# AN ENDEMIOLOGICAL STUDY OF ENTERIC VIRUS INFECTIONS

## Poliomyelitis, Coxsackie, and Orphan (ECHO) Viruses Isolated from Normal Children in Two Socio-Economic Groups*

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The refinement in recent years of tissue culture techniques has facilitated the isolation of a large number of viruses from the human intestinal tract. In addition to the polioviruses and certain members of the Coxsackie group which multiply in tissue culture, these techniques have led into another group of enteric agents, known as the orphan viruses. Some of the latter are being classified as "enteric cytopathogenic human orphan (ECHO) viruses" (15). This paper deals with the enteric viruses isolated by repeated samplings obtained over a 29 month period, from 136 healthy preschool children of two socio-economic groups in Charleston, West Virginia. Related morbidity data concerning the incidence of acute minor illness, and serum antibody studies among these individuals and their families, are also presented.

These findings are part of a larger, longitudinal study (1, 2), of normal households, employing serum antibody measurements, virus isolation techniques, and data on the incidence of minor illness, thus providing information unobtainable in epidemic situations. Such endemiological studies aid in defining the extent of unrecognized infection which normally occurs; insight is gained into the variety and infectious properties of "immunizing" viruses excreted under natural conditions and the scope, duration, and intensity of antibody response can be ascertained. Other studies of this nature are being carried out by Dingle and his group who have had a number of families under observation, chiefly from the point of view of respiratory infections (3), and more recently by Fox (4) who is also investigating enteric viruses.

In the course of our study, viruses belonging to the poliomyelitis, Coxsackie, and orphan groups were isolated. Orphan viruses, as the term is used here, are a heterogenous group, not neutralized by poliomyelitis or Coxsackie antisera, and non-pathogenic for monkeys or suckling mice (5, 15). Findings in this

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study suggest that they are commonly present in the intestinal tract, and related immunologic observations demonstrate that many type-specific orphan viruses exist. Their relationship to illness is still poorly defined. This study provides additional information as to their seasonal distribution in man.

## *Materials and Methods*

*The Population under Study.*—Two relatively uniform socio-economic areas in Charleston, West Virginia, were delineated geographically (1). District I is composed of 1143 persons in 239 households in the north central part of the city where environmental sanitation is poor and the economic status is generally low. District IV has a population of 1929 persons in 521 households of middle to upper middle class socio-economic status living in the southeastern part of the city. Hygiene is generally good in this area.

Monthly visits to families were made for collection of fecal specimens from children, and for the gathering of information on the incidence of illnesses, including those of acute minor nature from the entire family. Sera for antibody determinations were collected from families semiannually, in the spring and fall, over the course of the study.

A total of 1558 stool specimens, 592 in District I and 966 in District IV, were collected from 136 healthy children of preschool age between June, 1951, and October, 1953. Fifty-one of the children resided in District I in 39 households, and 85 in District IV in 80 households. The children ranged from 2 weeks to 4 years of age when the initial specimen was obtained. Specimens were collected monthly when possible. The number of children participating varied slightly during the course of the study. The mean average number of stools collected by month for the 29 month period was 20.4 and 33.3 for District I and IV, with standard deviations of 8.3 and 12.3 respectively.

*Collection and Testing of Stools.*—Fecal specimens were recovered from commodes or diapers, placed in small metal circular containers, and kept on ice until collected 24 to 48 hours later. Fecal specimens were then stored at −20°C. until thawed for virus testing. Suspensions were prepared by transferring 3 gm. of feces to a pyrex bottle containing 27 ml. of sterile distilled water. The bottle was then placed on a shaker for 1 hour in the cold. The resulting suspensions were transferred to lusteroid tubes and centrifuged at 4000 R.P.M for 1 hour. The supernatant fluid was placed in lusteroid tubes and a mixture of antibiotics added to yield concentrations of 500 units of penicillin, 500 μg. of streptomycin, and 25 μg. of tetracycline per ml. The supernates were then held at −20°C. until thawed for tissue culture (TC) inoculation.

The tissue culture methods, and the criteria for recognizing virus-induced cytopathic changes, as used in this laboratory, have previously been described (6). Four trypsinized monkey kidney cultures grown in a nutrient medium consisting of 0.5 per cent lactalbumin hydrolysate, 2 per cent calf serum, and Hanks's salt solution were drained, and 1 ml. of fecal supernate was added to each tube. The mixture was incubated for 1 hour at 37°C. to allow virus to be adsorbed to the cells, following which the fluid was discarded, and the cell layer on the glass washed with 2 ml. of salt solution. 1 ml. of fresh lactalbumin medium in Earle's salt solution was added, and the cultures returned to the incubator, to be examined on the 2nd day, and every 3 days thereafter to the 14th day. Culture fluid of all tubes that showed cytopathic changes resembling those of viral infection were pooled and 0.2 ml. passed into each of 4 trypsinized kidney cultures and observed for virus-induced degeneration.

*Identification of Viruses.*—Infectivity titers ($TCD_{50}$ per ml.) of the viruses thus demonstrated in their early TC passage was determined in tube cultures using the cytopathic end point. Neutralization of 100 $TCD_{50}$ of virus was then attempted, using 1:25 dilutions of poliomyelitis monkey antisera, each of which had titers of over 1000 against 100 $TCD_{50}$ of homologous virus. Typing of the non-poliomyelitis viruses was attempted, using four pools of

Coxsackie hyperimmune antisera prepared, as already described, against 16 known types (7). Monkey hyperimmune serum, representative of type A9 (strain Grigg) was also used by itself, this type having been recovered from human stools on several occasions.

To determine whether the non-poliomyelitis viruses were pathogenic for infant mice, a property of the Coxsackie group, each virus in this category was inoculated into 4 litters of newborn mice, the inoculum of 0.03 ml. of TC fluid being divided between the subcutaneous and intracerebral routes. The mice were observed daily for 2 weeks and those developing paralysis, ataxia, or dying after the 2nd day following inoculation were harvested for histological examination and passage in infant mice. If a virus pathogenic for infant mice was obtained, it was considered to belong to the Coxsackie group. To determine the comparative infectivity of these viruses in TC and in infant mice, fluid from infected cultures and suspensions of the tissue of infected mice (eviscerated torso plus brain) were titrated simultaneously in monkey kidney cultures and in infant mice.

Viruses not neutralized by poliomyelitis or Coxsackie antisera, and not pathogenic for mice, were considered to belong to the orphan group. They were found to be non-pathogenic for monkeys by the intracerebral, intraspinal, intraperitoneal, and intramuscular routes. Further studies of the orphan viruses recovered in this study will be described elsewhere (8).

## RESULTS

*Types of Viruses Isolated and Their Seasonal Distribution.*—As shown in Table I, 77 virus isolations were obtained from 1558 stools examined; 15 were polio virus, 14 being Type I, and one Type II; 29 were Coxsackie viruses, and 33 were orphan viruses. During the months of June to October, 92 per cent of the virus isolations were made. Of the stools collected in District I, the lower socio-economic group, 8 per cent yielded virus in contrast to 3 per cent of those collected in District IV. In District I, 3 per cent, 6 per cent, and 14 per cent of the stools examined were positive in the years 1951 to 1953 respectively, as compared to 5 per cent, 3 per cent, and 3 per cent positive for virus during the comparable years in District IV.

The pattern of virus isolations, according to their classification, is shown in Figs. 1 and 2, for Districts I and IV, respectively. In District I, a total of 10 polioviruses were isolated, one during February, 5 during July, 2 during August, and 2 during September. Of 13 Coxsackie viruses obtained, 12 were isolated in the months of June to September, and 1 during December. Of the 25 orphan viruses, 2 were obtained during March and April, and 23 from July to October. In District IV, 5 polioviruses were isolated in July, August, and October, 16 Coxsackie viruses during the months of July to November, and 8 orphans during July to November.

Fig. 3 illustrates that the composite monthly percentages of the total number of stools collected are approximately equal ranging from 6.6 per cent to 10.1 per cent. In contrast the large majority of isolations over the 29 month period occurred from June to October. Fig. 4 shows the monthly percentage of stools yielding virus and the number of specimens collected per month over the 29 month study period. It demonstrates that the curves of Fig. 3 are made up of a cycle of events which repeats itself each year.

*Number of Viruses Isolated per Child.*—In District I, 18 children yielded

single virus isolations. Although 2 viruses were not isolated from a single specimen, 9 children yielded more than one virus, from 2 to 6 in number, in

TABLE I

*Stool Virus Isolations Obtained From Children Residing in Charleston, West Virginia*

| Month of specimen collection | 1951 | | 1952 | | 1953 | | 1951–1953 | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. tested | Per cent positive | No. tested | Per cent positive | No. tested | Per cent positive | No. tested | No. positive | Per cent positive |
| **District I** | | | | | | | | | |
| Jan. | | | 26 | 0.0 | 31 | 0.0 | 57 | 0 | 0.0 |
| Feb. | | | 27 | 0.0 | 26 | 3.8 | 53 | 1 | 1.9 |
| Mar. | | | 25 | 0.0 | 31 | 3.2 | 56 | 1 | 1.8 |
| Apr. | | | 23 | 0.0 | 19 | 5.3 | 42 | 1 | 2.4 |
| May | | | 29 | 0.0 | 9 | 0.0 | 38 | 0 | 0.0 |
| June | 6 | 0.0 | 29 | 3.4 | 16 | 0.0 | 51 | 1 | 2.0 |
| July | 6 | 16.7 | 33 | 21.2 | 17 | 29.4 | 56 | 13 | 23.2 |
| Aug. | 9 | 22.2 | 19 | 31.6 | 15 | 46.7 | 43 | 15 | 34.9 |
| Sept. | 12 | 0.0 | 31 | 12.9 | 14 | 28.6 | 57 | 8 | 14.0 |
| Oct. | 11 | 0.0 | 29 | 0.0 | 10 | 70.0 | 50 | 7 | 14.0 |
| Nov. | 19 | 0.0 | 21 | 0.0 | | | 40 | 0 | 0.0 |
| Dec. | 26 | 0.0 | 23 | 4.3 | | | 49 | 1 | 2.0 |
| All months... | 89 | 3.4 | 315 | 6.0 | 188 | 13.8 | 592 | 48 | 8.1 |
| **District IV** | | | | | | | | | |
| Jan. | | | 35 | 0.0 | 50 | 0.0 | 85 | 0 | 0.0 |
| Feb. | | | 36 | 0.0 | 38 | 0.0 | 74 | 0 | 0.0 |
| Mar. | | | 36 | 0.0 | 43 | 0.0 | 79 | 0 | 0.0 |
| Apr. | | | 29 | 0.0 | 40 | 0.0 | 69 | 0 | 0.0 |
| May | | | 42 | 0.0 | 23 | 0.0 | 65 | 0 | 0.0 |
| June | 6 | 0.0 | 39 | 0.0 | 50 | 0.0 | 95 | 0 | 0.0 |
| July | 9 | 11.1 | 36 | 11.1 | 51 | 11.8 | 96 | 11 | 11.5 |
| Aug. | 12 | 8.3 | 30 | 16.7 | 44 | 6.8 | 86 | 9 | 10.5 |
| Sept. | 16 | 0.0 | 38 | 2.6 | 42 | 2.4 | 96 | 2 | 2.1 |
| Oct. | 18 | 16.8 | 41 | 0.0 | 48 | 4.2 | 107 | 5 | 4.7 |
| Nov. | 25 | 4.0 | 31 | 3.2 | | | 56 | 2 | 3.6 |
| Dec. | 30 | 0.0 | 28 | 0.0 | | | 58 | 0 | 0.0 |
| All months... | 116 | 5.2 | 421 | 2.6 | 429 | 2.8 | 966 | 29 | 3.0 |

different samples over the course of the study (Table II). Two children yielded poliovirus, and 2 children orphan viruses for 2 consecutive months (Table III). One child yielded Type I poliovirus in July, and again in September of the same year, and one child excreted Coxsackie virus in June and August

of the same year. The remainder of the multiple isolations were of mixed types. As shown in Table IV, 15 households in District I yielded single isolations; 7 families yielded multiple isolations. In 5 of the 7 households in District I

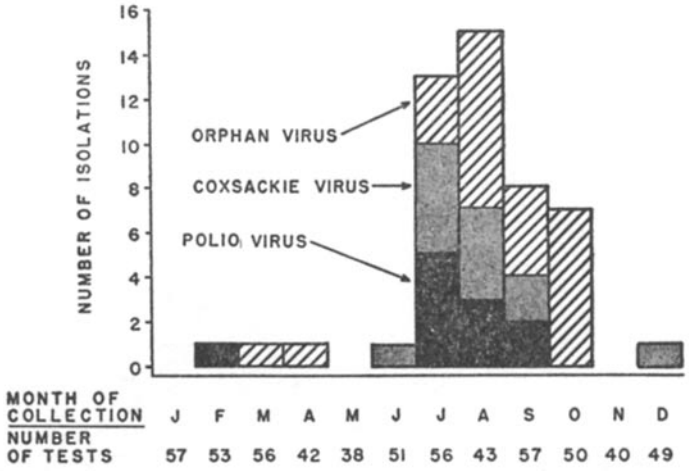


FIG. 1. Number of enteric viruses isolated in District I by month and virus type. 1951–53.

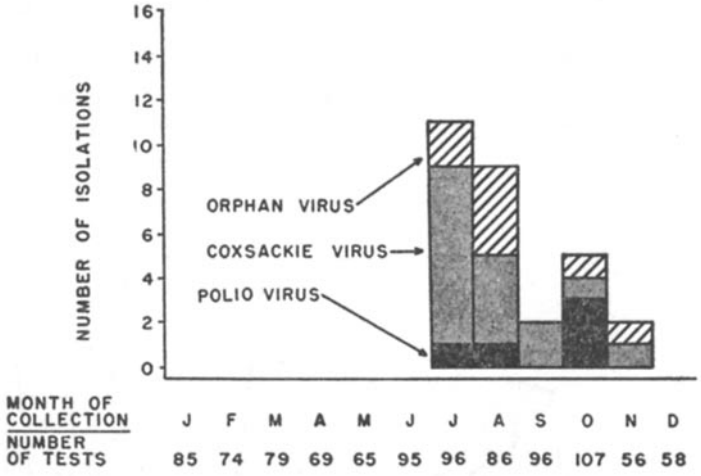


FIG. 2. Number of enteric viruses isolated in District IV by month and virus type. 1951–53.

with multiple isolations, there was simultaneous excretion of virus by more than one household member (Table V). Three children in one household yielded Type I poliovirus at the same time.

In District IV, 19 children had single virus isolations, while 4 children yielded more than one isolation over the 29 month study period. The data are

summarized on Table II. One child in District IV yielded a Coxsackie virus in 2 consecutive monthly stool specimens. The remainder of the multiple isolations in District IV were of mixed types. Fifteen households in District IV

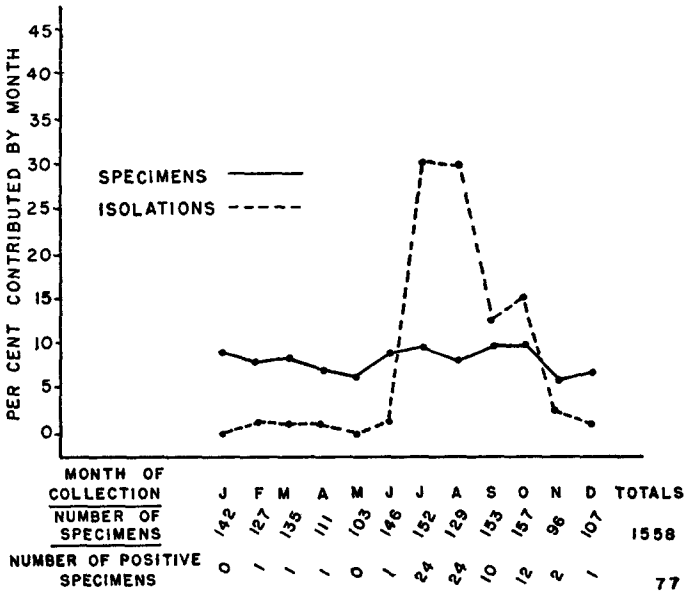


FIG. 3. Monthly percentages of the total number of stools collected and the total number of enteric virus isolations for Districts I and IV combined. 1951–53.

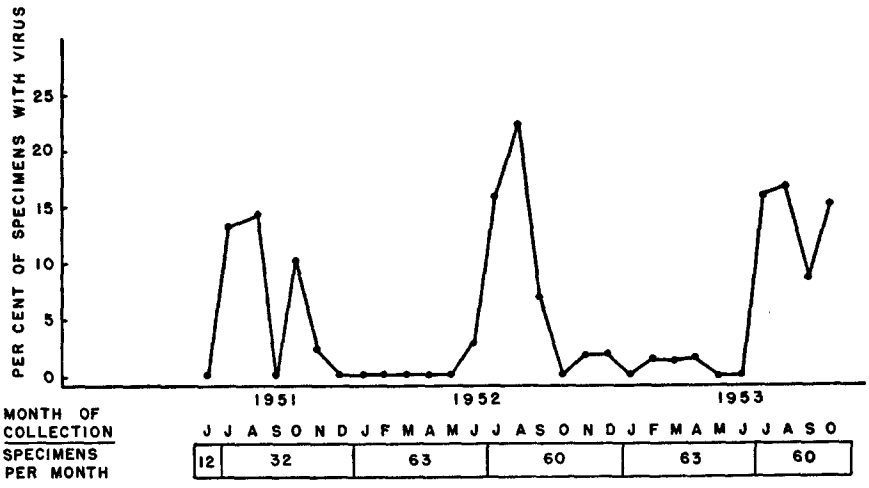


FIG. 4. Monthly percentages of enteric viruses isolated in Charleston, West Virginia, over the 3 year study period.

TABLE II

*Number of Virus Isolations Obtained from Individuals during the Three Year Study Period*

| No. of virus isolations | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| District I, No. of individuals | 18 | 3 | 2 | 3 | 0 | 1 |
| District IV, No. of individuals | 19 | 2 | 2 | 0 | 0 | 0 |
| Total No. of individuals | 37 | 5 | 4 | 3 | 0 | 1 |
| Total No. of virus isolations | 37 | 10 | 12 | 12 | 0 | 6 |

TABLE III

*Consecutive* Monthly Isolations of Viruses of the Same Kind*

| District | | Polio | Coxsackie | Orphan |
|---|---|---|---|---|
| I | No. of individuals | 3 | 1 | 2‡ |
| IV | No. of individuals | 0 | 1 | 0 |

* Consecutive isolations refer to 2 or 3 month periods.

‡ One child yielded orphan viruses for 2 months and 3 months on two separate occasions.

TABLE IV

*Number of Virus Isolations Obtained from Households*

| No. of virus isolations | 1 | 2 | 3 | 4 | 5 | 6 | 14 |
|---|---|---|---|---|---|---|---|
| District I, No. of households | 15 | 2 | 1 | 3 | 0 | 0 | 1 |
| District IV, No. of households | 15 | 1 | 4 | 0 | 0 | 0 | 0 |
| Total No. of households | 30 | 3 | 5 | 3 | 0 | 0 | 1 |
| Total No. of isolations | 30 | 6 | 15 | 12 | 0 | 0 | 14 |

TABLE V

*Types of Virus Found in Simultaneous Multiple Isolations within Households*

| District | | Isolations of the same kind | | | Isolations of different types | | |
|---|---|---|---|---|---|---|---|
| | | Polio | Coxsackie | Orphan | Polio and Coxsackie | Polio and orphan | Coxsackie and orphan |
| I | No. of households | 1* | 1 | 2 | 0 | 0 | 0 |
| IV | No. of households | 1 | 0 | 0 | 0 | 0 | 1 |

* Type I poliovirus isolated from 3 individuals in one family. All other viruses were isolated from 2 individuals within each family.

yielded single virus isolations. In 5 households, multiple virus isolations were obtained (Table IV). Simultaneous isolations of Type I poliovirus were obtained from 2 children in one household, and, similarly, simultaneous isolations of Coxsackie and orphan viruses were obtained from 2 children in another household (Table 5).

*Minor Illnesses Reported in Children Yielding Viruses.*—Table VI lists the kinds of virus isolated, dates of isolation, and illnesses in all children yielding more than one virus isolation. No unusual symptom patterns were discernible in children having Coxsackie or orphan virus isolations as compared to a control group of children whose stool specimens did not yield virus during the same months. The control group was of the same age and lived in the same districts as the group with virus isolations.

Seven of 12 children with poliovirus isolations, and 10 of their 29 family contacts gave a history of minor illness within 3 weeks prior to or after virus isolation. Four individuals with isolations and 7 contacts complained of nasal symptoms or colds; 4 individuals yielding virus and 3 contacts had diarrhea; 1 individual yielding virus and 4 contacts complained of vomiting; 2 family contacts noted headache, and 2 children yielding virus and 4 contacts noted temperature elevation. The illnesses of two of the individuals with isolations and one contact confined them to bed. Two of the individuals with isolations and 2 contacts had illnesses, the severity of which kept them semiambulatory about the home. Among 40 individuals in control family groups in the same districts from whose children no virus was obtained, 9 persons complained of minor illnesses during comparable periods of time consisting of mild colds, coughs, diarrhea, and in 2 individuals fever in various combinations. The 2 individuals with "fever" were kept at home in bed during their illness. The remaining individuals maintained normal activity. The above multiplicity and greater severity of symptoms in the poliomyelitis group involving a larger percentage of individuals may be significant and associated with poliomyelitis infection. The last column of Table VII summarizes the above morbidity data on individuals with poliovirus isolations and their family contacts.

*Homotypic and Heterotypic Neutralizing Antibody Response againsl Poliovirus.*—Sera were obtained from 10 of the children from whom poliovirus isolations were obtained, and 15 of their family contacts. Specimens were collected in the spring and fall from 1951 to 1953. However, many of the above individuals were taken into the study at varying times after its onset, and in several instances initial sera were obtained only after virus isolation.

Neutralizing antibodies against 100 $TCD_{50}$ of poliovirus were determined by the colorimetric method (9), as modified in this laboratory using versene-treated monkey kidney cells in styrene panels (6). Each serum was run at the following dilutions, using 2 cups per dilution for each virus type: 10, 50, 250. Serum antibody levels were grouped as negative, $<10$; low, 10; medium, 50; high, 250 or greater. Among the 10 virus carriers from whom sera were obtained, 9 yielded Type I and one Type II polioviruses. Eight showed a conversion from no detectable antibody to medium or high titers of antibodies. Two individuals had antibodies in medium or high titer on the first blood specimen obtained after virus isolation. In 6 of the above individuals, in addition to the development of type-specific antibodies, heterotypic re-

TABLE VI

*Individuals with Multiple Virus Isolations*

| District No. | Person No. | Date of specimen | Virus type | Symptoms and family diagnosis | Severity* |
|---|---|---|---|---|---|
| IV | 17 | 10–18–51 | Polio I | None | |
| | | 7–17–52 | Coxsackie | Cough, nasal, fever; "chest cold" | 2 |
| | 47 | 8–15–51 | Polio II | None | |
| | | 7–24–53 | Coxsackie | None | |
| | | 10–16–53 | Orphan | None | |
| | 62 | 10–15–51 | Coxsackie (A9) | None | |
| | | 11–15–51 | Coxsackie (A9) | None | |
| | | 7–28–52 | Coxsackie | None | |
| | 66 | 8–24–52 | Orphan | Nasal; "head cold" | 1 |
| | | 7–29–53 | Polio I | None | |
| I | 90 | 7–28–53 | Polio I | None | |
| | | 9–22–53 | Orphan | None | |
| | 91 | 8–16–51 | Orphan | None | |
| | | 7–22–53 | Polio I | None | |
| | | 8–21–53 | Polio I | None | |
| | | 10–13–53 | Orphan | None | |
| | 93 | 9–10–52 | Coxsackie (A9) | Nasal; "head cold" | |
| | | 7–24–53 | Polio I | None | |
| | | 10–13–53 | Orphan | None | |
| | 94 | 7–24–52 | Orphan | None | |
| | | 8–18–52 | Orphan | Nasal; "head cold" | 1 |
| | | 7–24–53 | Polio I | Diarrhea | 1 |
| | | 8–19–53 | Polio I | Vomiting, Diarrhea | 1 |
| | | 9–16–53 | Polio I | Vomiting, Diarrhea | |
| | | 10–13–53 | Orphan | None | |
| | 96 | 8–16–51 | Orphan | Cough, nasal; "cold" | 1 |
| | | 8–20–52 | Orphan | None | |
| | | 7–19–51 | Orphan | Fever | 2 |
| | | 2–4–53 | Polio I | Nasal, cough, fever, diarrhea; "intestinal flu" | 3 |
| | | 8–21–53 | Coxsackie | None | |
| | | 10–15–53 | Orphan | Headache, vomiting | 2 |
| | 105 | 8–18–52 | Orphan | Diarrhea | 1 |
| | | 8–19–53 | Coxsackie | Diarrhea, fever, vomiting | 1 |
| | | 10–15–53 | Orphan | None | |
| | 109 | 8–13–53 | Polio I | Nasal; "cold" | |
| | | 9–13–53 | Polio I | None | |
| | 121 | 6–4–52 | Coxsackie | None | |
| | | 8–27–52 | Coxsackie | None | |
| | | 3–11–53 | Orphan | None | |
| | | 4–9–53 | Orphan | None | |

* 1, normal activity; 2, confined to home; 3, bedridden.

## TABLE VII

*Poliovirus Isolations, Neutralizing Antibody Response, and Illnesses in Children from Whom Isolations Were Obtained and in Family Contacts*

| Family No. | Person No. | Age* | Poliovirus type | Type I | | | | | | Type II | | | | | | Type III | | | | | | Illnesses‡ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | '51 | | '52 | | '53 | | '51 | | '52 | | '53 | | '51 | | '52 | | '53 | | |
| | | | | S | F | S | F | S | F | S | F | S | F | S | F | S | F | S | F | S | F | |
| 1 | 1 | 2 mo. | I | § | | | | H | H | § | | | | 0 | 0 | § | | | | 0 | 0 | Nasal, diarrhea |
| | 2 | 2 | I | 0§ | L | H | | H | H | 0§ | L | 0 | | 0 | 0 | 0§ | L | 0 | | 0 | 0 | |
| | 3 | 20 | | 0 | H | H | H | H | H | H | H | H | H | H | H | H | H | H | H | H | H | |
| | 4 | 23 | | | | | | | | | | | | | | | | | | | | Headache, cold, vomiting |
| 2 | 1 | 2 | II | | § | | | 0 | 0 | § | H | | | H | H | § | 0 | | | 0 | 0 | |
| | 2 | 5 | | | | 0 | 0 | 0 | 0 | | | H | H | H | H | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 3 | 8 | | | T | T | T | 0 | 0 | 0 | H | H | H | H | H | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 4 | 34 | | L | H | H | H | H | H | L | H | H | H | H | H | L | L | L | L | L | L | |
| | 5 | 41 | | | | | | | | | | | | | | | | | | | | Fever, general aches |
| 3 | 1 | 1 | I | 0 | 0 | 0 | 0 | 0§ | 0‖ | 0 | 0 | 0 | 0 | 0§ | 0 | 0 | 0 | 0 | 0 | 0§ | 0 | Nasal, cough |
| | 2 | 4 | I | | | | | | | | | | | | | | | | | | | |
| | 3 | 31 | | 0 | | | | | | M | | | | | | M | | | | | | |
| | 4 | 27 | | 0 | 0 | 0 | 0 | 0 | 0¶ | | 0 | L | L | 0 | 0 | M | M | M | M | M | M | |
| 4 | 1 | 2 | I | | | 0 | 0 | 0§ | H | | | 0 | 0 | 0§ | M | | | 0 | NT | H§ | H | Fever |
| | 2 | 19 | | | | H | H | H | H | | | M | M | M | M | | | L | L | L | L | Fever, sore throat, cough, |
| | 3 | 1 | I | | | | | | | | | | | | | | | | | | | headache, vomiting, |
| | 4 | 25 | | | | | | | | | | | | | | | | | | | | diarrhea, cold |
| 5 | 1 | 1½ | | | | | | | | | | | | | | | | | | | | Nasal, cough, fever |
| | 2 | 3 | I | 0 | 0 | 0 | 0§ | H | H | 0 | 0 | 0 | 0§ | 0 | 0 | 0 | 0 | 0 | 0§ | 0 | 0 | Nasal, cough, fever, diar- |
| | 3 | 10 | | 0 | 0 | 0 | 0 | M | M | M | | M | M | H | H | | | 0 | L | L | L | rhea |
| | 4 | 6 | | 0 | 0 | 0 | 0 | M | H | H | H | H | H | H | H | | | | | M | L | Nasal, sore throat, cough, |
| | 5 | 31 | | | | | | | | | | | | | | | | | | | | fever |
| | 6 | 31 | | M | M | M | M | M | M | M | M | M | M | M | M | M | M | M | M | M | M | |
| 6 | 1 | 5 | I | 0 | 0 | 0 | 0 | 0§ | H | 0 | 0 | 0 | 0 | 0§ | L | 0 | 0 | 0 | 0 | 0§ | 0 | Nasal, cold |
| | 2 | 3 | | | | | | | | | | | | | | | | | | | | Nasal, vomiting, skin |
| | 3 | 23 | | L | | M | M | M | M | M | M | | M | M | M | M | | M | M | M | M | eruption |
| 7 | 1 | 3 | I | | | 0 | 0 | 0§ | H | | | 0 | 0 | 0§ | M | | | 0 | 0 | 0§ | M | |
| | 2 | 6 | | | 0 | 0 | | 0 | H | | | | | | M | | 0 | 0 | | 0 | M | |
| | 3 | 23 | | | | | | | | | | | | | | | | | | | | |
| | 4 | 28 | | | | | | | | | | | | | | | | | | | | |
| 8 | 1 | 3 | I | | | | | 0§ | | | | | | 0§ | | | | | | 0§ | | Diarrhea |
| | 2 | 4 | I | 0 | | 0 | 0 | 0§ | H | 0 | | | | NT§ | H | 0 | | 0 | 0 | 0§ | 0 | |
| | 3 | 3 | I | | | | | 0§ | H | | | | | 0§ | H | | | | | 0§ | 0 | Vomiting, diarrhea |
| | 4 | 1 | | | | | | | | | | | | | | | | | | | | Vomiting, diarrhea |
| | 5 | 6 | | H | H | H | H | H | H | H | H | H | H | H | H | M | M | M | L | L | NT | |
| | 6 | 9 | | M | M | M | M | M | H | M | M | M | M | M | H | M | H | H | H | H | H | Diarrhea |
| | 7 | 11 | | | | | | | | | | | | | | | | | | | | |
| | 8 | 16 | | H | M | | | | | M | M | | | | | M | | | | | | |
| | 9 | 17 | | H | H | H | H | H | H | M | H | H | H | H | H | M | H | M | H | H | H | |
| | 10 | 35 | | | | | | | | | | | | | | | | | | | | Nasal, sore throat, cough, |
| | | | | | | | | | | | | | | | | | | | | | | cold |
| | 11 | 39 | | M | M | M | M | M | H | M | | M | M | H | H | H | H | H | H | H | H | Nasal cold |

S, spring serum specimen.
F, fall serum specimen.
0 = < 10; L = 10; M = 50; H = 250; NT = no test; T = toxic at 10 serum dilution.
* Age at time of virus isolation.
‡ Illness within 3 weeks prior to or following virus isolation.
§ Time of virus isolation in relation to time of serum specimens.
‖ Blood drawn 4 days after stool containing virus was collected.
¶ Blood drawn 1 day before stool containing virus was collected.

sponses, *i.e.* antibody responses to types other than the infecting types, were also noted, (in confirmation of a finding first described by Sabin (10) using the Lansing mouse neutralization test in patients infected with Type I virus). The child having a Type II polio isolation also demonstrated transient low titer antibodies to Type I, and another of the above individuals yielding a Type I poliovirus developed transient low titer Type II and III antibodies. Among the remaining 4 of the above children with Type I infections who developed heterotypic responses, the number of specimens were not sufficient to determine the duration of response. Two of these individuals had antibody responses to Type II, and 2 to Type II and III in addition to Type I. Table VII summarizes the data on polio virus isolations, neutralizing antibody responses, and associated illnesses in children from whom the isolations were obtained, and their family contacts.

*Neutralizing Antibody Response in Contacts of Poliovirus Carriers.*—Among 15 family contacts, 6 developed type-specific poliomyelitis antibodies in the specimen following virus isolation from the sentinel child. Four contacts showed

TABLE VIII

*Poliovirus Type-Specific Neutralizing Antibody Response of Family Contacts of Virus Carriers*

| Age group of contact | No. of persons in group | Developed new homotypic antibody | Homotypic antibody rise | Homotypic antibody present but level unchanged | Homotypic antibody absent |
|---|---|---|---|---|---|
| 0–6 | 4 | 3 | 0 | 1 | 0 |
| 7–15 | 5 | 2 | 2 | 1 | 0 |
| >15 | 6 | 1 | 2 | 2 | 1 |
| Total..... | 15 | 6 | 4 | 4 | 1 |

a rise in type-specific antibodies; 4 contacts maintained the same antibody titer, and in one contact no antibodies appeared. Table VIII lists the antibody response of family contacts according to age groupings. Two contacts of a child excreting Type II poliovirus developed antibodies to Type II, and also low and medium levels to Type I. The latter appeared transiently lasting up to 6 months. Two contacts of children excreting Type I virus also developed antibodies to Type II and III respectively. These appeared in the last specimens collected, too close to the time of virus isolation to determine the duration of the response. The significance of transient heterotypic neutralizing antibodies following single known poliomyelitis infection, both as to mechanism of production and relationship to immunity, is not known. The low titers and transient nature of the heterotypic response in contrast to the higher titers and more permanent nature of the homotypic poliomyelitis antibody response suggest that factors other than simultaneous infection are involved.

*Orphan and Poliomyelitis Antibody Responses in Two Families with Virus*

*Carriers.*—Table IX presents data on orphan and poliomyelitis antibody responses, among the members of 2 families from whom orphan viruses were isolated from one and three of their members respectively. The 3 children

TABLE IX

*Orphan and Poliomyelitis Antibodies (AB) Present in Two Families Yielding Orphan Viruses and Type I Poliovirus*

| Family No. | Person No. | Virus type and date of specimen | Age | Orphan neutralization antibody* | | Polio neutralization antibody | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | May | Nov. | May | | | Nov. | | |
| | | | | 1953 | 1953 | 1953 | | | 1953 | | |
| | | | | | | I | II | III | I | II | III |
| 113 | 4 | | 16 | 0 | | H | | M | H | L | M |
| | 5 | | 13 | 0 | 0 | M | H | H | M | H | M |
| | 6 | | 11 | 0 | 0 | M | M | M | H | M | M |
| | 7 | | 8 | 0 | 0 | H | L | L | H | M | L |
| | 8 | | 5 | 0 | 0 | NT | NT | H | H | L | H |
| | 10 | Orphan 10/8/53 | 3 | | 0 | | | | H | 0 | 0 |
| 91 | 2 | | 41 | M | H | M | H | H | H | H | H |
| | 3 | | 18 | 0 | M | H | H | H | H | H | H |
| | 6 | | 12 | 0 | 0 | | | | | | |
| | 7 | | 11 | 0 | H | M | M | H | H | H | H |
| | 8 | | 7 | L | | H | H | L | H | H | NT |
| | 9 | Polio-I 7/22/53 | 4 | | M | 0 | NT | 0 | H | H | 0 |
| | | 8/21/53 | | | | | | | | | |
| | | Orphan 10/13/53 | | | | | | | | | |
| | 10 | Polio-I 7/24/53 | 3 | 0 | | 0 | 0 | 0 | | | |
| | | Orphan 10/13/53 | | | | | | | | | |
| | 11 | Polio-I 7/24/53 | 3 | 0 | H | 0 | 0 | 0 | H | H | 0 |
| | | 9/16/53 | | | | | | | | | |
| | | Orphan 10/13/53 | | | | | | | | | |

We are indebted to Dr. Richard A. Ormsbee for carrying out the neutralization tests against the orphan viruses.

* Orphan virus antibodies for family 91 were tested against virus isolated from person 9 on 10/13/53 and for family 113 against virus isolated from person 10 on 10/8/53.

0 = < 10; L = 10; M = 50; H = 250 or greater; NT = No test.

in family 91 from whom orphan viruses were simultaneously obtained also yielded Type I polio viruses during the same period of time. Two extremes of responses to orphan virus are shown. The virus obtained from family 113 was not considered to be present as a result of contamination from non-human sources since it could be regrown from the original stool preparation easily and was not neutralized by normal monkey serum. Further details of the

antibody responses in families in which there was an orphan virus carrier are described elsewhere (8).

None of the individuals in family 113 developed specific orphan antibodies, and no marked changes in poliomyelitis antibody patterns were noted prior to, and following, orphan virus isolation from one of the children in the family.

In family 91, two of the three orphan viruses tested, numbers 10 and 11, were found to be antigenically identical (8). Three individuals developed antibodies after the virus isolation, including one of the individuals from whom the orphan virus was isolated. In addition two family contacts already had specific antibodies against the family virus prior to the present isolation. Type I poliovirus was also recovered from individuals 9, 10, and 11 in family 91 at varying times from July to September, 1953. Two of the poliomyelitis-infected individuals from whom sera were obtained showed the development of Type I antibodies. Three family contacts showed rises in Type I poliomyelitis antibodies. The complete data on this family, including the record of minor illness at the time of the virus isolations, are summarized in Table VII.

*Some Properties of the Isolated Viruses.*—Typing of the non-polioviruses was attempted in monkey kidney TC using hyperimmune sera against 16

TABLE X

*Average Titers (Log $ID_{50}$ per ml. or per gm.) of Coxsackie Viruses in Tissue Culture and Infant Mice*

| | TC passage virus | | Mouse passage virus | |
|---|---|---|---|---|
| | TC test | Mouse test | TC test | Mouse test |
| Type A-9 Coxsackie viruses | 6.8 | 3.9 | 7.5 | 5.5 |
| Coxsackie viruses other than A-9 | 6.0 | 3.7 | 6.7 | 5.8 |

known Coxsackie types. Eight of the viruses were neutralized by Grigg type A9 antiserum, and one by a pool of immune sera containing antibodies against 5 Coxsackie viruses. However, 20 mouse-pathogenic viruses were not typable in TC with known antisera. For 15 of these strains histological examinations of muscles demonstrated myositis in 3 mice, myositis combined with steatitis in 8 mice, and no evident lesions in the sections examined in 4 mice. The myositis and steatitis produced by these viruses in mice, and the cellular degeneration observed in TC did not appear to differ from the lesions produced by the typable Coxsackie viruses.

The average titer of the 8 strains of type A9 Coxsackie virus using first or second TC passage material was $10^{6.8}$ in trypsinized monkey kidney culture, and $10^{3.9}$ in infant mice. This compares with an average titer of $10^{6.0}$ and $10^{3.7}$ for other Coxsackie viruses in TC and infant mice respectively (Table X). The average titers of Type A9 first passage mouse virus was $10^{7.5}$ in TC and $10^{5.5}$ in mice as compared to $10^{6.7}$ in TC and $10^{5.8}$ in mice for the other Coxsackie viruses, in their first mouse passage after tissue culture. The first mouse passage virus of all Coxsackie types generally showed a slight increase

in TC infectivity and a more marked increase in mouse infectivity as compared to the TC-passed Coxsackie viruses.

The log of the average titer of the polio virus strains in TC was 6.5 with a range of 4.7 to 7.0. The corresponding determination in TC for the Coxsackie viruses gave an average of 6.1, the range being 5.0 to 7.5. The orphan viruses showed an average of 5.7 with a range of 3.5 to 7.5. Among the orphan strains, at least 8 antigenic types were identified; they are described in a separate communication (8).

## DISCUSSION

During the period of study which extended for 2½ years, a repeatable seasonal incidence of enteric virus excretion was noted, over 90 per cent of isolations occurring in the months of June to October. This pattern was true for all 3 virus groups: poliomyelitis, Coxsackie, and orphan. It is noteworthy that the viral agents which inhabit the human intestinal tract have certain properties in common: seasonal occurrence, ether resistance, and size (6).

The frequency of virus isolation (8.3 per cent) in District I, the lower socio-economic group, was almost 3 times that (3.1 per cent) in District IV, a middle to upper middle class district with good environmental sanitation. The average number of persons per household in District I was 4.9 as compared to 3.7 in District IV. The average number of persons per room in District I was about one as compared to an average of 0.5 in District IV. The larger family size, and crowding in the lower socio-economic group as well as poor personal hygiene in the presence of closer personal contact may account for a greater spread and recovery of enteric viruses from District I (2).

Seven of the 12 children with poliovirus isolations, and 10 of their 27 family contacts exhibited an associated increase in minor illnesses consisting of cold or nasal congestion, fever, diarrhea, vomiting, and headache. The possibility that strains of polioviruses which are non-pathogenic for nervous tissue were isolated from mildly symptomatic or non-symptomatic children is being investigated by evaluating their infectivity, and pathogenicity for nervous tissue when inoculated into monkeys. In confirmation of Ramos-Alvarez and Sabin who isolated naturally attenuated strains from normal Cincinnati children (11), at least 3 such Type I strains have been detected among the 10 Charleston strains studied (12).

Among the 10 children with poliovirus isolations, and their 15 family contacts from whom sera were obtained, two of the individuals with isolations, and two of the contacts, developed transient heterotypic responses. (Four individuals with poliovirus isolations and two family contacts developed heterotypic responses, which could not be followed for duration of response for lack of specimens.) All individuals demonstrating a heterotypic response likewise developed a homotypic antibody response which persisted. The simultaneous heterotypic appearance of antibody at low levels which are

transient in nature (10) is compatible with more recent evidence of group antigens within the polioviruses (13, 14).

Excretion of enteric virus does not necessarily imply a causal relationship to concurrent illness. The association between virus excretion, infectivity, and symptomatology can be established only when significant numbers of individuals are studied, correlating virus excretion, appearance of antibodies, and development of symptom patterns in virus carriers and their contacts as well as in non-infected control populations.

The relatively large number of orphan virus isolations, *i.e.* 33 found among 25 of 136 normal children studied, suggests that they are commonly present in the intestinal tract. This is in agreement with the recent observations of Ramos-Alvarez and Sabin (11) who found that 25 of 31 cytopathogenic agents recovered in monkey kidney tissue cultures from the rectal swabs of 1566 healthy American children belonged to a hitherto unidentified group of enteric viruses. The fact that no unusual incidence of associated illness was noted in individuals yielding this particular group of orphan viruses, further suggests that they are mildly pathogenic or non-pathogenic for humans. However, many type-specific orphan viruses exist, and possible associations with human illness of other orphans of greater virulence may become evident. An appreciable number, for example, have been isolated from patients with aseptic meningitis (5), and this has been especially true for patients in the New England area in 1954.

## SUMMARY

An endemiological study of enteric viruses was conducted among 136 normal children, living in households in two socio-economic groups, over a 29 month period in Charleston, West Virginia.

A repeatable seasonal incidence of enteric virus excretion was noted with over 90 per cent of isolations occurring in the months of June to October. Of 592 stools examined in District I, a lower socio-economic group, 8.3 per cent yielded virus as compared to 3.1 per cent of 966 stools examined in District IV, an upper middle class district with good environmental sanitation. Among the 77 viruses isolated in tissue cultures of monkey kidneys, 44 per cent were ECHO or orphan viruses, 37 per cent Coxsackie viruses, and 19 per cent poliomyelitis viruses.

Among poliovirus carriers, and 15 family contacts, 10 individuals had simultaneous heterotypic and type-specific antibody responses. The heterotypic ones were usually present at low levels and were transient in nature.

Family infection with certain orphan viruses was also evident from antibody development which occurred following isolation of virus in the sentinel child.

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