Intestinal Viral Flora of Healthy Children Demonstrable by Monkey Kidney Tissue Culture

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Some of the complexities of laboratory support or confirmation of epidemiologic studies in poliomyelitis will be revealed in this exploratory research.

Many viruses known to be responsible for specific diseases can occur in the alimentary tract of healthy individuals. Among those that may be mentioned are poliomyelitis, hepatitis, Coxsackie, herpes simplex, mumps, influenza, ARD (acute respiratory disease), or APC (adenoido-pharyngo-conjunctival) viruses. The poliomyelitis, herpes simplex, mumps, and some members of the Coxsackie and APC groups of viruses are known to produce cytopathogenic effects in monkey kidney tissue cultures. However, the use of monkey kidney tissue cultures has revealed the existence of a whole new group of viruses which have been encountered not only in routine tests on stools of patients with suspected poliomyelitis, but also in large numbers among healthy children.

The purpose of this communication is to present the data recently accumulated in our laboratory on the viruses recovered by means of monkey kidney tissue cultures from the rectal swabs of 3,337 healthy children in this country and in Mexico. Thirty-one strains were recovered from the 1,566 specimens obtained from children aged one to 17 years during July and August, 1953, in Cincinnati,[1] and 334 strains from the 1,771 specimens obtained from children aged one to four years during June, 1954, in Mexico City and Veracruz.

Table 1 shows the frequency of poliomyelitis and other viruses recovered from children of different ages in Cincinnati. Table 2 shows the comparative incidence of poliomyelitis and other viruses among the one- to four-year-old children in Cincinnati, Mexico City, and Veracruz. Although the children in all

Table 1—Incidence of Poliomyelitis and Other Viruses in Rectal Swabs of Healthy Children of Different Ages in Cincinnati, Ohio

Age Group Years	No. of Children Tested	Poliomyelitis Viruses Per cent	Other Viruses Per cent
1– 4	154	0.6	5.2
5– 9	537	0.3	2.6
10–14	683	0.2	0.2
15–17	154	0	0
Unknown	38	0	3.4
1–17	1,566	0.3	1.6

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Table 2—Incidence of Poliomyelitis and Other Viruses Among One to Four-Year-Old Healthy Children in Cincinnati and Mexico

City	No. of Children Tested	Poliomyelitis Viruses Per cent	Other Viruses Per cent
Cincinnati	154	0.6	5.2
Mexico City	1,491	3.4	15.6
Veracruz	280	8.2	10.0

considerably in climate during the month of June—Veracruz, at sea level, is hot and humid, whereas Mexico City, at about 7,000 feet above sea level, is warm and dry.

The data shown in Table 4 indicate that among the poliomyelitis viruses recovered during these particular non-epidemic years in Mexico and Cincinnati, Type 1 was only rarely encountered and Type 3 predominated. To obtain some idea of the quantities of

Table 3—Incidence of Poliomyelitis and Other Viruses in Rectal Swabs of Healthy Mexican Children by Individual Years in One to Four-Year Age Group

Age Year	Mexico City No. Tested	Mexico City Poliomyelitis Per cent	Mexico City Other Viruses Per cent	Veracruz No. Tested	Veracruz Poliomyelitis Per cent	Veracruz Other Viruses Per cent
1	431	4.4	17.8	34	11.7	8.8
2	460	3.6	16.3	56	12.5	16.0
3	311	3.2	12.5	54	7.4	16.6
4	289	1.7	14.5	136	5.8	5.2
1–4	1,491	3.4	15.6	280	8.2	10.0

three cities derive from the lowest economic groups, it is evident that the incidence of these viruses was much higher in the Mexican cities than in Cincinnati. Table 3 shows the incidence of these viruses by individual years among the one- to four-year-old children in Mexico City and Veracruz. Most noteworthy is the approximately 12 per cent carrier rate of poliomyelitis viruses among the one- and two-year-old children in Veracruz. Although the poliomyelitis carrier rate was found to be much higher among all one- to four-year-old children in Veracruz than in Mexico City, the total carrier rate for viruses recovered by monkey kidney tissue cultures was not significantly different in these two cities which vary

poliomyelitis virus that may be present in the relatively minute amounts of feces picked up by the rectal swabs titrations were performed on 56 of the Mexican specimens. The data shown in Table 5 indicate that in approximately half the swabs the amount of virus was in excess of 10^3 TCD_{50} and occasionally as high as 10^5 to $10^{5.9}$ TCD_{50}.

Table 4—Incidence of Each Type of Poliomyelitis Virus Among 78 Strains Isolated from Healthy Children

City	Type 1	Type 2	Type 3
Mexico City	4	9	37
Veracruz	0	7	16
Cincinnati	0	3	2

Table 5—Quantitative Determination of Poliomyelitis Virus in Rectal Swabs from 56 Healthy Mexican Children

Type of Virus Recovered	No. of Rectal Swabs Tested	No. of Rectal Swabs Containing Indicated Log TCD_{50} of Virus					
		$< 10^1$	10^1–$10^{1.9}$	10^2–$10^{2.9}$	10^3–$10^{3.9}$	10^4–$10^{4.9}$	10^5–$10^{5.9}$
1	4	0	0	2	0	0	2
2	10	0	0	4	3	3	0
3	42	2	3	18	13	3	3
1 + 2 + 3	56	2	3	24	16	6	5

Characteristics of the Nonpoliomyelitis Viruses

Among the 26 nonpoliomyelitis viruses recovered from the Cincinnati children only one proved to be pathogenic for suckling mice and by serologic tests was identified as a Coxsackie, Type B_4. The remaining 25 viruses were found to belong to five distinct antigenic types. In view of the possibility that some of the naturally occurring Coxsackie viruses may not be pathogenic for suckling mice we tested our five distinct prototype viruses with antisera against the five Group B (Types 1–5) and seven Group A (Types 7, 9, 11, 13, 14, 15, and 18) Coxsackie viruses which have been found to produce cytopathogenic effects either in monkey kidney or HeLa cell cultures, or in both. All these Coxsackie antisera were kindly supplied by Dr. Gilbert Dalldorf and were used in a dilution of 1:5 in the neutralization tests, but none neutralized 100 TCD_{50} or less of our five new prototypes. It was also established that our five prototype viruses are different from the six reported [2] and unreported antigenically distinct "orphan" viruses ("Farouk," "Cornelis," and "Morrisey," "Pesascek," "Noyce," and "D'Amori") currently available in Melnick's laboratory, from the "Mack" and "Kentucky" strains of Steigman,[3] and the 2, as yet unreported, antigenically distinct viruses ("2–85" and "2–188") recovered by Hammon in the Philippines.[4]

In cynomolgus monkey kidney tissue cultures our Types 1, 2, 3, and 5 produce cytopathogenic effects which are indistinguishable from those exhibited by the poliomyelitis viruses. The Types 3 and 5 viruses had longer incubation periods in the initial passages, but after four passages in tissue culture they produced the cytopathogenic effect as rapidly as the others. Our Type 4 virus can be distinguished from all the others, as well as from the poliomyelitis, herpes, and mumps viruses, by the distinct cytopathogenic effect, consisting of a clumpy degeneration of the cells which characteristically appears after an incubation period of from two to ten days depending on the amount of virus.

It is noteworthy that only the Type 4 virus was capable of producing a cytopathogenic effect in cultures of kidneys derived from Cebus capucinus monkeys, which were resistant to standard strains of the three types of poliomyelitis virus as well as to our four other prototypes. We have been informed by Drs. Irving Gordon and William Jordan that our five prototype viruses can be propagated in HeLa cells, although the yield of virus is low. We have also been told by Dr. Robert Huebner that our five prototype viruses do not belong to the APC group and by Dr. Robert N. Hull that they are distinct from the cytopathogenic agents that he recovered from normal monkeys. It

should be noted here that human gamma globulin was found to contain antibody for all but our Type 2 prototype virus. However, antibodies for the Type 2 viruses have been demonstrated in individual human sera and its absence from gamma globulin in titers of 1:100 or more appears to be a reflection of the low incidence or low titers (or both) of antibody against this particular type.

Our five new prototype viruses are not pathogenic for suckling mice nor for adult mice by the intracerebral or spinal routes. They are not pathogenic for rabbits by the intracutaneous, intramuscular, or intravenous routes. The Types 1, 2, and 3 viruses were not pathogenic for cynomolgus monkeys by the spinal route but antibody developed as a result of the inoculation. All five prototype viruses pass gradocol membranes with an APD of about 300 mμ, but only the Type 4 virus has been tested more completely and found to have a size of about 60 to 90 mμ.

The frequency distribution of our five prototype viruses among the nonpoliomyelitis cytopathogenic agents recovered from healthy children in Cincinnati and Mexico is shown in Table 6. It is noteworthy that the Type 1 virus, representing 32 per cent of the Cincinnati strains, was not found at all among the 261 Mexican strains, and that the Type 2 virus, with an incidence of 44 per cent in Cincinnati, constituted only 8.1 per cent of the Mexico City strains. On the other hand, the Type 5 virus which had the lowest incidence in Cincinnati in 1953 had the highest incidence in Mexico City in 1954. It is, furthermore, noteworthy that only Type 5 virus was found in Veracruz and that only in an incidence of 7.1 per cent. Preliminary neutralization tests on the unclassified Mexican strains using pools of Coxsackie, as well as of five Melnick and two Hammon prototype antisera, indicate that the incidence of Coxsackie viruses is very low and that about 90 per cent of the strains still remain unclassified.

Only preliminary serologic surveys have thus far been carried out on the incidence of infection with the five Cincinnati prototype viruses among human beings. The results shown in Table 7 indicate that among the 61 Cincinnatians the incidence of antibody was much higher among those 20 to 30 years of age than among those one to five years of age. It is of interest that the total incidence of antibody among 19 children from India, mostly under four years of age, was as high as among those 20 to 30 years of age in Cincinnati. It is, furthermore, noteworthy that only 3 per cent of the Cincinnatians had

Table 6—Incidence of Each of the Five Types of Cincinnati "HE" Viruses Among the Nonpoliomyelitis Cytopathogenic Agents Recovered from Healthy Children in Cincinnati, Mexico City, and Veracruz

"HE" Virus Type	Cincinnati 25 Strains Per cent	Mexico City 233 Strains Per cent	Veracruz 28 Strains Per cent
1	32	0	0
2	44	8.1	0
3	12	2.6	0
4	8	1.3	0
5	4	15.4	7.1
Unclassified	0	72.6	92.9

Table 7—Incidence of Neutralizing Antibodies for Five Types of Cincinnati "HE" Viruses Among Human Beings in Cincinnati and India

Population	Age Group Years	No. Tested	Per cent Positive for Indicated Type of "HE" Virus					Per cent Positive for One or More Types	Per cent Positive for All 5 Types
			1	2	3	4	5		
Cincinnati	1–5	31	13	6	29	10	13	48	0
	20–30	30	30	13	40	63	40	87	3
India	1–4	19	53	10	37	26	42	84	5

antibody against all five types by 20 to 30 years of age.

The ultimate significance of these new viruses in the etiology of human disease still is a problem for future study. It is clear, however, that they are not the viral counterpart of the normal bacterial flora of the human enteric tract because they are found most frequently during the early years of life, and at least in Cincinnati were found only rarely after the 10th year of life. Some of the "orphan" viruses recently recovered by Melnick and his associates [5] from the stools of patients with a diagnosis of either nonparalytic poliomyelitis or the aseptic meningitis syndrome were found to be identical with our Types 1 and 3 prototype viruses and their relation to this type of illness must be investigated further. One of us (A.B.S.) recently found that a virus that was associated with an epidemic of rhinitis in chimpanzees, as well as another virus that was associated with a family outbreak of an acute "steatorrheic" enteritis were antigenically related but not identical with the Cincinnati Type 4 virus.

This observation suggests that at least some of the new viruses, which are demonstrable by monkey kidney tissue culture in the human enteric tract, may ultimately prove to be related to infections of both the respiratory and enteric tracts.

Appendix

Since presentation of this paper, the so-called "orphan" and HE viruses have been grouped together under the name of ECHO (enteric cytopathogenic human orphan) viruses [see Science (Dec. 16), 1955, 122:1187–1188]. In accord with this new classification the five Cincinnati HE viruses are now known as ECHO viruses, Types 7 to 11.

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