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Role of the echo viruses in the etiology of gastrointestinal disease

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THE ROLE OF THE ECHO VIRUSES IN THE ETIOLOGY OF GASTROINTESTINAL DISEASE

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Introduction

The ECHO (enteric cytopathogenic human orphan) viruses, having been originally isolated in most part from nonparalytic poliomyelitis patients, were "orphans" in the sense that their relationship to human disease was unknown. Indeed, as recently as 1960, Smith and Conant write in Zinsser's Microbiology that these viruses "...do not appear to be associated with any demonstrable disease...." (1) Since then, they have been clearly shown to be definite causes of a variety of clinical syndromes; these will be mentioned later.

However, the relationship of ECHO virus infection to gastrointestinal disease is not clear. It is true that in many syndromes definitely associated with ECHO virus infection, such as paralytic disease and pleurodynia, diarrhea may be a symptom. Also, enteroviruses are frequently isolated from the contents of the normal gastrointestinal tract. However, the primary point to be considered in this paper is whether the ECHO viruses can primarily infect the GI tract with the production of gastroenteritis alone and, if so, how important are they in the production of diarrheal disease.

The general nature of ECHO virus infections (e.g. biological characteristics, pathogenesis, clinical manifestations), as found in four excellent reviews (2,3,4,5) will be discussed first. This will be followed by a review of the literature dealing with

studies attempting to link ECHO viruses with gastrointestinal disease, and finally a general summary of the available evidence and conclusions.

History

Twenty-seven antigenic types of ECHO viruses have been recognized; at least six more await approval as new serotypes. ECHO viruses, along with poliomyelitis and Coxsackie viruses, comprise a group known as the Enteroviruses; these share such properties as size, tissue culture cell affinities, excretion from the human gut, and epidemiologic patterns. ECHO viruses were first isolated in 1951 when Robbins (6) and associates isolated two agents that were not polioviruses; one demonstrated cytopathogenicity, yet was not pathogenic for suckling mice. In 1955, a special committee was formed by the National Foundation for Infantile Paralysis to consider this new group; they were named "ECHO" at this time. (It is interesting to note that originally it was planned that when a virus' relation to a specific disease was established, it was then no longer "orphan" and would be removed from the ECHO group. However, this has not been done.) Thirteen prototype virus types were recognized then.

Biological Characteristics

The ECHO viruses thus far measured have had sizes ranging from 20 to 30 millimicrons. They are probably spherical, and are relatively stable; optimum preservation demands storage at

-20 degrees Centigrade or lower. They are not inactivated by ethyl ether. Data suggests that ECHO viruses, like polioviruses, consist of an RNA core surrounded by a protein covering, although RNA-fractions have been reported for only several serotypes. ECHO viruses are optimally isolated in tissue cultures of rhesus or cynomologus monkey kidney cells.

Host Range and Pathogenesis

In general, ECHO viruses are infectious only for man. They are non-pathogenic for embryonated hens' eggs, mice, hamsters, rabbits or guinea pigs. ECHO viruses are transitory (not constant, as are enteric bacteria) inhabitants of the human alimentary tract, and their presence may produce various diseases or go unrecognized. However, it is believed that asymptomatic infections due to ECHO virus are uncommon, with 85% of virus isolations associated with illness (7). Present in the alimentary tract as pathogens rather than commensals, at least as far as is known, they give rise to apparent or inapparent infections.

Generally, the incidence of enteroviruses in the stools of healthy persons varies with age (higher in young children than adults), season (higher in summer months), and socio-economic status (higher in persons of lower economic status). Also, dominance of a particular serotype is a general rule, and different serotypes predominate in different years. Apparently, certain of the ECHO virus serotypes are found in healthy persons, and

others are more commonly associated with disease; thus, they vary in their pathogenicity for human beings.

Fecal carrier rates have ranged from 1.5 to 20%, depending on time and place. In temperate climates carriers are encountered more often during summer and autumn. In semitropical or tropical areas the carrier rate is independent of season. Since infants and children are the primary hosts, carrier rates may exceed 50% in selected population groups such as orphanages or nurseries (8).

Among healthy children, relatively high carrier rates for ECHO viruses have been found. In children aged one to four years, Ramos-Alvarez and Sabin (9,10) found an incidence of five percent in Cincinatti during summer, and of ten and 16% respectively in two Mexican cities during early summer. Considering these figures, one must conclude that ECHO viruses frequently, perhaps usually, produce subclinical infections.

As with the Coxsackie and polioviruses, ECHO viruses usually enter the human host through the oropharynx and are shed in oropharyngeal secretions and the feces. All 27 serotypes have been detected in feces. They have also been found in the nasopharynx. The virus is usually confined to apparent target cells in the alimentary tract, but may pass through the blood and metastasize to secondary target organs. The incubation period ranges from two to 20 days, usually five to 10 days. Knowledge of pathology is scant because of the few deaths attributed to ECHO virus

infections.

ECHO viruses are distributed worldwide, and based on recovery of prevailing serotypes and on antibody surveys, these agents have been found in the United States, Mexico, the Philippine Islands, India, Germany, Hungary, Scotland, and Egypt. Epidemics are most common in temperate zones during the (late) summer and fall months; indeed, all outbreaks in these zones have occurred at these times. Discrete outbreaks with accumulation of clinical cases have not been encountered in the tropics. ECHO virus infections occur mainly in children six to 15 years of age, presumable because of their antigenic inexperience, and in young adults. The decreased incidence of illness and possibly infection among older persons is dependent upon previous active immunization, and possibly more restricted exposure. There is no significant difference regarding sex incidence.

Dissemination of virus from the infected human into the community occurs via the stool and oropharyngeal secretions, apparently predominantly by the former. The virus may be shed in the stool as long as about seven weeks after onset of disease. Extra-human vectors have not been found to play a significant role in the dispersal of ECHO viruses in the human population. Throat swabs, except during the acute phase of the illness, contain little or no virus.

Clinical Manifestations

A broad spectrum of clinical manifestations is produced by ECHO viruses. A biphasic character of onset is common with ECHO infections. A first phase, with fever, anorexia, vomiting, and sore throat, is often followed by a short period in which the patient is asymptomatic, this being followed by abrupt onset of the major phase.

(a) Constitutional findings such as fever (100%), headaches (95%), myalgia (41%), anorexia, and nausea and vomiting (66%) are common.

(b) Central nervous system manifestations include an aseptic meningitis syndrome with headache, fever, neck and back stiffness, and positive Kernig's and Brudzinski's signs. This is the most common clinical picture associated with ECHO virus infections (presently 17 serotypes have been associated with this disease) and usually runs a benign course.

(c) Exanthematous eruptions, usually characterized by a rubelliform rash consisting of discrete pink to red macules or maculopapules, are a large part of the clinical picture.

(d) Regarding respiratory manifestations, sore throat is a common finding, with coryza, conjunctivitis, myringitis, and cough observed infrequently.

(e) Gastrointestinal manifestations are the subject of this paper.

As indicated previously, ECHO viruses are easily isolated from throat swabs taken during the acute stages of the disease and from rectal swabs or stool specimens up to seven weeks after onset of the disease. Cerebrospinal fluid specimens will also yield virus when taken in the acute stages of patients with central nervous system manifestations. Convalescent serums demonstrate a rise in neutralizing antibodies (which generally are type specific) and complement-fixing antibodies; there is no common complement-fixing antigen for the ECHO group.

ECHO virus infections usually have a good prognosis, and are seldom associated with serious residua. Persistent paralysis or death was seen only in the early cases reported by Kibrick (11) and others and by Steigman (12) and associates. As they are not susceptible to antibacterial drugs, treatment of mild ECHO virus infections is symptomatic. There is no available active immunizing agent for any ECHO virus, and no specific control measures are available.

Early Evidence of Viruses as Cause of Gastrointestinal Disease

The large number of cases of diarrheal disease to which no definite bacterial or protozoan pathogen could be assigned led for many years to an assumption that viruses must play an important part in the etiology of this syndrome. There was sufficient evidence available to support this etiologic relationship. Light and Hodes (13), in 1943, produced diarrhea in calves by feeding

a filtrate of stool from cases of epidemic diarrhea in newborn infants. Serial passage was made 29 times in calves. The agent produced the disease in calves by parenteral inoculation of blood from sick calves and cross-infection occurred.

Gordon (14) and others, in 1947, reported the production of diarrheal disease in human volunteers following oral administration of filtered stool suspension and throat washings from patients suffering from epidemic diarrhea in a State Hospital. The disease was carried through three passages in h uman volunteers.

In 1945, Reimann (15,16) and his group, reported on several epidemics of diarrheal disease in which a stool filtrate, after nebulization and inhalation, produced similar diarrhea in human volunteers; oral feeding was not effective in reproducing the disease. Several relevant reports also appeared from Japanese workers.

Even though there was no positive evidence as yet to prove the relationship, largely because viruses had not yet been grown and studied, there was sufficient presumptive evidence at hand to prompt Higgins (17) to write in an editorial in The American Journal of Medicine in 1956 that "...there is impressive evidence that filtrable agents, or viruses, may be the proximate cause of several clinical syndromes which are frequently characterized by diarrhea."

Diagnosis of Enterovirus Infection

A virus infection may be diagnosed by the isolation of virus from the patient, and/or by demonstration of a rise in specific serum antibodies during the illness. von Magnus (18) points out that for the enteroviruses, the study of the patient's sera unfortunately is not very suitable for diagnostic purposes. He gives the following reasons: The antibodies may have reached their peak by the time the first signs of illness are recognized. Secondly, because of lack of knowledge concerning group antigens, each one of the twenty-odd antisera has to be included in the tests if one wants to look for a rise in ECHO antibodies. Thirdly, heterologous antibody responses are quite common with the enteroviruses. He goes on to state that even though a reliable diagnosis of an enterovirus infection depends on the isolation and identification of the virus itself, this isolation does not necessarily mean that the causative organism has been found. For example, adenoviruses may be found in the stools for several months after the initial infection. Also, as stated previously, subclinical infections with enteroviruses are very common, especially in childhood.

Huebner (19) has recommended that viruses must satisfy the following conditions in order to be considered as the cause of illness: (a) The virus must be well established by animal inoculation or tissue culture and passage; (b) Its h uman origin must

be proved by repeated isolation; (c) It must be shown to produce infection as evidenced by the development of antibody; (d) It must be constantly associated with a well-defined clinical syndrome; (e) It must produce illness corresponding to the natural disease in man following experimental inoculation.

Investigations which have dealt with the subject of ECHO viruses and their relationship to gastrointestinal disease have, in general, been of three main types. One concerns the epidemiology of the enteroviruses in healthy people; samples of findings in this category have already been mentioned. Another type has been controlled studies of large numbers of patients with sporadic diarrhea, and a third type of study has to do with sharply defined outbreaks of gastroenteritis. There have also been experiments in which human volunteers were inoculated with ECHO viruses, after which the clinical response was noted and attempts made to isolate the virus and detect a rise in antibody titre.

A thorough perusal of all the available investigations on this subject has been made. This literature summary will now be presented, reviewing articles both supporting and questioning ECHO viruses as a cause of gastrointestinal disease.

The Case For ECHO Viruses as Etiological Agents in Gastrointestinal Disease

Eichenwald (20) and associates documented the very first study in which a virus isolated in the laboratory was shown to be a cause of an outbreak of epidemic diarrhea. This actually

consisted of two separate outbreaks of diarrhea, and occurred in New York Hospital in the summer of 1956.

Mild diarrhea (which persisted one to five days and was treated with supportive therapy) developed in 12 out of 21 infants in a premature nursery. Of these 12 cases, ECHO 18 virus was cultured from rectal swabs in ten cases, and was not recovered from the well infants. All infants with this diarrhea showed a significant rise in antibody to ECHO 18, and this rise in titre was related, in time, to the course of the illness. It is also significant that the virus was found only during the outbreak--it was not present before or after.

Shortly after this epidemic, diarrhea developed in five infants on another ward in the same hospital; this occurred following exposure to a nurse who was known to be excreting ECHO 18 virus. The virus was cultured in all of these five cases, and was not found in ten other infants on the same ward.

The validity of this study is strengthened by the number of patients involved, the fact that the study was controlled, and that virus isolation studies were supported by serological data.

Another series, which also meets these criteria, was published just two months later (also in the J.A.M.A.) by Ramos-Alvarez and Sabin. (21) This study, from Cincinnati, presents what is generally considered probably the best evidence of association

of enteroviruses with diarrheal disease, and is especially significant with regard to the ECHO group.

In the summer of 1955, out of 56 rectal swabs taken from infants and children with diarrhea, viruses were isolated in 28 (50%). In the summer of 1956, viruses were isolated in 48% of 97 swabs taken from diarrhea cases; 100 matched controls also studied in 1956 showed a 20% isolation rate. In all, 42 ECHO viruses were recovered from the children with diarrhea. The incidence of ECHO viruses was six times greater in the diarrhea group than in the control group, while the incidence of polio and Coxsackie viruses was similar in both groups.

A study by Cramblett (22) and associates was carried on from July of 1960 through September of 1961. During this period, ECHO 19 was recovered from specimens of 30 patients. Twelve of these were infants with upper respiratory infections (in several of these patients, there were "loose and malodorous" stools; however, the significance of this is questionable).

In two of the 30 patients, diarrhea was the chief manifestation. One of these was a laboratory technician who, while working with the virus, became accidently infected and 48 hours later developed nausea, abdominal pain, and diarrhea, with no other symptoms; this persisted for 36 hours.

The virus was neutralized by both the patients' sera and by ECHO 19 rabbit antisera, and precipitins against ECHO 19 were

present in some of the convalescent sera of the patients.

Thus, although the number of patients involved here is small (only two of the 30 had solely gastrointestinal disease), and there are no control studies, the incident regarding the laboratory technician might be comparable to a human volunteer study.

Three outbreaks of gastrointestinal disease which were etiologically attributable to ECHO and polioviruses are presented by Giovanardi and Bergaurini (23). Diarrhea was the major symptom in these epidemics, and they occurred in northern Italy in 1959 and 1960.

In the first series of cases, tests were conducted on 20 patients and on 18 healthy "contacts." All stools were studied for pathogenic enterobacteria and staphalococcus, and were negative. The viruses which were isolated were adenovirus type 3, polio types I and III, and ECHO types 9, 11, and 14. However, a significant number of patients showed serologic evidence of infection only for ECHO 14 (10), ECHO 9 (7), and polio type III (6). There was a relatively low incidence of isolation of these agents.

The second epidemic presents a better "case" for the ECHO viruses. This outbreak was associated only with ECHO 11 virus. Out of 13 infants who were quartered together, six became ill with gastrointestinal symptoms in a period of four days. The ECHO 11 virus was isolated from the stools of all but one of this

group of six; the only exception was an infant who died on the fourth day of illness and was not examined virologically. In all of the available paired sera there was a four-fold or greater increase in neutralizing antibody for the isolated virus. Again, as in the first epidemic, no enterobacteria or staph. were isolated from the stools.

The third epidemic was associated only with poliovirus type II.

Ramos-Alvarez (24) studied 56 children less than four years of age in Cincinnati during the summer of 1955. These children, which included both clinic and hospital cases, all had diarrhea as a chief symptom; fever, vomiting, abdominal pain, and blood and mucous in the stools were also present in many of the cases.

Of the 56 children tested, viruses were recovered from the rectal swabs of 24 (using monkey kidney tissue cultures). Of these 24 (considering only ECHO viruses), three were found to have poliovirus, seven (29%) had ECHO virus (including one patient with type 2, one with type 8, two with type 11, and three with type 12), and Coxsackie virus was isolated from four of the 24 patients.

Neutralization tests were done with the sera of 19 patients, against the virus which was recovered from their own rectal swabs. In 14 of these a significant increase in antibody was noted in the convalescent serum specimen. Six of these 19 had grown out ECHO viruses. Two of the six showed low antibody response (acute

to convalescent antibody titre was 0 to 8 and 2 to 10). The responses of the other four "ECHO virus patients" were good (less than 10 to 32, less than 10 to 100, and two showing response of less than 10 to 320-plus).

Again, considering data such as the above, case reports such as this do not, in themselves, prove that a causal relationship exists, but, quoting the author, "...the fact that most of the patients from whom a virus was recovered developed antibody shortly after disappearance of clinical manifestations suggests that the virus infections and the disease were at least concurrent and could have been etiologically related."

Lepine (25) and associates, from the Institut Pasteur in Paris, present data which suggest that ECHO 14 may be highly contagious in a nursery. In July of 1959, three babies in a nursery had gastroenteritis with bloody stools. Using rectal swabs, 13 strains of ECHO virus type 14 were isolated in the three children and in 20 contacts. (This shows one of the few instances of virus isolation in epidemic infantile diarrhea.) No pathogenic bacteria were found in the stool cultures.

Also using rectal swabs, ten other strains of ECHO 14 were isolated from 20 children in the same nursery. The carriers had no symptoms or signs except for a stationery weight for three or four weeks. Antibodies to ECHO 14 were demonstrated in two of the three infants with diarrhea and in seven of the ten healthy

carrier infants.

From Ruchill Hospital in Glasgow comes a report by Sommerville (26), the data having been collected from April 1957 to 1958. During this one year period, from the stools of 338 children (all of whom were five or less years old) admitted to the hospital with diarrhea, 75 enteroviruses were isolated, i.e., an isolation rate of 22%. Seventeen enteroviruses were isolated from a control group of 115 children admitted, during the same time period, with respiratory infections (isolation rate of 14%).

The proportion of polioviruses and Coxsackie viruses were similar in both groups. However, ECHO viruses were isolated in 8.5% of the group with diarrhea, and in 2.5% of the control group with respiratory illness. Thus, both with the 22 vs. 14 percentages, and with the 8.5 vs. 2.5 percentages, we do have a difference, but one that is not significant.

The enteroviruses isolated included three types of poliovirus, three types of Coxsackie, and ECHO types 6, 7, 9, 11, and 13. Those most frequently isolated were ECHO 7, Coxsackie A9, and poliovirus type I.

This report again suggests a causal relationship between ECHO viruses and diarrheal disease. However, it is obvious that the results are not markedly significant, and are not supported by serological data. The latter point is a glaring sin of ommission in this experiment.

Previous to a report by Berkovich and Kibrick (27) in April of 1964, infection with ECHO 11 in the newborn infant had not been recorded. They describe a late summer outbreak of illness involving newborn infants (seven) and mothers (five), which, besides ECHO 11, also involved ECHO 18 and Coxsackie A9 viruses. Besides diarrhea, clinical signs also included fever, upper respiratory disease, and aseptic meningitis.

Of the 12 patients studied, five had gastrointestinal symptoms; these are summarized in Table I:

CASE	PT.	DATE OF SERUM SPEC.	SYMPTOM	STOOL ISOLATE	NEUT. ANTIBODY TITRE			H-I AB. TIT.
					ECHO 11	ECHO 18	COX.A9	ECHO 11
2	Baby boy	-----	Mild diarrhea	None				
3	Baby girl	None	Fever, "red throat", diarrhea	None				
4	Mother	9/25/59	Diarrhea,	-----	1:2	1:8	1:512	1:128
		10/21/59	severe		1:16	1:8	1:512	1:256
		2/27/61	headache		1:2.8		1:178	1:32
5	Baby	9/23/59	Fever,	ECHO	1:11	1:8	1:64	1:512
	girl	10/21/59	diarrhea	11	1:128	1:8	1:32	1:512
8	Baby	9/22/59	Fever, di-	ECHO	1:128	1:8	1:8	1:256
	boy	9/26/59	arrhea, a-	11	1:64		1:8	1:5120
		10/23/59	septic meningitis		1:512	1:8	1:8	1:1024

TABLE I

It is obvious that the material here is quite "weak" in a number of ways. The study was not aimed primarily at ECHO viruses and diarrheal disease. Perhaps, in case number eight, the association of diarrhea along with the fever and aseptic meningitis is coincidental. The "mild diarrhea" reported for case number two appears suspicious. None of the stools were studied

for possible bacterial pathogens. There were no actual stool isolates in three of the cases. Serological evidence also appears weak in places, and obviously this is where the authors placed most of their emphasis.

Cramblett (28) and associates present six infants, all five to 14 months of age, from whom the virus isolated was the JV-1 virus, a relatively newly recognized virus. It was first recovered from these infants in the time period between two days preceeding and the day after onset of symptoms.

The symptoms consisted mainly of a mild clinical respiratory illness involving coryza, pharyngitis, and fever of short duration. However, in all of the patients, the stools were abnormal during the first two or three days of illness. In four of the six infants, frank diarrhea, with copious, foul, watery stools was present. The stools of the other two patients were also of this description, but of normal frequency. Vomiting occurred in five of the six infants.

The acute and convalescent sera showed a rise in titre of both neutralizing and complement-fixing antibodies against the JV-1 virus. Except for one case, there were no rising antibodies against adenoviruses, Coxsackie A viruses, ECHO type 8, and influenza A', B, and C viruses.

Following a discussion on general properties, pathogenic properties, and diagnosis of ECHO viruses, Verlinde (5) presents

1553 patients from whom one of the enteroviruses was isolated, and what clinical syndromes were seen in these patients. This study was done over the period 1955 to 1961. Fifty-eight of the 1553 patients were classed as having "diarrheal disease." The rest of the clinical entities were grouped as pharyngeal gastrointestinal illness, aseptic meningitis, encephalitis, and paralytic disease.

Of the 58 patients with diarrhea, poliovirus was isolated from 12%, Coxsackie from 44%, and ECHO virus from 44%. There were no controls in this study, no serological investigation was carried out, and there is no mention of the types of ECHO virus which were isolated. The author made no attempt to reach any particular conclusion. It was apparent that this was not an experiment aimed at establishing a relationship between ECHO virus and gastrointestinal disease.

Klein (29) and others, from Boston City Hospital, show some evidence implicating ECHO 11 virus in acute gastroenteritis in adults. The gross weakness of the study appears to be that only three patients were involved. They had symptoms, of abrupt onset and brief duration, which included watery diarrhea, severe vomiting, chills, and abdominal cramping.

ECHO 11 virus was recovered from the rectal swab and acute serum of two of these patients. One of the patients had a twofold rise in neutralizing antibody, the other had no rise. In

a third case with the same symptoms, no virus was isolated, but a four-fold rise in neutralizing antibody to ECHO 11 was found.

Inoculation Experiments

There have been two reports of inoculation transmission experiments using ECHO viruses, with resultant gastrointestinal disease. Buckland (30) and associates write on the "Experimental infection of human volunteers with the U-virus--A strain of ECHO virus type 11." In the first part of this article, the authors confirm that the "U-virus" is, indeed, a strain of the ECHO group. They go on to describe two transmission experiments in which the dose of virus--10^5 TCD_{50} in 1 ml.--was instilled as nasal drops.

In the first experiment, nine test subjects received the virus culture fluid from the third tissue culture passage of human embryo-lung culture, while 11 controls received the culture fluid without the virus. Eight of the test subjects developed definite illness one to three days after inoculation; in four of these there was abdominal pain, three of whom also had diarrhea. The eight patients who became sick had other symptoms including malaise, sore throat, fever, etc. However, the symptoms were mainly gastrointestinal in nature. Of the 11 controls, nine remained completely asymptomatic, one had some abdominal pain, and one a slight fever. In the test group, virus was recovered from the stools of all nine subjects and from the throats of all but one.

In the second transmission experiment, the inoculum was

culture fluid from the eleventh passage of the virus in monkey kidney tissue culture. Here, none of the eight test subjects developed any definite illness, and the seven controls also remained well. Virus was recovered from the feces of all eight test subjects, and from the throats of five of the eight.

Rather extensive serological investigations were also done. Summarizing the two series, experimental infection induced an average antibody rise of about 30-fold as judged by hemagglutination-inhibition or about 100-fold according to neutralization studies.

Besides the significance of this study as regards ECHO viruses as causes of GI disease, one can note the significance concerning immunization potential. For example, passage in human embryo-lung culture resulted in clinical symptoms (and also antibody response) but passage in monkey kidney tissue culture with subsequent experimental inoculation resulted in a significant rise in antibody titre but no clinical illness. This would make the latter more desirable for immunization investigations.

Buckland (31) and his group describe another human inoculation experiment, using ECHO virus type 20 as nasal washings or tissue culture fluids. Forty-three volunteers, who lived in isolation, were inoculated; 27 of these became ill. The predominate features observed were headache, malaise, aching limbs, sore throat, and fever. Only eight of the group had abdominal symptoms. The virus was easily recovered in the throat and feces of 25 of the

volunteers, and antibody responses four-fold or greater were noted in 20 of these 25.

The Case Against ECHO Viruses as Etiological Agents in Gastrointestinal Disease

Rather extensive enteroviral and bacterial studies were made on 390 infants (age range 0-2) with diarrhea as the sole symptom, and 384 controls (ages 0-2) matched as to time, age, race, and socio-economics, by Yow (32) and associates. The study was done over the period January 1959 to November 1961, in the Houston area.

Forty-six of the test group were newborns involved in five separate nursery outbreaks of diarrhea. No enteroviruses were isolated from this group, nor from 46 well controls.

Enteroviruses were isolated from 5.6% of the 390 infants with diarrhea, and from 4.4% of the controls. ECHO viruses were isolated 1.5 times more frequently in the study group (15/390) than in the controls (10/384). However, since P equals 0.35, by Chi-square, this is not statistically significant. Even by including only patients studied from May through October, when the chance of isolating enteroviruses would be greatest, the isolation rate in the study group was 9.4% and in the control group 9.1%.

Regarding pathogenic bacteria, the isolation rate in diarrhea patients was 22% and only 2% in controls.

It is to be noted that in this investigation only single

stool or rectal swab specimens were collected. Also, no antibody studies of any kind were carried out. However, the number of patients studied was large, the study was well controlled, and the authors concluded that enteroviruses did not play a significant role in the production of infantile diarrhea in the Houston area in the three year period of investigation.

A study also involving strictly virus isolations without serological work was done by McLean (33) and others. They report on three epidemics of gastroenteritis between May 1960 and March 1961, involving 80 infants. Stools of the two of the infants yielded ECHO 9 virus in August of 1960. However, the significance of this is questionable since the same serotype was isolated from a control infant during the same month.

Stools from 13 of 356 infants without diarrhea yielded enteroviruses. These included two strains of ECHO 9 and one strain of ECHO 14. None of the "control" group of 356 had aseptic meningitis or pleurodynia (which might have explained the viral isolations from these cases). However, these were not "well" controls.

Nevertheless, this report shows virtual absence of detectable viruses from stools of infants with acute gastroenteritis during this time period.

Very similar conclusions are reached in a report by Walker (34) and associates, also dealing strictly with viral isolations without serological work. During 1959 and 1960, they studied

five epidemics of acute gastroenteritis in infants at the H ospital for Sick Children in Toronto. No virus whatever was isolated from the stools of 208 children with diarrhea. On the other hand, from April 1959 to April 1960, 27 strains of enterovirus were isolated from 420 stools of "control" infants; these all had symptoms unrelated to the gastrointestinal tract. Included in these isolations were one ECHO type 6 and five ECHO type 14.

Working at the Montreal Children's Hospital, from July 1958 to May 1959, Joncas and Pavilanis (35) had a group of 74 infants and children with "acute self-limited episodes of diarrhea and vomiting." Using rectal swabs, 14 viruses were isolated from this group; this included one ECHO type 10 and one ECHO type 22. In a control group of 62 children, five viruses were isolated (including one ECHO type 9). The authors, when comparing their results with those of Ramos-Alvarez and Sabin (21) and Sommerville (26), find their low isolation rates difficult to explain, and stated that this was probably due to "technical" difficulties. One wonders how great an effect these difficulties might have in any of the series discussed in this paper, as viral isolation studies and serological work are, at best, tedious and exacting.

Tuckman (36) and co-workers made a survey of acute gastrointestinal illness in a general practice involving over 8000 patients in London from 1957 to 1958. Using children less than five years of age, feces from 49 study cases and 29 healthy controls

were tested for the presence of ECHO viruses. A strain of type 4 virus was isolated from (only) one patient, but from none of the controls.

Lepow (37) and his group, in their report on an epidemic of ECHO 9 infection, stated that vomiting and diarrhea were unusual and tended to be mild and that abdominal pain was uncommon.

Summary and Conclusions

Following an introduction, the history, biological characteristics, host range and pathogenesis, and clinical manifestations of ECHO virus infection have been considered. This has been followed by a discussion on early evidence of viruses as a cause of gastrointestinal disease, and by a consideration of the diagnosis of enterovirus infection.

Papers which appear to have provided the best evidence for ECHO viruses as etiological agents in gastrointestinal disease are those by Eichenwald (20) and associates, Ramos-Alvarez and Sabin (21), and Cramblett (22) and others. Eichenwald's study, besides being the first in which an isolated virus was shown to cause epidemic diarrhea, also dealt with a large number of patients, was well controlled, and was supported by serological data. These criteria are also met by the work of Ramos-Alvarez and Sabin, and their study is generally considered the best evidence of association of enteroviruses with diarrheal disease. The outstanding feature of Cramblett's work is the similarity of the laboratory

technician incident to a human volunteer study.

The work done by Giovanardi and Bergaurini (23), Ramos-Alvarez (24), and Lepine (25) and his group also present good evidence for this association, although not as strong as the three discussed above. Lepine's group present one of the few instances of virus isolation in epidemic infantile diarrhea.

The next five articles cited in this paper contain evidence, although rather weak, supporting ECHO viruses. Sommerville (26) presents results which are not markedly significant, but furthermore, his work is not supported by serological data. The latter is emphasized by Berkovich and Kibrick (27), but their results are not particularly striking. Verlinde's (5) study is weak in that there were no controls, no serological work, and there is no mention of any specific types of ECHO viruses. Klein (29) and associates present only three patients in their study.

It is worth noting that, of the six reports presented which showed ECHO viruses as insignificant in the etiology of gastrointestinal disease, in none of these was serological work carried out. References numbered 32 through 35 do, however, deal with large numbers of patients and are fairly well controlled. The work by Tuckman (36) and by Lepow (37) and others, however, appears poor and ill-defined.

Considering both the quantity and the quality of available reports, therefore, it would appear that evidence at this time

favors ECHO viruses as having a definite role in the causation of gastrointestinal disease. In time, perhaps this role will be outlined more precisely as papers dealing with this subject become more abundant in the literature.

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