubation, it was passaged through another tissue culture before titration. A calculated dose of 100 TCD₅₀ was used in all neutralization tests. As a rule the virus was first typed against polio antisera, and if not neutralized, further typings were carried out by Coxsackie and ECHO combination antiserum pools as described by Lim and Benyesh-Melnick(8). Confirmation was made by typing with antiserum indicated in results of the combination pool tests, both by neutralization and (for many of the strains) by complement fixation test.†

Results. Of the 36 families tested for presence of enteroviruses, 27 yielded positive

TABLE I. Viral Isolation in Test and Control Series.

Group	No. of families	No. of specimens	No. of isolations—		
			Total	ECHO 11	Other
Study	18	74	33	19	14*
Control	18	83	19	5	14†

* There were 7 isolations of ECHO 4, 1 of ECHO 6, 1 of ECHO 9, 2 of polio 1, and 3 of polio 3.

† There were 3 isolations of ECHO 5, 4 of ECHO 13, 2 of ECHO 15, 2 of Coxsackie B2, 2 of Coxsackie B3, and 1 of Coxsackie B5.

isolations, 16/18 (89%) in the study group and 11/18 (61%) in control group. As shown in Table I, of 74 specimens in the study group, 33 were positive (45%) in contrast to 19 positive isolations from 83 specimens (23%) in the control group, the difference being significant ($P < 0.01$).

The most prevalent virus isolated in the study group was ECHO 11, 19 of 33 isolations; the same agent was found among viruses isolated from control children, but to a lesser extent (5 of 19 isolations). Of the 12 families in the study group in which individuals were excreting ECHO 11 virus, 8 were infected with this virus alone. Three families were infected with both ECHO 11 and ECHO 4, and one family with ECHO 11 and ECHO 6.

Of 5 children and one mother admitted to the hospital with symptoms of aseptic meningitis, 2 were from families yielding ECHO 11 alone, one from an ECHO 4 family, one from a family infected with both ECHO 4 and ECHO 11, and one from a family infected with ECHO 9 and poliovirus type 1. Virus from the patient was of the type isolated from the family, except for the ECHO 9 patient. In this instance, ECHO 9 was isolated from patient's cerebrospinal fluid and feces, but 2 weeks later when family specimens were taken, 2 siblings were carrying poliovirus type 1. Spinal fluid was tested from 2 ECHO 11 patients but no virus was isolated.

The number of individuals with symptoms in the study group was 61% (45/74) in contrast to only 18% (15/83) in control series (Table II). In the study group, ECHO 11 was isolated from 22% (10/45) of individuals with symptoms but inapparent infection was common, the same virus being isolated from

† The CF tests were kindly carried out by Dr. M. Shingu, Research Fellow in this laboratory.

TABLE II. Total Population Studied in Relation to Illness and Isolations of ECHO 11 and Other Enteroviruses.

	Total No. individuals	With symptoms*			Without symptoms		
		Total	ECHO 11	Other†	Total	ECHO 11	Other†
Study	74	45	10	10	29	9	4
Control	83	15	2	3	68	3	11

* These included any of the following, usually being multiple in a single patient: headache, fever and malaise, stiff neck, backache, muscle pains, sore throat, abdominal pain, vomiting, nausea, anorexia, diarrhea, swollen glands.

† See footnote to Table I.

31% (9/29) of asymptomatic patients. Of 19 individuals in the study group excreting ECHO 11 virus 53% had symptoms, the more frequent complaints being headache, fever, malaise, vomiting, abdominal pain, sore throat and stiff neck.

In the control group, ECHO 11 was isolated less often; only 5 individuals in 3 families yielded the virus. Only one of these families reported an illness and in the 3 members tested the virus was found. In 2 of these individuals the only symptom was a recent attack of diarrhea, whereas in the study group this symptom was not associated with isolation of ECHO 11. In the other 2 cases, ECHO 11 was found to be excreted together with Coxsackie B2, but in neither case were any symptoms reported.

Symptoms accompanied ECHO 11 infection in older children more frequently than in those under 5 years of age. In those under 5 years of age, only 1 of 7 virus carriers became ill, whereas 6 of 7 children over 9 years old had symptoms similar to those described above (Table III).

Discussion. Studies of aseptic meningitis outbreaks have usually shown that although there is a predominant virus associated with the disease, other enteroviruses may be concurrently isolated from individuals associated with the epidemic(1-3). This study has again

revealed that it is possible for overlapping infections of many enteroviruses to occur in a limited area at the same time, making it difficult from a single isolation to determine the etiological relationship of the agent to the outbreak. The association of ECHO 11 with this epidemic is noteworthy. In comparing test and control populations, there was a higher degree of illness compatible with aseptic meningitis reported among individuals in the former than in the latter group ($P < 0.01$). The symptomatology associated with these cases appeared more varied than that usually found in "classical" aseptic meningitis outbreaks.

Summary. In a study of an outbreak of aseptic meningitis, ECHO 11 was the most prevalent virus isolated (24 of 52 isolations). Other enteroviruses associated with cases in the outbreak but to a lesser degree were ECHO 4 and polioviruses 1 and 3. Children over 5 years of age infected with ECHO 11 were more apt to have clinical symptoms of aseptic meningitis than younger children.

TABLE III. Occurrence of Symptoms According to Age in Children of Study Group Excreting ECHO 11.

	Age group (yr):				Total
	0-4	5-9	10-15	>15	
ECHO 11 with symptoms	1	3	5	1	10
ECHO 11 without symptoms	6	2	1	0	9
Total	7	5	6	1	19

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