EXPERIMENTAL STUDIES ON COXSACKIE VIRUS INFECTIONS IN WISCONSIN

by

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INTRODUCTION

Although poliomyelitis has become an important illness in our society and has been intensively studied during the past few decades there are still certain phases of its epidemiology with incompletely resolved problems. Wickman in 1913 (74) observed abortive cases which, together with healthy carriers, he postulated were of prime importance in the production of epidemics. This theory was developed by Frost (29) who believed that paralysis was the exception in this disease and that the majority of our population became immune by the end of childhood through such nonparalytic infection. Extensive research has substantiated these earlier observations and poliomyelitis is now considered to be primarily a nonparalytic infection frequently associated with minor, indefinite symptoms and occurring one or more times in the majority of our population (8, 34, 57).

Several determinants influencing the production and severity of paralysis are now recognized. Among these are fatigue, chilling, and overheating (44), tonsillectomy (4), immunization (46, 32, 1), pregnancy, and endocrine function (73). Recently Ingalls and Aycock (39) presented evidence from an outbreak at a boys' school that the incidence of upper respiratory illness predisposes to the clinical forms of poliomyelitis.

The spread of poliomyelitis is considered by many to be by person to person contact and much evidence supports

this theory (2, 9, 10, 23, 24, 32, 58). Other modes considered likely are via food, milk, water, and arthropod vectors. It would appear that any of these may produce the illness in a given individual or epidemic but that personal contact is the most likely route of spread in the majority of cases. Whether this spread is primarily through nasopharyngeal secretions or fecal contamination remains a highly controversial question.

The variables which seem to influence the spread of this virus are poorly understood. The immunity of the population with respect to the strain of virus which is prevalent and the degree of contact between infected and susceptible persons are probably very important (32, 58). Armstrong (2) has postulated that the seasonal incidence may in part be due to concomitant variations in atmospheric humidity.

The recent developement of complement fixation tests for detection of infections and tissue culture technique for isolation of poliomyelitis virus from stools of infected humans will undoubtedly facilitate more extensive study of poliomyelitis epidemiology (8, 64).

In 1948 Dalldorf and Sickles (21) first reported the isolation of a new infectious agent from two boys diagnosed as cases of poliomyelitis. The agent was identified as a virus characterized by the production of necrotic muscle lesions and death when inoculated into newborn mice. Since this initial isolation was from Coxsackie, New York,

that name was adopted to identify the virus. Since then numerous reports have appeared describing the new virus from several aspects.

The Coxsackie viruses have been found in several states in this country (13) as well as Alaska (5), Canada (3), England (25), Denmark (45), France (43), and Israel (28). Several types of illnesses have been attributed to this virus including herpangina (13, 38, 62), epidemic myalgia or pleurodynia (25, 26, 42, 74), aseptic meningitis (56), and "three-day fever" (72). The virus has also been associated with epidemics of poliomyelitis and with individual cases of paralytic and nonparalytic poliomyelitis as well as with indefinite illness resembling the latter (13, 17, 18, 20, 29, 35, 40, 54, 56, 59, 66). The most intensively studied illness is herpangina which was first described by Zahorsky in 1920 (75) and is now believed to be a specific symptom complex caused by certain strains of the Coxsackie viruses.

The virus has been isolated from human feces, sewage, flies, nasopharyngeal washings and other materials (12, 35, 37, 72, 74). The isolation of the virus from stools of patients and their contacts and the developement of type specific antibodies in increasing titers together with the correlation of these laboratory tests with clinical data establish the fact that this virus is a definite human pathogen. The numerous reports of coincident occurrence of Coxsackie and poliomyelitis virus suggest

a possible relationship although the validity and significance of these interpretations are not established (13, 20, 47, 51).

The contents of this thesis is based on two summers work with the Wisconsin State Board of Health. The first summer was spent in the northern part of the state collecting epidemiological data and laboratory specimens under the supervision of Dr. Frances Cline, District Health Officer. The second summer was spent in the laboratories of the Department of Microbiology at the University of Wisconsin Medical School under the direction of Dr. A. F. Rasmussen performing virus isolations on the specimens previously collected. The latter work was supported by a State Grant from the United States Public Health Service.

The primary purpose of this project was to study poliomyelitis in an entire group where it is naturally occurring and can be studied to best advantage. Initially there were two objectives; the first was to obtain serial fecal specimens and pharyngeal washings before and during infections of poliomyelitis in groups such as families, small communities, or in children's summer camps. The second was to obtain clinical information concerning the relation of indefinite summer illnesses to poliomyelitis. Following the discovery of Coxsackie virus and reports of its occurrence with poliomyelitis virus in humans, it was decided to test the specimens for the presence of this virus.

The purpose of the Wisconsin State Board of Health in

employing medical students in such projects as will be described, is not only to obtain additional summer personnel for the study of specific problems but also to give interested students an opportunity to gain first hand experience and information about public health in this state. Appreciation is expressed to Dr. Arthur R. Zintek, former Director, Section on Preventable Diseases, Wisconsin State Board of Health, for making this study possible and to the many other personnel of the State Board of Health who were of assistance. The author is also grateful to Dr. K. P. Newcomb of Woodruff, Wisconsin, for cooperation and assistance in the procurement of data and materials there and also to personnel of the Dept. of Microbiology at the University of Wisconsin Medical School for assistance in the virus isolations.

MATERIALS AND METHODS

The community of Woodruff, Wisconsin, was the first site of this study, chosen because of its size (population 475), a reportedly high incidence of presumably virus infections and also the assurance of wholehearted cooperation by the local physician in such a study. It was also hoped that laboratory material and information pertinent to the problem could be obtained from the many local summer camps for children. To begin with, all of the households in Woodruff were visited and information was gathered about each family. This included the ages of all occupants, occupations, history of recent acute illnesses, contacts with other families, milk and water supply, and type of sanitation. Families with three or more children were then revisited and requested to furnish stool specimens and throat washings from each child. In view of the fact that there was no epidemic of acute illness, much less poliomyelitis, there was often a considerable degree of reluctance to cooperate. It was also inadvisable to make reference to poliomyelitis in discussing this request for fear of initiating harmful rumors in the community.

During this survey several summer camps for children were visited with the local physician who also served as camp doctor in a number of such camps. Many of the camps were experiencing epidemics of relatively mild infections characterized by fever and sore throats.

Throat cultures from some of the more severe cases showed no evidence of beta hemolytic streptococcus on blood agar plates. The clinical observations together with the apparent resistance of these infections to the antibiotics and sulfonamides suggested that the etiology was viral in nature.

Camp A, a girls' camp, was first visited about four days after opening its second four week session. An outbreak of illness characterized by fever and sore throats began almost as soon as the incoming group of campers arrived with two of their number being ill on arrival. When it was learned that the brother of a newly arrived camper was diagnosed as a case of paralytic poliomyelitis it was decided to study this group as well as possible without disrupting the purpose of the camp. In this case it was possible to obtain daily oral temperatures of each girl as well as histories of any illness and conduct weekly superficial examination of the entire camp. An attempt was made to obtain duplicate throat washings and stool specimens at least one week apart from each girl. Of added interest was the fact that five days after returning home one girl was diagnosed as having nonparalytic poliomyelitis.

Camp B, for boys, was visited during the last week of its final camp period after two cases of paralytic poliomyelitis were reported there. At this camp there had been a severe outbreak of the indefinite illness similar to that in Camp A. All of the infirmary records of illness

were made available and stool specimens were obtained from nine of the cases which were ill in the infirmary, several of which had symptoms indistinguishable from nonparalytic poliomyelitis. No specimens were obtained from the cases of poliomyelitis since they were transferred immediately after diagnosis.

In both of the camps the sanitary and food handling facilities had all been checked by personnel of the State Board of Health and had been found to meet required standards. Additional inspection in the camps did not show any appreciable variation in the environment of the two camps.

Laboratory procedures: All specimens were frozen as quickly as possible and stored at -20° C.

Stools were prepared, as described by Howitt (35), for inoculation into mice by suspending them in four times their weight of sterile distilled water. This was followed by centrifugation at 16,000 RPM for 40 minutes at 6° C. Part of the clear supernate was pipetted into small tubes for immediate inoculation, the remainder stored at -20° C. Streptomycin and penicillin were added about half an hour before inoculation in quantities of 5 mg. and 1000 Units per cc. of inoculum, respectively. Random samples of these relatively bacteria-free suspensions produced neither growth on blood agar nor evidence of suppuration when inoculated into infant mice.

Infant mice, less than 48 hours old, were used to detect the presence of Coxsackie virus. Intracerebral

and dorsal subcutaneous inoculations of 0.01 and 0.03 cc. respectively were those generally used. One complete litter was used to test a single stool specimen and usually consisted of six to ten mice. In litters larger than seven one was usually marked and left to serve as a normal control. The young were checked daily after inoculation for ten days for evidence of lethargy, paralysis and death. If paralysis occurred in a mouse it was killed with ether, skinned, eviscerated, and decapitated, then either frozen or ground up in saline as a 20% suspension for further inoculation or preparation as purified antigen.

Antibodies were developed in adult mice by the intraperitoneal injection of paralyzed mouse carcass suspension. Three doses were injected at weekly intervals and the mice were bled two weeks after the last inoculation. The serum was separated and kept frozen until ready for use in complement fixation tests.

To prepare antigen for complement fixation tests the paralyzed muscle suspension was frozen and thawed three times and allowed to remain at 6° C. overnight (35). This created a floc which was removed by centrifugation. The clear supernate was then passed through a Seitz filter and stored at -40° C.

Throat washings consisting of normal sterile saline, which had been gargled about thirty seconds, were obtained from many of the individuals studied. These were cleared by centrifugation and injected with penicillin and streptomycin into infant mice as were the stool suspensions.

Failure to detect any Coxsackie virus in throat washings, including those from individuals who had the virus in their stools, led to the discard of this method of testing.

Histologic sections were prepared from several paralyzed mice using either entire limbs or carcasses cut sagitally. Mice were sacrificed for this purpose at various stages in the developement of paralysis and in cases where recovery from paralysis had occurred. These sections were used to confirm the presence of skeletal muscle necrosis and the absence of central nervous system and visceral lesions. Detailed descriptions of the pathogenesis and histological pathology have been recently reported by Melnick and Godman (50).

RESULTS

Village of Woodruff: This community consisted of 121 households including 419 persons who consented to personal interview. The incidence of all illnesses reported during interviews between July 15 and September 1, 1949 is shown in Table 1 as occurring in various age groups and expressed in percentages of the total of each group. In Table 2 the same illnesses are analyzed as to the symptoms which occurred in various age groups expressed in percentages of the total illnesses. Worthy of note is the higher incidence in the younger age groups and also the higher percentage in the 20-39 age group which includes the majority of parents of children in the study. In studying the compiled figures it was noted that more adults were ill when there were children present in the household. The total number of homes with children (0-10 years) was 67 with illness occurring in 38 or 57%. Of these 38 homes there was indefinite illness of adults in 14 homes which is 37% of this number or 21% of the 67 total homes with children. The total number of homes with no children during the study was 54 and of these illness, of the upper respiratory type commonly noted, occurred in only 4 homes or 7%.

Of the households with three or more children, 14 were studied more intensively and stool and pharyngeal washing specimens were obtained from them. A summary of the illnesses which occurred in this group is shown on the

Table 1. Incidence of indefinite illness in Woodruff, Wis., during summer of 1949 on the basis of age groups.

Age group	No. in group	No. ill	Percentage ill
0-4 years	57	20	35
5-9 years	44	16	36
10-14 years	30	7	23
15-19 years	34	2	6
20-39 years	135	18	13
40 and over	119	3	3
Total	419	66	13

Table 2. Incidence of illness in Woodruff during summer of 1949 on the basis of symptoms, expressed in percentage of total illness in a given age group.

Age group Symptoms	0-4	5-9	10-14	15-19	20-39	40 plus
"Colds"	45	25	14	100	44	67
Sore throats	55	56	43	50	44	33
Fever	35	31	29		22	
G. I. upsets	30	19	14		6	33
Headaches	5	25	29		16	33
Neuromuscular	5	25	29		28	33

Total homes with children (0-10 yrs.) during study	67	
Of these homes indefinite illness occurred in 38, or	57%	
Of these 38 there was adult illness in 14 homes, or	37%	
Total number of homes with no children during study	54	
Indefinite illness occurred in 4 of these homes, or	7%	

Table 3. Incidence of illness on the basis of age groups among the 14 households studied intensively in Woodruff, July 15- Sept. 1, 1949.

Age group	No. in group	No. ill	Percentage ill
0-4 years	18	10	63
5-9 years	19	9	47
10-14 years	7	1	14
15-19 years	1	0	
20-39 years	25	7	28
40 and over	3	0	
Total	73	27	37

Of total households, 13 had one or more cases of illness.

Table 4. Percentage of illnesses demonstrating various symptoms among the 14 households intensively studied in Woodruff, July 15-Sept. 1, 1949.

Age group	0-4	5-9	10-14	15-19	20-39	40 plus
Symptoms						
"Colds"	70	11			43	
Sore throats	60	88	100		71	
Fever	40	55	100		57	
G. I. upsets	50	22				
Headaches	10	33			43	
Neuromuscular	10	44	100		14	

basis of age groups in Table 3 and on the basis of symptomatology in Table 4. Of the total, 13 households or 93% had one or more cases of indefinite illness and it is noted that in the adult group (parents) the incidence is 28%, considerably higher than the incidence of illness among the adult population of the village as a whole (Table 1). The age group with the most frequently occurring illness is the 0-4 group as would be expected. It is realized that some of the apparently significant differences may have been largely due to more intensive interviewing and more conscientious reporting of adults who were parents.

Inoculation of infant mice was done with 12 stool specimens from 8 children, represnting 6 families in which there had been illness during the course of the survey. All of the individuals so tested had been, or were ill, at the time the specimens were obtained except in the case in which an entire family of three children were tested (see below). A Coxsackie type of virus was found in only this one family, one of which was diagnosed as having nonparalytic poliomyelitis and presented the following clinical picture:

Karen S., age 5. On 8-30 and the preceding several days she had played with a boy, age 3, who had been ill with the "flu" for the previous three days. This was at the home of a family who operated a home bakery half a mile from the village and where the patient's older sister did housework daily. That afternoon Karen was unusually tired and slept several hours.

8-31: Fever of 102° F. orally, complained of headache, pain in back and neck, nausea and diarrhea.
9-1: Examination revealed a normally developed girl, acutely ill. 101° fever; the pharynx was diffusely inflamed and there was moderate cervical lymphadenopathy. Ventral bending of the spine with the knees extended could be done to just less than 90°. Ventral bending of the neck painful with palpable nuchal spasm. Neurological otherwise grossly normal. Application of hot packs gave considerable relief.
9-2: Temp. 99.2° F. Complains of pain in legs and paresthesias in feet which make walking difficult. Stiffness of back and neck but tendon reflexes hyperactive. Lumbar puncture revealed normal pressure, clear fluid, 5 cells, and a strongly positive Pandy test for globulin.
9-3: Temp. 99.6° F. Still complains about stiff back and neck. Tendon reflexes not as active as preceding day.
9-6: Temp. normal. Recovery from symptoms was uneventful and complete by 9-11 when ventral bending of spine could be done to about 120° without discomfort.

The older sister, Vivian age 12, had an episode of stiffness of the neck and pain in her right leg on 9-2. James, age 16, disclosed no history of illness although mother stated that he had not felt well on a couple of days a few weeks earlier. Tests for Coxsackie virus in the stools revealed:

	Age	Date 8-11	8-19	9-2	9-9
Karen	5	-	-		
Vivian	12	-			-
James	16		-		

It is of interest to note that Karen was found to have the virus present in her stools only after her symptoms began, having been negative two and three weeks previously. James, on the other hand, denied having any symptoms yet had a positive stool on 8-11.

Camp A: In studying this group of 77 girls, age 9-14, it was decided to divide them into two age groups in order to determine any difference in attack rates or symptoms. Table 5 shows the distribution of symptoms in the two groups with a higher incidence of most symptoms in the 9-11 age group. The temperature variations were divided into those above 99.0°, 99.6°, and 101.0° F. to show the degree of fever encountered. Although the 99.0 temperatures are often considered to be within the range of normal variation, they were included because it was felt that the total elevation for the entire camp might be of interest. Also, no individual showed persistent elevations to that level without other evidence of illness. The daily temperatures were taken at the same time each day at the end of a strictly enforced rest period, giving relatively standard conditions.

The records were also divided on the basis of cabin groups which ranged from 5 to 16 girls in each. There was neither apparent difference in the time of onset of illness in the cabin groups nor any specific pattern of spread through them. This may possibly be explained by the extensive intentional mixing of these groups at meals and during recreational activities.

Table 5. Camp A, July 23-August 19, 1949. Incidence of various symptoms occurring in the two age groups.

Symptoms	Juniors: 30 girls (9-11 years) No.	% of group	Seniors: 47 girls (12-14 years) No.	% of group
Sore or inflamed throat	28	93	45	95
99.0° temperature	30	100	35	74
99.6° temperature	15	50	14	30
101.0° temperature	6	20	6	13
Infirmary patients with sore throat	12	40	15	31
Cervical adenopathy	14	47	20	42
Headache	5	16	6	13
Stiff back or neck	6	20	6	13
G. I. upset	2	6	4	8

Table 6. Incidence of Coxsackie virus in stool specimens from Camp A, July 23-August 19, 1949.

Total campers	77	
Total counselors	15	
Total	92	
Total stools tested	152	
Campers' (108 paired, 21 single)	133	
Counselors' (14 paired, 5 single)	19	
Number of stools having virus	20	13%
Number of individuals having virus	14	15%
Campers	13	17%
Counselors	1	7%
Number of positive girls (having virus) with paired stools	11	
Number of above having virus present in both stools	6	56%

A more productive approach was to divide the camp into those who had just arrived on 7-23-49 and those who had been in the camp the previous four weeks also, designated as four and eight week campers respectively. The former group had come primarily from the Chicago area on one train with considerable mixing and close contact enroute. On entry the routine superficial physical examination showed two girls to be febrile and to have sore throats characterized by diffuse redness, edematous appearance and numerous lymphoid papules of the soft palate and pharyngeal walls. There was also moderate anterior and submandibular lymphadenopathy. If it is assumed that these incoming illnesses were of the same type as those responsible for the later epidemic of sore throats, then the girls who were on the train undoubtedly had a more intense and earlier exposure than those already at camp. In Fig. 1 is shown the summation curve of those who had temperatures of 99.0° and greater, divided into the two groups of 4 and 8 week campers. Fig. 2 shows the summation curve of temperatures elevated to 99.6° and more with division into the same groups. There is noted a considerable lag in the summation curve for the 8 week group in both these figures. This supports the assumption that this illness was introduced into camp by the incoming group and that the spread was on a person to person basis. It is realized that the results from this analysis are only speculative since the necessary daily laboratory studies to substantiate this

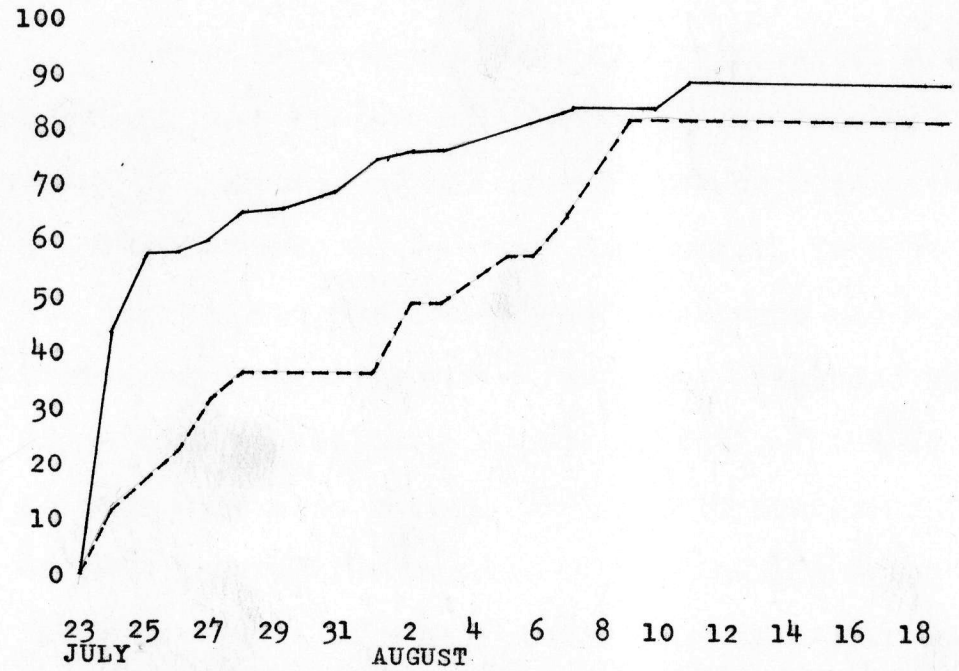


Fig. 1. Camp A, 1949. Summation curve of campers having had temperatures of 99.0° or above, expressed in percentage of total group. —% of 4 week group;---% of 8 week group.

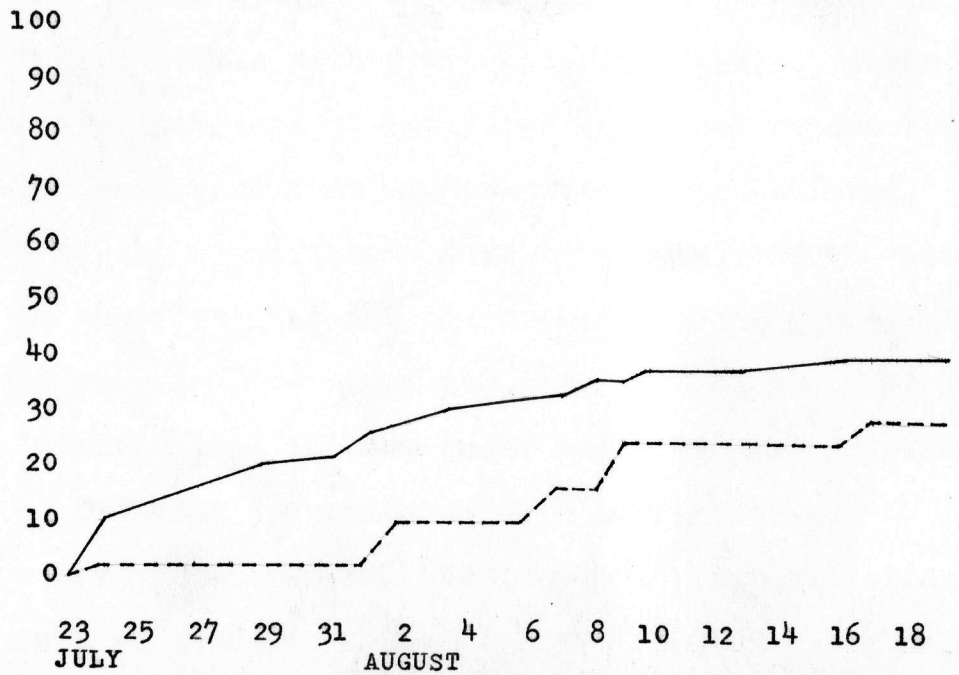


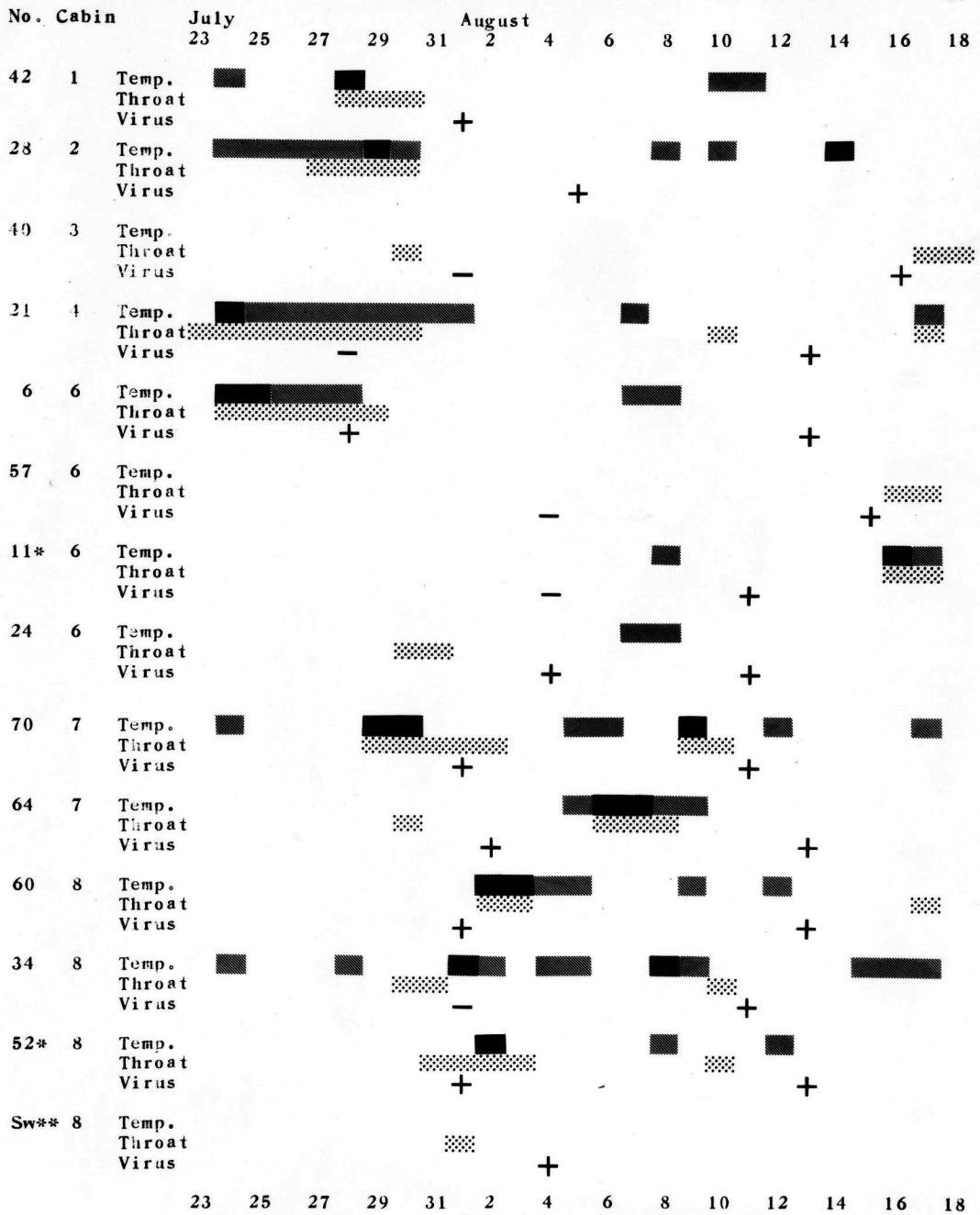
Fig. 2. Camp A, 1949. Summation of campers having had temperatures of 99.6° or above, expressed in percentage of total groups. —% of 4 week group.--- % of 8 week group.

supposition are lacking.

Stools were examined for the presence of Coxsackie virus as described in the laboratory procedures. Of the total 77 campers, stools from 75 were tested with duplicate specimens, seven to fourteen days apart, from 54 girls. Two campers did not furnish any specimens and 4 specimens (which were negative for virus) were discarded because of defective labeling. Stools from 12 of the 15 counsellors were also tested, with paired specimens from 7 of that group. The results of the testing are shown in Table 6. It is of significance that the virus was recovered twice from more than half of the positive group with duplicate fecal specimens but from only 15% of the total group.

In an attempt to correlate the incidence of virus in the stools with symptomatology, Table 7 shows the daily incidence of fever and any other evidence of illness for each girl from whom the virus was isolated. It is of apparent significance that in no case was the virus found in the first but not the second specimen, while the reverse is true in five cases (40, 57, 11, 21, 34). It is of interest that in some cases there was only minimal evidence of clinical infection as with campers 24 and 57. Conversely the symptoms preceded the virus isolation by as much as 22 days in the case of No. 21. This would tend to influence one against attributing the symptoms to the virus which was isolated. However, it must be pointed out that, considering the technical difficulties in making

TEMPERATURE ELEVATION AND SYMPTOMS IN GIRLS HAVING COXSACKIE VIRUS IN THEIR STOOLS



sore or inflamed throat; 99.0°F.+ temperature; 99.6°F.+ temperature; + = virus present in stool; − = no virus present; * 8 week camper; ** Counselor.

Table 7. Camp A, 1949.

such an isolation, the failure to isolate the virus is of less significance than is the recovery of the virus.

Table 7 also shows the typical course of the illnesses which were characteristically mild although some were rather prolonged. There were fewer complaints about soreness of the throat in this camp than in the other one to be described, although inspection usually revealed a moderately inflamed pharynx with many lymphoid follicles and occasional focal ulcerations. Penicillin injections were used almost routinely for the patients in the infirmary but seemed to have little effect on the course of the illnesses.

Of the girls who had the C virus in their stools it is seen in Tables 7 and 8 that cabins No. 6 and 8 had the highest incidence, as well as having a greater incidence of fever (99.6°) than the other cabin groups. Of the 12 counselors tested for the presence of virus, only one, from cabin No. 6, had the virus in her stools.

TABLE 8

Cabin No.	Number of campers	Number having 99.6° temp.	%	Number having C virus	%
1.	14	1	7.1	1	7.1
2.	11	4	36.4	1	9.1
3.	6	1	16.6	1	16.6
4.	6	2	33.3	1	16.6
5.	5	1	20.0	0	
6.	16	7	43.7	4 (5)*	25.0 (26.3)
7.	10	3	30.0	2	20.0
8.	9	6	66.6	3	33.3

* 1 counselor with virus, 26.3% of total of 19 persons.

In further attempting to correlate the presence of virus with incidence of illness it is difficult to say who was actually ill or not, since the temperature elevations may, in part, have been due to normal variations and the observations of supposed throat infection due to different interpretations of the clinical observers. If all the campers who were admitted to the infirmary beds (exclusive of injuries) are selected and the incidence of virus in this group is determined, the following results recorded in Table 9 are found. Likewise, if the girls who had at one time or another a 99.6° temperature are selected, approximately the same percentage of these harbored the virus. The percentages of those having virus in these groups are considerably higher than those for the camp as a whole (15.2%) and for the remaining "well" group of 63 campers from which the virus was isolated four times (6.4%).

TABLE 9

Total infirmary cases	27
Infirmary cases having C virus in stools	9
Percentage of above	33.3%
Campers with 99.6° or higher temperature	29
Number of these with C virus in stools	10
Percentage of above	34.5%

An attempt was made to determine whether the viruses isolated were all of one type. Adult female mice were immunized with 0.5 cc. of paralyzed mouse muscle suspension intraperitoneally two times at weekly intervals, shortly before impregnation. Viruses isolated from three different

individuals were used for this immunization and the infant mice born to these females were challenged with virus from the same or other individuals by intracerebral and subcutaneous inoculations of 0.01 and 0.02 cc. of 20% infected muscle suspensions. The suspensions used were found to produce death in 100% of infant mice inoculated with dilutions up to 10^{-5}. The results of these cross-protection tests, the technique for which was first described by Melnick, et al, (49), are shown in Table 10.

TABLE 10

Immunizing virus	Challenge virus	Result (fraction of litter dead or paralyzed)	Control
235	33	1/8 dead	10/10
235	60	1/9 dead	10/10
235	234	0/9	9/9
235	92	0/9	8/8
60	33	1/8 dead	10/10
60	60	0/10	10/10
33	33	0/7	10/10
33	60	0/9	10/10
33	92	1/8	8/8
33	235	0/10	6/6

Limited complement fixation tests, using type specific antisera*, indicated that of the 10 viruses tested all belonged to Group A, Dalldorf type III. Not all of the viruses have been tested and there were some conflicting results probably traceable to unsatisfactory antigens.

Second passage of virus from paralyzed mice was attempted in 13 of the 19 stools which were considered positive for

* Antisera were obtained from Dr. J. E. Smadel of the Dept. of Virus and Rickettsial Diseases, Army Medical Service Graduate School, Washington, D. C.

Coxsackie virus. One of these produced no paralysis but there was no opportunity to re-test this result. Three produced paralysis in only a part of the litter inoculated. The remaining 9 produced paralysis in all of the mice inoculated which was repeated two or more times with 7 of these. Second passage was attempted using muscle suspensions from 10 separate groups of mice not paralyzed by stool inoculations. In none of these did paralysis occur.

In an attempt to rule out errors in the initial tests, 20 of the 133 negative stools were inoculated a second time with uniformly negative results. 17 of the 19 positive stools were reinoculated one or more times with repeated paralysis in 14 and questionable results in three cases. The latter may have been due to attenuation from repeated thawing since they produced either positive results on second passage or positive complement fixation tests (as antigens).

Questionable results were all repeated when possible, although in not all such cases was a definite result obtained. The questionable result of a stool inoculation was considered negative unless definite paralysis resulted on subsequent inoculation of the stool or second passage of the virus from the questionably paralyzed mouse tissue. As was pointed out by Howitt (36), more reliance can be placed upon the primary isolation of the virus from a stool specimen than on that from second or third passage

of the mouse tissue with the possibility of spontaneous or human contamination.

Limited testing for poliomyelitis virus, using stool specimens from Camp A, was done on three macaca mulatta monkeys. Paired specimens from two girls were inoculated into separate monkeys and a pool of 9 specimens from 5 individuals was inoculated into a third monkey. The pool of specimens from the five girls and one of the individuals' specimens produced distinct neurological symptoms of poliomyelitis in those two monkeys. Microscopic examination of the brain and cord showed characteristic histological changes with marked destruction of anterior horn cells in the spinal cord. The third monkey died three days after inoculation and showed gross and microscopic evidence of diffuse cerebral inflamation probably due to viable bacteria or toxic effect of the inoculum. The pool of five individuals' specimens also produced paralysis in mice and later tests showed all five of the individuals to have Coxsackie present. The two individuals' specimens produced negative and questionable results for C virus and are not included in the group of positive results.

III. Camp B. This camp of 118 boys also was operated for two consecutive 4 week periods with 89 boys remaining for the full 8 weeks. Since this camp was studied for only the final week following the diagnosis of two cases of paralytic poliomyelitis, the clinical records analyzed are largely those of the camp infirmary. Table 11 shows

the division of the camp population, the incidence of illness and the frequency of the more common symptoms. There had been 32 cases of upper respiratory infections during the first four weeks but the greatest incidence took place about half-way through the second period. The following is a description of one of the characteristic cases.

Henry M., age 12. Came to infirmary with sore throat. Oral temp. 100.0°. Pharynx diffusely red. Later temp. rose to 103.0° and he complained of headache, nausea, malaise and difficulty in swallowing. Kept in bed and given penicillin injections daily.
8-17: Temp. 103.0°. Throat is more inflamed, with marked papule formation. Complains of headache and stiffness of the dorsal neck.
8-18: Temp. 103.4°. Throat appears to have slightly ulcerated areas. Other symptoms persist. Examination revealed tightness of the left pectoralis and sterno-mastoid and both trapezii muscles. Ventral bending with knees extended to 75°, can't put chin on chest. Assumes tripod position on sitting up.
8-19: Temp. 99.2°. Feels better but has very sore throat and has ulcerated, necrotic appearing lesions in pharynx. Stiffness of back and neck the same. Stool specimens yielded Coxsackie type of virus. Progressive improvement occurred and patient was discharged on 8-24.

The histories and also the appearance of the patients personally observed at this camp showed that they were considerably "sicker" than the children in the other camp. The two cases diagnosed as having poliomyelitis had symptoms similar to many of those in camp prior to the onset of paralysis. Their cases are briefly presented.*

J. C., age 11. 7-25 to 8-3 had a sore throat and low grade fever ranging from 99.4° to 100°, reported daily to infirmary.
8-5: Temp. 101.6°. Throat red, complains of headache, malaise and coryza. Kept in infirmary, given penicillin.
8-6: Temp. 101.6°. Throat more sore and red with lymphoid papules on post. pharynx.
8-7: Temp. 101°. Condition the same. Given Aureomycin.

* Gratitude is expressed to the attending physicians and St. Mary's Hospital of Rhinelander for portions of the following clinical data.

Table 11. Camp B, July 23-August 19, 1949. Incidence of illness among campers and the percentage of illnesses demonstrating various symptoms.

Total number of boys	118	
8 week campers	89	
4 week campers	29	
Total infirmary patients	36	31%
Total number that had symptoms	68	58%
8 week campers ill	54	60%
4 week campers ill	14	48%

Symptoms	No.	% of total group	% of total illnesses
Sore throat	45	38	66
99.0° temperature	44	37	65
99.6° temperature	30	25	44
101.0° temperature	23	20	34
G. I. upset	17	14	25
Headache	16	13	24
Paralytic poliomyelitis	2		

Comparison with the period of June 26-July 22, 1949. Illness among the 8 week campers.

Total number of boys in group	89	
Total number which were ill	32	36%

Symptoms	No.	% of total group
Sore throat	22	25
99.6° temperature	13	15
G. I. upset	10	11
"Colds"	9	10
Headache	4	5

8-8: Temp. 100°. Complains of headache, malaise, abdominal pain. Speaks with nasal twang and regurgitates fluids through nose. Sent to St. Mary's hospital in Rhinelander. Physical examination revealed the following: throat red, left soft palate parlysis, moderate cervical lymphadenopathy. Neurological otherwise negative.
8-9: Physical exam unchanged. Laboratory findings: Urine: 4 plus albumin and occasional WBCs. Blood: Hb. 12.6 gms%, WBCs 13,000 with 64% PMNs and 26% lymphocytes. Lumbar puncture revealed clear fluid under normal pressure, 36 cells, primarily lymphocytes.
Transferred by plane to a Chicago hospital.
Diagnosis: Acute bulbar poliomyelitis.

CRC, age 31. Sports director and counselor at Camp B. Usual occupation, high school coach.
8-7 and 8-8: Sore throat with papules on post. pharynx.
8-10 to 8-14: Wife noted unusual irritability.
8-14: Participated in water carnival. Became very fatigued and sunburned. Noted unusual constipation.
8-15: Woke up early with a bad headache. Was in bed off and on all day but directed all of sports. Had a 101° fever by noon and noted vertigo when upright. By 9:30 PM he had a "splitting" headache and severe stiffness and pain in the neck and back. Unable to sleep during night.
8-16: Neck and back muscles extremely stiff making any movements painful. Dull headache continued and eyes hurt on moving them. Was sent to St. Mary's hospital where physical examination revealed the following: Temp. 101°, rigidity of the back and neck, inability to sit upright with knees extended, and normal reflexes except for absent abdominal response. Laboratory findings: Urine normal. Blood: Hb. 16.9 gms.%, WBCs 11,800 with normal differential. Lumbar puncture revealed a ground glass appearing fluid under normal pressure with 395 cells, predominantly PMNs. Globulin test positive; no organisms present on smear.
8-17: Temp. 101.2°. Spasm in back and neck slightly reduced. Patient progressively improved so that by 8-25 there was only slight stiffness and spasm remaining in the back. He was discharged at this time with the diagnosis of acute nonparalytic poliomyelitis.
12-29-49: Examination of patient in his home revealed:

1. Slight weakness of left quadriceps femoris.
2. Weakness of right anterior tibial. Unable to stand on the right heel.
3. Subjectively he feels that strength in legs is not normal and has had frequent cramps on any effort. Until recently could not dorsi-flex the left great toe. Weakness interferes with coaching.

Stool specimens were collected from nine infirmary patients and attempts were made to isolate Coxsackie virus from them. Of these, four, or 45% yielded the

virus, including the first case cited above. No attempts were made to isolate poliomyelitis virus. Unfortunately, no leucocyte counts or bacteriological studies could be done except in the cases of the two paralytic disease.

The summation of new cases of illness is shown in Fig. 4 for the 4 and 8 week groups of campers, using fever and symptoms as criteria for new cases. In this camp it would appear that the smaller group which entered camp on 7-23 had both a lower attack rate and a lag in developement of new cases. However, since more complete clinical data is lacking on each boy, many of the milder cases may have been missed.

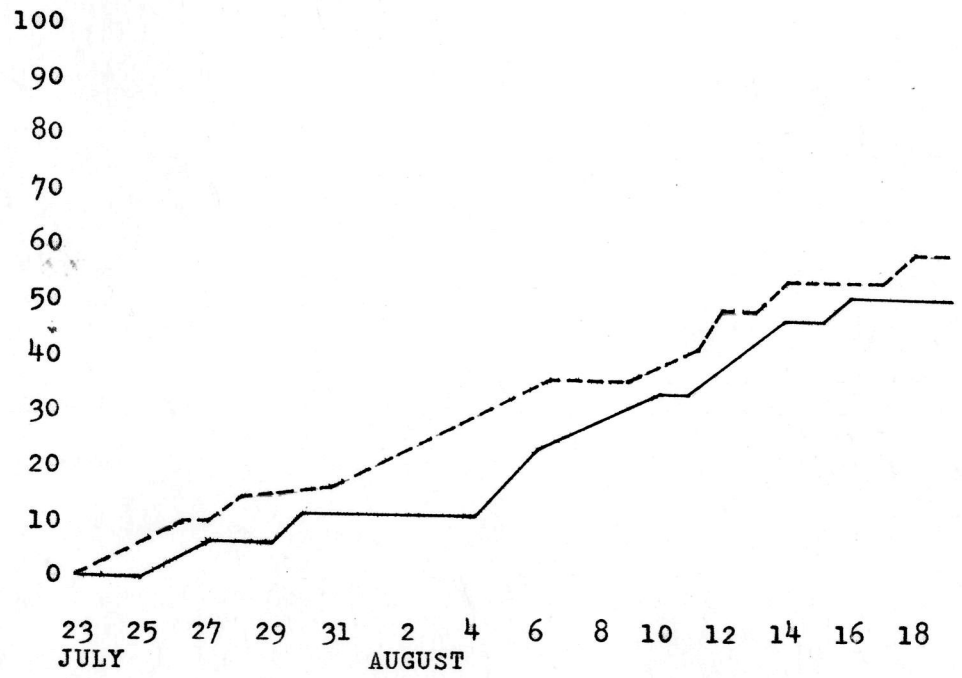


Fig. 4. Summation of new cases of illness in Camp B, on the basis of fever and symptoms. July 23-August 19, 1949. —— 4 week campers. --- 8 week campers. Expressed in percentage of total number in group.

DISCUSSION

I. Characterization of illnesses.

In Camp A, sore and inflamed throats with frequently occurring lymphoid papule formation was about the most common feature of the outbreak described. Fevers were of a low grade, less commonly over 101°, which occurred in 16.5% of the total group (Table 5). Over a third of the camp was confined at some time to the infirmary with fever and sore throat. Other findings were the cervical lymphadenopathy in 44.5% and the muscle stiffness and pain in 16.5% of the campers. Also of note was the fact that the previous camp period had been relatively free of illness with few admissions to the infirmary and few episodes of elevated temperature. On the following year (1950), in a comparable period, less that 15% of two similar groups of girls in the same camp (many were the same ones) had temperature elevations to 99.6°. The camp managers were very certain in their opinion that the group studied in 1949 had well above average incidence of illness.

In Camp B more ulcerations of the inflamed throats were observed. 37.5% of all the boys had at one time or another an inflamed, sore throat. Since weekly inspection of the entire group was not done a great many such cases may have been missed. Fevers tended to be higher in this camp with 19.1% of the patients having 101° or above and a 102° fever occurred in 15 of the campers. 30% of the camp was ill in the infirmary during the four week period. Both the histories and appearance of the patients gave the

impression of a much more severe illness.

There is perhaps no significant difference in the total incidence of illness in the two groups. No actual comparison of the two camps can be made because of the difference in the methods of collecting data. However, there is an apparent lag in the summation curves and daily total incidence of the 4 week campers in Camp B (Fig. 4), as opposed to Camp A where the reverse is true.

In the village of Woodruff the group which was followed more intensively (households with 3 or more children) had a rather high total incidence of illness, with over one-third of all persons having been sick during the six week period. (Table 3) It is seen that sore throat was a predominant complaint in many illnesses as was the case in the camps studied.

The higher incidence of illnesses in adults from homes with children is in agreement with the observations of Hammon (32) in immunological testing of large groups. This is a variable which would seem to be important in evaluating various factors which influence the age distribution and incidence of poliomyelitis.

II. Etiology.

The etiology of illnesses observed in Woodruff can not be determined from the scant laboratory observations presented. However, it may be stated that the Coxsackie group of virus was present in one family and may have caused illness in the youngest member of that family.

This village was in the same general area as the

camps studied. Since the Coxsackie virus was present in the village and camps and since the clinical picture of illnesses in all three groups resembled each other, a possible epidemiologic relationship may have existed. However, in Camp A some of the campers were ill on arrival, presumably bringing the infection with them and initiating the observed outbreak of illness. On the other hand, in Camp B the outbreak seemed to begin with the 8 week group about half-way through their first camp period. This may well have represented a type of illness originating in the native population of the area. It was the opinion of some observers that this type of illness was probably present in several areas of northern Wisconsin as well as numerous other foci throughout the country.

The fact that Coxsackie virus was isolated from stool specimens as described does not prove it to be the etiologic agent in the outbreaks of illness observed. Noticeably lacking are immunologic tests of acute and convalescent sera. Failure to obtain such sera was partly because of unawareness of their usefulness at the time as well as difficulties encountered in obtaining them in private camps and homes. There is, however, considerable evidence for the assumption that the virus was of significance. The large number of negative results in testing specimens from two summers and several separate groups of people excludes the possibility that this may have been the normal constituent of all stools. That the paralyzing agent was a virus was shown by the serial

passage in mice, its resistance to antibiotics, and its passage through Seitz filters.

Concerning the reliability of the laboratory results, the question arose as to whether the testing missed a significant share of the stools containing Coxsackie virus in low concentrations. There is some evidence of this in the small number of questionable results where the inoculated mice showed a temporary lethargy or partial paralysis. There also seemed to be a diminution in the ability of some specimens to produce paralysis when re-tested several months later a second or third time. Thus a number of isolations may have been missed either because of low concentrations of virus or because of the lethal effect on the virus by storing and processing. Furthermore, there may well have been clinical infections without the virus being excreted in the stool every day. However, the much more frequent isolation of the virus from persons who were ill than from those well together with the previous observations in other laboratories gives strong indication that the presence of Coxsackie virus was significant in this study.

Poliomyelitis was apparently present in both camps on the basis of laboratory studies in the one and the presence of paralytic disease in the other. The total incidence of the poliomyelitis virus in these groups with special reference to those persons with Coxsackie virus would be very valuable in further evaluating the data presented.

III. Epidemiology.

The children's camps are a unique group for studying illnesses within them. Children enter from a variety of locations, many undoubtedly carrying infectious agents common to their family or community. Once in the camp each individual is exposed more or less to the mixture of bacterial and viral agents brought by the others. Providing there is no serious pathogen to which there is susceptibility, each person becomes adapted to this new environment with minimal or no clinical symptoms. This phenomenon was observed in the camps of the Armed Forces (14) and presumably is true for these groups of children. In the case of these camps there was almost total isolation from the outside world, especially in the case of Camp A, where a two week voluntary quarantine was observed following the diagnosis of poliomyelitis in the brother of a girl who had just arrived (and was sent home).

In Camp A the entry of infection into the group was most likely via the incoming campers since there were two cases of illness among them on their arrival. As was mentioned there had been minimal illness in the previous four week period. The camp employees lived on the camp grounds and were inspected for communicable disease by the camp doctor. Pasteurized milk was obtained from a large dairy which supplied all of the surrounding area and camps. A milk or food borne epidemic would be anticipated to give a more explosive outbreak rather than

over the course of two or three weeks as was observed. The drinking water was from a safe well, tested at regular intervals with no pollution ever demonstrated. Another source of water borne infection may have been from the swimming area of the lake which, although meeting minimum requirements for safe swimming, probably showed a progressive pollution from the daily swimming as was shown in other camps with similar conditions. A vector might be suspected; insects (of which there were very few) were effectively excluded by screening and rodents were not observed. Considering all of the above possible methods of spread in the epidemic at Camp A, person to person contact seems the most likely. Initially, enroute to camp, there was widespread and close contact among the incoming group which was struck first by the illness. Exposure in the cabins was favorable for spread of a respiratory illness and the intentional mixing of cabin groups would further enhance this spread. An incubation period is difficult to arrive at since there were no daily laboratory studies and specific exposure could not be defined for any given individual.

In Camp B there was not the rigid isolation from the outside world since the staff had numerous contacts in sports and social events with other camps. Here the pattern of spread could not be as clearly defined since there had been considerable illness in the camp prior to the arrival of the new group of campers. Concievably, one might assume the outbreak of illnesses in the second period to

be a continuation of the one encountered in the first period. The intramural spread could have easily occurred as is postulated for Camp A.

One factor which must be mentioned was the general attitude towards the illnesses in the two camps. In Camp A the possibility of poliomyelitis became very evident to all concerned following the report of that illness int the family of one camper. Accordingly, fatigue and chilling were avoided with limited swimming, curtailment of the more strenuous activities, and increase in the rest hour from one to two hours. Camp B, on the other hand, considered the febrile illnesses less serious and did not restrict the activities for the camp, which in such an age group and under camp conditions may become very strenuous. One of the two paralytic cases had a definite history of over-exertion after the onset of symptoms which is generally considered an important determinant in the severity of paralysis (44). The variation of severity of both the sore throats and the poliomyelitis which were present in both camps may well have been due to strain variation of virulence in the causative organism but the difference in exposure to fatigue would appear to be significant.

In Woodruff the sanitary facilities were by no means as uniform as those described above. Although there was a municipal sewage disposal system and water supply, the lower income groups frequently utilized out-houses and shallow wells. The milk supply was from four dairies

with pastureization carried out by two of them. No correlation could be made relative to the incidence of illness and the source of milk or adequacy of sanitary facilities. Children playing together probably afforded the greatest source of exposure outside of the homes. Playmates as a rule were among children of homes close together. No apparent temporal sequence of spread was evident on a map made showing the incidence of illness in each household. It was found that there was frequently a history of exposure to illness in other communities during short trips made by families on weekends. However, in the case of the 5 year old girl cited no travel was undertaken and there was little contact with other children except for the case of indefinite illness related.

IV. The Coxsackie Virus.

The virus itself has been quite thoroughly studied as to physical properties (27, 33, 48), immunologic characteristics (7, 13, 20, 47), and the experimentally produced pathological lesions (48, 59). There are at the time of writing two groups of the Coxsackie virus. Group A comprises the majority of the types now known and produces lesions primarily in the muscles of infant mice, whereas the Group B virus may also cause lesions in other tissues, notably the central nervous system and pancreas (61). On the basis of immunologic typing and microscopic examination of the entire paralyzed mouse bodies, the virus encountered in the present study is placed in Group A. As indicated, there are numerous

(seven or more) immunologically distinct types of virus which have been isolated from various locations.

The most intensive study of illness caused by the C virus was on herpangina during outbreaks in the Washington, D. C. area (13, 38, 62). The illnesses were characterized by fever, sore throats with vesicular, ulcerated areas lasting usually one to four days. Group A Coxsackie virus was isolated and type specific antibody rise was observed in a large percentage of those ill and their contacts. There is considerable resemblance between herpangina and the illnesses observed in the two summer camps.

Another clinical entity associated with the incidence of this virus is epidemic pleurodynia or Bornholm's disease, reported from this country and Europe. Group B virus is the one generally found in patients with this symptom complex of fever and muscular pain which is often thoracic and pleuritic in nature. No evidence of this type of illness was observed in this study.

The age incidence reported for herpangina and the presence of Coxsackie virus is generally in children and young adults with the majority occurring in the 0-4 age group (15, 20). No such specific age group can be derived from this data.

No difference in sex incidence has been reported for Coxsackie virus (13, 20), and none could be ascertained in this series of cases. Seasonal variation has been reported elsewhere (13, 20) with the greatest incidence

in the late summer and early fall. All of the isolations reported here were from that period but no studies were performed during other seasons to substantiate this.

The virus has been shown to be quite contagious as seen in the reports of laboratory infections and the high incidence of virus in the contacts of clinical cases of herpangina (13, 25, 52, 66). The method of spread postulated has been one of person to person contact, probably via respiratory secretions. Experimentally the virus has been isolated from cockroaches (27), the urine and feces of mice (60), and the excreta of monkeys (52, 54). It has been found occurring naturally in flies, municipal sewage, human throat washings and feces and questionably in human tissues and blood (13). These findings make several routes of spread theoretically possible.

Laboratory infections have been reported by several observers with isolation of virus associated with symptoms and the development of type specific antibodies in increasing titers (25, 39, 53, 66). One instance of intentional infection of a human with similar results is reported (25). In several instances type specific antibodies to several strains of C virus were found in a single individual.

The study of Coxsackie infections in experimental animals has proved to be very interesting. The work on chimpanzees by Melnick and Kraft (52) demonstrated that although no signs of illness could be demonstrated, a

transient viremia, intestinal carrier state, and a prompt specific antibody rise resulted from oral feeding of the virus. This protected the animal from further infection by homologous virus but not against heterologous strains which in turn would produce the same response as the initial infection. This study and the observations of the development of numerous type specific antibodies in a given individual points to the fact that multiple infections by various strains of C virus can occur in humans. The simultaneous infection of a child by two strains of C virus was recently reported (6). The higher incidence of such virus infections with symptoms in the younger age groups may be explained on the basis of progressive development of immunity in the older age groups through frequent exposure and infection as maturity is attained.

Another interesting study was done by Melnick and associates (49) on the protection of infant mice against C virus by antibodies derived from the colostrum of immune mothers. Protection of the young was as effective in mice born of non-immunized mothers as in those born of immunized females. Both neutralizing and complement fixing antibodies are transferred this way. This type of cross-protection test was used in this study to differentiate antigenically similar types of virus.

The question of the relation of Coxsackie virus to poliomyelitis was initiated by the very first isolation of the virus from paralyzed patients. Since then several papers have reported the association of these two viruses

in both the paralytic and nonparalytic forms of poliomyelitis, (3, 16, 18, 20, 21, 22, 35, 51, 54, 56, 63). In a recent review by Cole and associates (13), including a large series of cases, it was proposed that the relation is merely one of coincidence and as a result of errors in sampling, since the two viruses occur together only as frequently as might occur by chance when adequate sampling is used. Dalldorf (20) in reviewing cases from three years did not find that the presence of C virus in the stools of poliomyelitis patients had any demonstrable effect either as to the presence or severity of paralysis. However, he did note an inverse relation between the frequency of C virus isolations and the frequency of paralysis for a given year. The fact that the Coxsackie virus is found in specific clinical entities unrelated to poliomyelitis also tends to discount any postulated direct relationship. Interference phenomenon have been demonstrated in laboratory experiments although nothing comparable has been found occurring naturally (19, 69).

Although it may well be true that the coexistence in the same patient by the two viruses is only the result of chance occurrence, they may well have an effect on the course of disease produced by each. If an interference effect occurs as has been shown inconclusively in laboratory animals, there might well be a beneficial effect. On the other hand, if the Coxsackie infections are considered as one of the upper respiratory diseases which may have a predisposing effect on the production of

paralysis, this virus might indeed be very deleterious in persons infected with poliomyelitis. This effect might be directly through the presence of the virus itself or via resulting secondary bacterial invaders. The type of throat lesion seen in herpangina and in Camp B of this series could conceivably be the same type of predisposing factor as is the pharyngeal trauma of tonsillectomy. It would thus appear that there are several possible mechanisms through which Coxsackie virus might affect the course of human poliomyelitis infections.

Further study of this virus which resembles the poliomyelitis virus in physical and epidemiological properties may lead to a clearer understanding of some of the unsolved problems in various aspects of poliomyelitis infections. The relative easy and inexpensive isolation of this virus and the above described tests make this an ideal agent to study as it occurs in humans.

SUMMARY

The results of epidemiological and laboratory studies of indefinite summer illness in a village and two children's summer camps are presented. The minor illnesses described were characterized by sore throats, fever, and variable other symptoms. In the one camp paralytic poliomyelitis was observed in two persons. In the other camp the virus of poliomyelitis was isolated in two groups of stool specimens. Clinical evidence of nonparalytic poliomyelitis was observed in the village.

In each group studied Coxsackie virus was isolated from stool specimens from twenty individuals. The virus was tentatively identified as belonging to Group A, type Dalldorf II. An apparent relation of the virus to the illness was demonstrated, with the virus found more frequently among children with sore throats and fever than among apparently well children. The significance of the above findings is discussed in the light of recent studies on poliomyelitis and Coxsackie viruses.

BIBLIOGRAPHY

1. Anderson, G. W., and Skaar, A. E. Poliomyelitis occurring after antigen injections. Pediatrics 7:741-759, 1951.

2. Armstrong, C. Seasonal distribution of poliomyelitis. A. J. P. H. 40:1296-1304, 1950.

3. Armstrong, M. P., Wilson, F. H., McLean, W. J., Silverthorne, N., (Clinical observations); Clark, E. M., Rhodes, A. J., Knowles, D. S., Ritchie, R. C., and Donahue, W. L. (Laboratory investigations). Studies on poliomyelitis in Ontario. II. Isolation of the Coxsackie virus in association with poliomyelitis virus: A preliminary report. Canad. J. P. H. 41:51-59, 1950.

4. Aycock, W. L. Tonsillectomy and poliomyelitis. I. Epidemiologic considerations. Medicine 21:65-94, 1942.

5. Banker, D. D., and Melnick, J. L. Isolation of Coxsackie virus (C virus) from North Alaska Eskimos. Am. J. Hyg. 54:383-390, 1951.

6. Beeman, E. A., Parritt, R. H., and Cole, R. M. Simultaneous occurrence of two immunologic types of Group A "Coxsackie" virus in a case of Herpangina. Proc. Soc. Exp. Biol. and Med. 78:295, 1951.

7. Casals, J., and Olitsky, P. K. Complement fixation tests with some of the viruses in the Coxsackie group. Federation Proc. 9:570-573, 1950.

8. Casals, J., Olitsky, P. K., and Anslow, R. O. A specific complement-fixation test for infection with poliomyelitis. J. Exp. Med. 94:123-137, 1951.

9. Casey, A. E. Place of contact and radial spread of epidemic poliomyelitis. Am. J. Dis. Child. 69:152-156, 1945.

10. Casey, A. E., Fishbein, W. I., Schabel, F. M., and Smith, H. T., Jr. Incidence of sub-clinical poliomyelitis in an urban area according to age groups. A. J. P. H. 40:1241-1250, 1950.

11. Casey, A. E., Fishbein, W. I., and Bundesen, H. N. Transmission of poliomyelitis by patient to patient contact. J. A. M. A. 129:1141-1145, 1945

12. Clark, E. M., Knowles, D. S., Shimada, F. T., Rhodes, A. J., Ritchie, R. C., and Donahue, W. L. Coxsackie virus in urban sewage. Canad. J. P. H. 41:1342-1358, 1951.

13. Cole, R. M., Bell, J. A., Beeman, E. A., and Huebner, R. J. Studies of Coxsackie viruses: Observations on epidemiological aspects of Group A virus. A. J. P. H. 41:1342-1358, 1951.

14. Commission on Acute Respiratory Diseases. Acute respiratory disease among new recruits. A. J. P. H. 36:439-450, 1946.

15. Curnen, E. C. Human disease associated with Coxsackie virus. Bull. New York Acad. Med. 26:335-342, 1950.

16. Curnen, E. C., Shaw, E. W., and Melnick, J. L. Disease resembling nonparalytic poliomyelitis associated with virus pathogenic for infant mice. J. A. M. A. 141:894-901, 1949.

17. Dalldorf, G. Coxsackie group of viruses. Science 110:594, 1949.

18. Dalldorf, G. Coxsackie viruses. Bull. New York Acad. Med. 26:329-335, 1950.

19. Dalldorf, G. The sparing effect of Coxsackie virus infection on experimental poliomyelitis. J. Exp. Med. 94:62-72, 1951.

20. Dalldorf, G., and Gifford, R. Clinical and epidemiologic observations of Coxsackie virus infections. New Eng. J. Med. 244:868-873, 1951.

21. Dalldorf, G., and Sickles, G. M. Unidentified filtrable agent isolated from feces of children with paralysis. Science 108:61, 1948.

22. Dalldorf, G., Sickles, G. M., Plager, H., and Gifford, R. Virus recovered from feces of "poliomyelitis" patients pathogenic for suckling mice. J. Exp. Med. 89:567-582, 1949.

23. Faber, H. K., Dong, L., and Silverberg, R. J. A search for poliomyelitis virus in house dust and a study of the effects of drying on the viability of the virus. J. Infectious Dis. 88:180-186, 1951.

24. Faber, H. K., Silverberg, R. J., and Dong, L. Studies on the entry and egress of poliomyelitis infection. IV. Atraumatic oral entry: distribution of lesions and virus during the incubation period with notes on asymptomatic poliomyelitis. J. Exp. Med. 94:455-470, 1951.

25. Findlay, G. M., and Howard, E. M. Coxsackie viruses and Bornholm disease. Brit. M. J. 1:1233-1236, 1950.

26. Finn, J. J., Jr., Weller, T. H., and Morgan, H. R. Epidemic of pleurodynia: clinical and etiologic studies based on one hundred and fourteen cases. Arch. Int Med. 83:305-321, 1949.

27. Fischer, R. G., and Syverton, J. T. The Cockroach as experimental vector of viruses. Bact. Proc. 1951:95.

28. Foreign Letters. J. A. M. A. 145:1215, 1951.

29. Frost, W. H. Epidemiological studies of acute poliomyelitis. Papers of Wade Hampton Frost. Pp. 120-170. New York, The Commonwealth Fund, 1941.

30. Geffen, D. H. Incidence of paralysis occurring in London children within four weeks after immunization. The Medical Officer 83:137, 1950.

31. Geffen, T. Bornholm disease. Brit. M. J. 4716:1185, 1951.

32. Hammon, W. McD., Sather, G. E., and Hollinger, N. Preliminary report of epidemiological studies on poliomyelitis and streptococcal infections. A. J. P. H. 40:293-306, 1950.

33. Himmelweit, F., Findlay, G. M., and Howard, E. M. The size of Coxsackie viruses as estimated through Gradocol membranes. Brit. J. Exp. Path. 31:809-812, 1950.

34. Howe, H. A. Epidemiology of poliomyelitis in the light of modern research. Symposium on Poliomyelitis, J. R. Paul, Editor. Am. J. Med. 6:537-550, 1949.

35. Howitt, B. F. Recovery of Coxsackie group of viruses from human sources. Proc. Soc. Exp. Biol. and Med. 73:443-448, 1950.

36. Howitt, B. F. Isolation and differentiation of the Coxsackie group of viruses. Federation Proc. 9:574-580, 1950.

37. Huebner, R. J., Armstrong, C., Beeman, E. A., and Cole, R. M. Studies of Coxsackie virus in southern Maryland community. J. A. M. A. 144:609-613, 1950.

38. Huebner, R. J., Cole, R. M., Beeman, E. A., Bell, J. A., and Peers, J. H. Herpangina. J. A. M. A. 145:628-633, 1951.

39. Ingalls, T. H., and Aycock, L. W. Upper-respiratory infection as a factor influencing susceptibility to poliomyelitis. New Eng. J. Med. 245:197-203, 1951.

40. Kilbourne, E. D. Diverse manifestations of infection with a strain of Coxsackie virus. Federation Proc. 9:581-584, 1950.

41. Kraft, L. M., and Melnick, J. L. Immunological reactions of the Coxsackie viruses. II. the complement fixation test. J. Exp. Med. 92:483-497, 1950.

42. Lazarus, A. S., Johnson, E. A., and Galbraith, J. E. Association of Group B Coxsackie viruses with outbreak of epidemic pleurodynia. Bact. Proc. 1951:95-96.

43. Lepine, A. S., Thieffry, S., Beine, L., Saulter, V., and Martin, Mme. Presence en France d' infections Humaines du group Coxsackie (Virus C). Ann. Inst. Pasteur 80:200-203, 1951.

44. Levinson, A. O., Milzer, A., and Lewin, P. Effect of fatigue, chilling and mechanical trauma on resistance to experimental poliomyelitis. Am. J. Hyg. 41:204-213, 1945.

45. Magnus, H. von Isolering af tre Virus-stammer af Coxsackie Gruppen fra Patienter med meningeale Symptomer. Ugesk. f. laeger 11:1451-1453, 1949.

46. McCloskey, B. P. Relation of prophylactic inoculation to onset of poliomyelitis. Lancet 1:659-663, 1950.

47. Melnick, J. L. Symposium on viral and rickettsial diseases. V. The poliomyelitis, encephalomyocarditis, and Coxsackie groups of viruses. Bact. Reviews 14:233-243, 1950.

48. Melnick, J. L. Studies on Coxsackie virus: properties, immunological aspects and distribution in nature. Bull. New York Acad. Med. 26:342-356, 1950.

49. Melnick, J. L., Clarke, N. A., and Kraft, L. M. Immunologic reactions of the Coxsackie virus. J. Exp. Med. 92:499-505, 1950.

50. Melnick, J. L., and Godman, G. C. Pathogenesis of Coxsackie virus infection. J. Exp. Med. 93:247-265, 1951.

51. Melnick, J. L., Kaplan, A. S., Zabin, E., Contreras, G., and Larkum, W. N. An epidemic of paralytic poliomyelitis characterized by dual infections with poliomyelitis and Coxsackie viruses. J. Exp. Med. 94: 471-492, 1951.

52. Melnick, J. L., and Kraft, L. M. Differentiation of immunological types among the Coxsackie viruses. Federation Proc. 9:585-591, 1950.

53. 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.

54. 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. Exp. Med. 91:185-195, 1950.

55. Melnick, J. L., Rhian, M., Warren, J., and Breese, S., Jr. The size of Coxsackie viruses and Lansing poliomyelitis determined by sedimentation and ultracentrifugation. J. Immunol. 67:151-162, 1951.

56. Melnick, J. L., Shaw, E. W., and Curnen, E. C. A virus isolated from patients diagnosed as non-paralytic poliomyelitis or aseptic meningitis. Proc. Soc. Exp. Biol. and Med. 71:344, 1949.

57. Paul, J. R. Recent trends in poliomyelitis research. Am. J. Med. 11:577-580, 1951.

58. Paul, J. R. The peripheral spread of poliomyelitis through rural and urban areas: Observations from a recent epidemic. Yale J. Biol. Med. 19:521-536, 1947.

59. Pappenheimer, A. M., Daniels, J. B., Cheever, F. S., and Weller, T. H. Lesions caused in suckling mice by certain viruses isolated from cases of so-called non-paralytic poliomyelitis and of pleurodynia. J. Exp. Med. 92:169-190, 1950.

60. Pappenheimer, A. M., Kunz, L. J., and Richardson, S. Excretion of Coxsackie virus in the urine of infected mice. Proc. Soc. Exp. Biol. and Med. 77:405-407, 1951.

61. Pappenheimer, A. M., Kunz, L. J., and Richardson, S. Passage of Coxsackie virus (Conneticut-5 strain) in adult mice with production of pancreatic disease. J. Exp. Med. 94:45-64, 1951.

62. Parrott, R. H., Ross, S., Burke, F. G., and Rice, C. E. Herpangina. New Eng. J. Med. 245:275-280, 1951.

63. Rhodes, A. J., et al. (Laboratory investigations); Armstrong, M. P., Wilson, F. H., McLean, W. J., and Silverthorne, N. (Clinical observations). Studies on poliomyelitis in Ontario. III. Further observations on association of Coxsackie and poliomyelitis viruses. Canad. J. Pub. Health 41:183-188, 1950.

64. Robbins, F. C., Enders, J. F., Weller, T. H., and Florentino, G. L. Studies on the cultivation of poliomyelitis in tissue cultures. Am. J. Hyg. 54:286-293, 1951.

65. Sartwell, P. E. Common respiratory disease in recruits. Am. J. Hyg. 53:224-235, 1951.

66. Shaw, E. W., Melnick, J. L., and Curnen, E. C. Infection of laboratory workers with Coxsackie virus. Ann. Int. Med. 33:32-40, 1950.

67. Sickles, G. M., and Dalldorf, G. Serologic differeces among strains of Coxsackie group of viruses. Proc. Soc. Exp. Biology and Med. 72:30, 1949.

68. Slater, E. A., and Syverton, J. F. Cultivation of Coxsackie virus. Proc. Soc. Exp. Biol. and Med. 74:509, 1950.

69. Sulkin, S. E., and Manire, G. P. Interference between poliomyelitis virus (Lansing) and Coxsackie virus. Texas Rep. Biol. and Med. 8:368, 1950.

70. Sulkin, S. E., Schwab, M., and Wallis, H. C. Isolation of Coxsackie virus. Litter differences among suckling mice. Proc. Soc. Exper. Biol. and Med. 77:354-356, 1951.

71. Sulkin, S. E., Manire, G. P., and Farmer, T. W. Cross-neutralization tests with Coxsackie viruses. Proc. Soc. Exp. Biol. and Med. 73:340, 1950.

72. Webb, C. H., and Wolfe, S. G. "Three-day fever" - An acute febrile disease of childhood (Further observations). Am. J. Dis. Child. 80:245-253, 1950.

73. Weinstein, L., Aycock, W. L., and Feemster, R. F. The relation of sex, pregnancy and menstruation to susceptibility in poliomyelitis. New Eng. J. Med. 245: 54-58, 1951.

74. 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.

75. Wichman, I. Acute poliomyelitis. Nervous and Mental Disease Monograph Series, No. 16, 1913. The Journal of Nervous and Mental Disease Publishing Co., New York.

76. Zahorsky, J. Herpetic sore throat. South. M. J. 13:871, 1920.

77. Zahorsky, J. Herpangina (A specific disease). Arch. Pediat. 41:181-184, 1924.

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