(4) Beeman, E. A., Huebner, R. J., and Cole, R. M.: Studies of Coxsackie viruses. Laboratory aspects of the group A viruses. Am. J. Hyg. 55: 83–107 (1952).
(5) Contreras, G., Barnett, V. H., and Melnick, J. L.: Identification of Coxsackie viruses by immunological methods and their classification into 16 antigenically distinct types. J. Immunol. 69: 395–414 (1952).
(6) Huebner, R. J., Cole, R. M., Beeman, E. A., Bell, J. A., and Peers, J. H.: Herpangina, etiological studies of a specific infectious disease. J. A. M. A. 145: 628–633 (1951).
(7) Curnen, E. C., Shaw, E. W., and Melnick, J. L.: Disease resembling nonparalytic poliomyelitis associated with a virus pathogenic for infant mice. J. A. M. A. 141: 894–901 (1949).
(8) Weller, T. H., Enders, J. F., Buckingham, M., and Finn, J. J., Jr.: The etiology of epidemic pleurodynia: A study of two viruses isolated from a typical outbreak. J. Immunol. 65: 337–346 (1950).
(9) Melnick, J. L., Kaplan, A. S., Zabin, E., Contreras, G., and Larkum, N. W.: An epidemic of paralytic poliomyelitis characterized by dual infections with poliomyelitis and Coxsackie viruses. J. Exper. Med. 94: 471–492 (1951).
(10) Rhodes, A. J., Clark, E. M., Knowles, D. S., Wilson, F. M., McLean, W. J., and Silverthorne, N.: Studies on poliomyelitis in Ontario, 111 further observations on the association of Coxsackie and poliomyelitis viruses. Canad. J. Pub. Health 41: 183–188 (1950).
(11) Melnick, J. L., and Ledinko, N.: Immunological reactions of the Coxsackie viruses. 1. The neutralization test: Technic and application. J. Exper. Med. 92: 463–482 (1950).
(12) Kraft, L. M., and Melnick, J. L.: Complement fixation tests with homologous and heterologous types of Coxsackie viruses in man. J. Immunol. 68: 297–310 (1952).
(13) Beeman, E. A., and Huebner, R. J.: Evaluation of serological methods for demonstrating antibody responses to group A Coxsackie (herpangina) viruses. J. Immunol. 68: 663–672 (1952).
(14) Melnick, J. L., and Ledinko, N.: Social serology: Antibody levels in a normal young population during an epidemic of poliomyelitis. Am. J. Hyg. 54: 354–382 (1951).
(15) Beeman, E. A., Cole, R. M., and Huebner, R. J.: Studies in man of neutralizing antibodies against group A Coxsackie (herpangina) viruses. Am. J. Hyg. 56: 215–231 (1952).
(16) Kraft, L. M., and Melnick, J. L.: Quantitative studies of the virus-host relationship in chimpanzees after inapparent infection with Coxsackie viruses. II. The development of complement-fixing antibodies. J. Exper. Med. 97: 401–414 (1953).

Isolation of a Coxsackie Virus During a Summer Outbreak Of Acute Minor Illness

By JOSEPH L. MELNICK, Ph.D., MARY WALTON, M.D., Dr.P.H., and IRA L. MYERS, M.D.

COXSACKIE VIRUSES have been isolated from patients with a variety of illnesses (*1*, *2*, *3*). However, the characterization of these viruses as the etiological agents of specific disease is not a simple matter. Extensive and detailed investigations were required before Huebner and his associates (*4*) were able to demonstrate that certain members of the Coxsackie group could induce the clinical entity known as herpangina. The fact that at least 16 antigenic types are included in the Coxsackie group (*5*) confuses the problem. Additional investigations combining epidemiological observations and laboratory study of specimens collected in the field are necessary to elucidate the role of these viruses in human illnesses. One such investigation is recorded here.

The observations described were made as a part of a study of the epidemiology of poliomyelitis which is being conducted in Charleston, W. Va. (See the preceding paper.) In the last week of July 1951 an unusual incidence of unclassified acute minor illness was noted in a block in one of the study areas in which households are regularly visited by in-

---

Dr. Myers, a commissioned officer of the Public Health Service, was assigned to the Communicable Disease Center poliomyelitis project, at Charleston, W. Va., when a part of this study was in progress. He is now with the Communicable Disease Center, Atlanta, Ga. Biographical data for Dr. Melnick and Dr. Walton will be found with the article by them on p. 1167 of this issue.

This study was aided by a grant from the National Foundation for Infantile Paralysis.

---

terviewers. The block is adjacent to one from which a case of poliomyelitis had been reported earlier in the week. The situation was investigated to determine whether or not the two observations were related.

Materials and Methods

Stools and paired serum samples were collected at times indicated in table 1. They were frozen at about −20° C. soon after collection and held at this temperature until tested. The methods used in the preparation of stools for testing for poliomyelitis and Coxsackie viruses, as well as the criteria for positive isolations, have been described in detail previously (*6*, *7*, *8*). For poliomyelitis virus one monkey was employed per sample, the animal being inoculated intracerebrally with a fecal extract which had been concentrated and partially purified by ultracentrifugation. Two litters of eight newborn mice each were inoculated for each test for Coxsackie virus.

Neutralization tests were carried out as described (*9*, *10*). For the paired serums, each serum was diluted 1 to 10 and run against varying dilutions of virus. The results in table 1 are listed as the log units of type A2 Coxsackie virus (Fleetwood strain) neutralized by this dilution of serum.

The viruses isolated in this study were typed first by the plate complement fixation test as adapted to Coxsackie viruses (*11*) and then by neutralization with the prototype serum in order to rule out the presence of more than one strain in the isolate (*5*).

The Outbreak

The poliomyelitis patient, a white male 6 years of age in household 8 (see table 1) became ill on July 18, 1951, with symptoms of fever, drowsiness, nausea, and epigastric pain. He developed a stiff neck the following day. A diagnosis of poliomyelitis was made on July 20 on the basis of increased lmyphocytes in the spinal fluid, stiff neck, and hamstring muscle spasm. Weakness of abdominal muscles was noted on July 21 and persisted until September 4. On July 21 his year-old brother developed fever and sore throat, at which time the attending pediatrician observed small vesicles in the throat (herpangina). On July 25 his mother reported a sore throat.

The 6-year-old poliomyelitis case played frequently with children in the adjacent study block and had attended a birthday party at the home of household 2 (see table 1) on July 4. There was no known contact with household 7.

Twelve families live in the study block. Seven were available for study. In 6 households 1 or more persons reported illness during July. Of the 10 children in these households 7 had symptoms between July 11 and 31. Six had fever and sore throat. In 2, small vesicles were seen on the first or second day of illness. The others had small superficial ulcers. The seventh reported only coryza. Morbidity reported in the block and laboratory findings are shown in table 1.

Laboratory Findings

Coxsackie virus was isolated from stools of each of six children in the study block and from the poliomyelitis patient and the younger child in that household (No. 8). By methods previously described (*5*) all eight strains were found to belong to type A2. The Charleston viruses reacted in the complement fixation test with type A2 serum but not with any of the other 14 prototype serums which were run simultaneously with each of the strains. Each strain was then found to be neutralized by antiserum to the prototype type A2 strain (Fleetwood). Over 100,000 doses of virus were neutralized by a 1 to 10 dilution of immune mouse serum.

One of four children from whom paired blood specimens were obtained showed a rise in titer of neutralizing antibody. No virus was isolated from stools of six adults in households with illness. All first blood specimens from the adults showed neutralizing antibody. None showed a rise in titer in convalescent blood.

Household 7 reported no illness during July. There was no known contact between children in this family and others in the block. No virus was isolated from stool specimens of 2 children and 2 adults in this family.

Poliomyelitis virus was isolated from a stool specimen from the poliomyelitis patient but not from the specimens of the sibling or from the

Table 1. Summary of morbidity and laboratory findings among certain householders in Charleston, W. Va., 1951

Household No.	Sex	Age	Symptoms		Serum				Stools		
					Dates		Log of type A2 neutralization index			Virus isolated	
			Date	Kind	1st	2d	1st	2d	Date	Type A2 Coxsackie	Poliomyelitis
1	M	30	6/23	Sore throat, fever, headache	7/27	9/6	4.8	4.8	8/8	0	
	F	28	7/24	Vomiting, diarrhea							
	F	5									
	M	3	7/11	Sore throat, fever					8/8	+	0
2	M	37									
	F	35			8/1	9/8	4.3	3.8	8/13	0	
	M	6	7/16	Sore throat,[1] fever [2]	7/27	9/8	5.3	5.3	8/13	+	0
	M	1	7/16	Sore throat, fever					8/13	+	0
3	M	37									
	F	34	7/25	Stiff neck [3]	8/1	9/6	4.3	3.8	8/13	0	
	F	8	7/29	Sore throat, fever, cough [4]					8/13	Inc.	
	M	5	7/31	Running nose	8/1	9/6	5.3	5.5	8/13	+	0
4	M	33									
	F	33	7/24	Sore throat					8/8	0	
	M	4									
	M	2									
5	M	33									
	F	33			8/1	9/8	3.0	2.8	8/8	0	
	M	5	7/22	Sore throat,[1] fever	8/1	9/8	5.0	5.8	8/8	+	0
	M	2	7/25	Sore throat, fever	8/1	9/8	1.3	>6.3	8/8	+	0
6	M	33									
	F	24									
	F	20	7/19	Headache, cough					8/8	0	
7	F	73							8/9	0	
	M	37									
	F	35			8/1	9/11	4.3	4.3	8/9	0	
	F	10			8/1	9/11	4.3	4.3	8/9	0	
	F	4							8/9	0	
8	M	34							8/8	0	
	F	27	7/25	Sore throat	7/27	9/6	1.8	2.5	8/8	0	
	M	6	7/18	Fever, drowsy, stiff neck, nausea [5]	7/24	9/4	4.3	>6.3	7/24	+	+
	M	1	7/21	Sore throat,[1] fever					8/8	+	0

[1] "Blisters in throat."

[2] Additional onset 7/21—fever, vomiting.

[3] Additional onset 8/9—fever, stiff neck, chest and eyes ached, diarrhea.

[4] Chest and eyes ached.

[5] Poliomyelitis case—additional symptoms: diarrhea, epigastric pain.

children in the study block. None of three pairs of serum run showed a rise in complement-fixing antibody to type A2 poliomyelitis virus.

Discussion

An investigation was made of an outbreak of minor illness which occurred coincidentally with the onset of poliomyelitis in a child during a period of low poliomyelitis incidence in Charleston, W. Va. Only three cases were reported in this area in 1951. The study was limited to one city block in which several cases of fever and sore throat (including some patients with herpangina) occurred during July 1951. Stool samples were collected as long as a month after symptoms were first noticed. If we assume that the illness was caused by type A2 Coxsackie virus, then the fact that every child tested who had been ill was found to be excreting this virus is an agreement with previous studies on the duration of Coxsackie virus carrier states. Because the first serum sample was usually taken several days after onset, it is not surprising that antibody rises could be demonstrated only in two instances. Neutralizing antibodies develop early in Coxsackie infections (*6*, *9*), and all patients excreting Coxsackie viruses either had a high titer of homotypic antibodies in their first serums or developed them by the time the second serums were taken.

It is noteworthy that only one household with children gave negative tests for Coxsackie virus and that the children of this household had no known contact with the children of the other families studied.

In the group of five households with children currently infected, none of the adults were found to be carriers. All adults who were tested had neutralizing antibodies to type A2 Coxsackie virus. The titer of antibody in the adults did not change during the period of observation. It was slightly lower than that in the children, which suggests that the infection of the adults might not have been of recent origin. Beeman, Cole, and Huebner have recently made extensive studies in this field and have come to conclusions with which we agree: Persons with type specific neutralizing antibody do not excrete virus when it is introduced into the household, nor is there a change in their neutralizing antibody level. Similar observations have been made on chimpanzees exposed to Coxsackie virus by natural routes (*12*).

There was little poliomyelitis in Charleston in 1951 and the data show how localized poliomyelitis can be in an urban area. The minor illness in the study area could not be related to poliomyelitis infection but rather to infection with one of the Coxsackie viruses. The patient who first called our attention to this area was found to be infected with both poliomyelitis and a Coxsackie virus. Although the contacts appeared sufficient for spread of Coxsackie virus within the block, no evidence could be obtained that poliomyelitis virus spread from the patient into the block. In 1950, which was also a year of low prevalence of clinical poliomyelitis, virological data also indicate the failure of the virus to spread widely through the community. Sewage tests were carried out in 1 circumscribed area of Charleston (4,000 people on the line) in which 2 cases occurred in 1 week during September. Weekly sewage samples were negative for 11 weeks before the cases occurred, and also during the week of onset. Poliomyelitis virus was isolated from the sewage the following week. After this, the tests became negative again.

Serological data on Coxsackie infections were obtained in Charleston during the summer of 1951 from normal children who were bled in the spring and again in the fall (see accompanying study). It was found that 30/83 (36 percent) of children under 10 years of age in this study area developed increases in complement-fixing antibodies to type A2 (Fleetwood), A4 (Texas-1), or B1 (Connecticut-5) viruses and to a much less degree (2/83) to B3 (Nancy). Of the 13 children with antibody to type B3 in the spring, 8 showed no antibody in the fall. Children developing Coxsackie virus antibodies had a significantly higher incidence of fever and sore throat than children who failed to develop these antibodies. Because of the heterotypic complement-fixing antibody response occurring in Coxsackie infections, it is not possible to say which virus was responsible for the antibody increases in the Charleston children. The isolation here of type A2 Coxsackie virus from children with similar illnesses during the same period sug-

gests that the type A2 virus may well have been one of the major etiological agents for the minor illnesses prevalent in Charleston during the summer of 1951.

Summary

A localized summer outbreak, presumably of herpangina, was investigated in Charleston, W. Va., during 1951.

Acute minor illnesses with symptoms of sore throat and fever were reported by the sibling of a case of poliomyelitis and by 6 of 10 playmate contacts under 10 years of age living in the block across the street. Some of the children had a syndrome compatible with herpangina. Both poliomyelitis virus and type A2 Coxsackie virus were isolated from a fecal specimen from the case of poliomyelitis, and a rise in neutralizing antibody titer to type A2 Coxsackie virus was demonstrated. Stool specimens from household and playmate contacts with symptoms were found to contain type A2 Coxsackie virus but not poliomyelitis virus. Adult members of the households possessed neutralizing antibodies to type A2 Coxsackie virus. None were found to be excreting virus.

ACKNOWLEDGMENT

We wish to express our thanks to Dr. M. V. Gallagher who reported the clinical observations in the study block and in the household of the poliomyelitis patient.

REFERENCES

(*1*) Melnick, J. L., and Curnen, E. C.: Coxsackie group [Review of literature]. *In* Viral and rickettsial infections of man. Ed. 2. Edited by T. M. Rivers. Philadelphia, Lippincott, 1952, pp. 338–358.

(*2*) Kilbourne, E. D.: The Coxsackie viruses and human disease. Am. J. Med. Sc. **224**: 93–102 (1952).

(*3*) Dalldorf, G.: The Coxsackie viruses: Isolation and properties. *In* Papers, etc., Second International Poliomyelitis Conference. Philadelphia, Lippincott, 1952, pp. 111–120.

(*4*) Heubner, R. J., Cole, R. M., Beeman, E. A., Bell, J. A., and Peers, J. H.: Herpangina, etiological studies of a specific infectious disease. J. A. M. A. **145**: 628–633 (1951).

(*5*) Contreras, G., Barnett, V. H., and Melnick, J. L.: Identification of Coxsackie viruses by immunological methods and their classification into 16 antigenically distinct types. J. Immunol. **69**: 395–414 (1952).

(*6*) Melnick, J. L., Ledinko, N., Kaplan, A. S., and Kraft, L. M.: Ohio strains of virus pathogenic for infant mice (Coxsackie group). Simultaneous occurrence with poliomyelitis virus in patients with "summer grippe." J. Exper., Med. **91**: 185–195 (1950).

(*7*) Paul, J. R.: I. Poliomyelitis. *In* Diagnostic procedures for virus and rickettsial diseases. New York, American Public Health Association, 1948, pp. 165–186.

(*8*) Melnick, J. L.: Chemical and physical methods for the preparation of clinical samples for virus study. *In* Proc., 4th International Congress of Tropical Medicine and Malaria, 1948, pp. 401–407.

(*9*) Melnick, J. L., and Ledinko, N.: Immunological reactions of the Coxsackie viruses. I. The neutralization test: Technic and application. J. Exper. Med. **92**: 463–482 (1950).

(*10*) Beeman, E. A., Huebner, R. J., and Cole, R. M.: Studies of Coxsackie viruses. Laboratory aspects of the group A viruses. Am. J. Hyg. **55**: 83–107 (1952).

(*11*) Kraft, L. M., and Melnick, J. L.: Immunological reactions of the Coxsackie viruses. II. The complement fixation test. J. Exper. Med. **92**: 483–497 (1950).

(*12*) Melnick, J. L., and Kaplan, A. S.: Quantitative studies of the virus-host relationship in chimpanzees after inapparent infection with Coxsackie viruses. J. Exper. Med. **97**: 367–400 (1953).