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Acute infectious nonbacterial gastroenteritis is a common self-limited, usually benign, disease; it occurs most frequently during the period from September to March and affects both institutionalized and dispersed populations. Outbreaks have a characteristically rapid epidemic spread, but isolated cases do occur. The clinical features of the illness last 24–48 hr and consist of combinations of diarrhea, vomiting, low-grade fever, nausea, abdominal cramps, and malaise. All or some of these features may be present with varying prominence in different outbreaks [1–4]. Specific treatment is not required, and sequelae have not been reported. The clinical syndrome has been transmitted to volunteers by the administration of bacteria-free filtrates and supernatants of stool by Riemann et al. [5], Gordon et al. [6], Jordan et al. [7], and Kojima et al. [8]. In the studies by Gordon et al. and Jordan et al., two clinically distinct forms of the disease were produced with inocula that provided homologous but not heterologous resistance to reinfection. However, despite extensive efforts, no etiologic agent has been detected by standard virologic techniques [9].

Since the last attempts to cultivate the agents of acute infectious nonbacterial gastroenteritis, several new techniques for detection of viruses have been developed [10, 11]. These recently developed methods encouraged us to reinvestigate the etiology of this syndrome. However, suitable material from earlier studies is no longer available; therefore, we sought to transmit the disease to volunteers under carefully controlled conditions, by administration of stool filtrates from well-defined outbreaks of acute infectious nonbacterial gastroenteritis. Administration of potentially infectious material was considered to be justifiable, since the courses of both the naturally occurring and experimentally induced disease are self-limited and without appreciable danger to the affected individual.

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Materials and Methods

Sources of inocula. Stools or rectal swabs were obtained from secondary cases of acute gastroenteritis from four separate outbreaks. The first outbreak took place aboard a U.S. Navy ship, the U.S.S. *Shenandoah,* in the Caribbean (E. S. Dunbar, 1966, unpublished data), while the other three occurred within the continental United States, at New Britain, Connecticut [12], Bethesda, Maryland (N. R. Blacklow, and R. Dolin, 1969, unpublished data), and Norwalk, Ohio. Each of the outbreaks conformed to the typical pattern of acute infectious nonbacterial gastroenteritis. In the outbreak in Norwalk reported by Adler and Zickl

[13], acute disease, consisting primarily of nausea and vomiting and to a lesser extent of diarrhea and low-grade fever, developed in 50% of students at an elementary school. A secondary attack rate of 32% and an estimated incubation period of 48 hr were noted. Illness lasted 24 hr and recovery was complete. Despite extensive efforts, no specific pathogen was detected in stools, throat washings, or sera of the patients who were studied.

Preparation of inocula. Each specimen was obtained from a secondary case during the outbreak. Rectal swabs from the outbreaks in Norwalk and New Britain, originally collected in 5 ml of Hanks' balanced salt solution, were obtained through the courtesy of Drs. Milford Hatch and Martin R. Ross. These specimens were diluted 1:100 in veal infusion broth supplemented with 0.5% bovine serum albumin. Stools from the outbreaks in Bethesda and on board the *Shenandoah,* obtained through the courtesy of Dr. J. Mills and Dr. A. Z. Kapikian, were prepared as 2% suspensions in veal infusion broth containing 0.5% bovine serum albumin. Each suspension was shaken with glass beads and centrifuged at 1,000 *g* for 2 hr, and the sediment was discarded. The fecal suspensions were filtered through a 1.2-μ Millipore filter, and streptomycin and aureomycin were added to final concentrations of 1,000 μg/ml and 33 μg/ml, respectively.

Diarrheal stools, from a volunteer who developed illness after receiving one of the Norwalk specimens, were made up to a 2% suspension in veal infusion broth containing 0.5% bovine serum albumin, and filtered as above.

Fluids harvested from a third passage (in human fetal intestinal organ culture) of the disease-producing Norwalk specimen were pooled, filtered, and prepared as above. Passage in organ culture was initiated with 0.6 ml of a 1:10 dilution of the original fluid from the rectal swab, and the intestinal organ cultures were maintained as described previously [14]. The estimated final dilution of the original material from the rectal swab, after three passages, was $10^{-4.9}$.

Safety tests. The inocula were found to be free of detectable microbial agents by the animal, bacterial, and tissue culture safety test techniques that have been described previously [15]. In addition, each inoculum was administered orally in a 5-ml volume to each of four rhesus monkeys, which showed no evidence of toxicity or disease. Although there was no evidence that clinical hepatitis was present in the donors of the inocula, these inocula, as well as acute and convalescent sera from all donors, were found to contain neither Australia antigen (HAA) nor antibody (anti-HAA) by complement-fixation tests [16]. Levels of glutamic-oxalacetic and glutamic-pyruvic transaminases in sera were within normal limits.

The stools of all donors of the inocula, including passaged material, were free of known enteric pathogens. Coliform organisms, isolated from these stools, did not produce bacterial enterotoxins when assayed in rabbit ileal loops [17]. The inocula were free of bacterial enterotoxins by the same determination.

Subjects. Volunteers participating in the study were healthy male prisoners, 18–45 years of age, at the Maryland House of Correction, Jessup, Maryland. In addition, normal volunteers at the Clinical Center of the National Institutes of Health took part in the study. Informed consent was obtained from all volunteers prior to study. Each volunteer received a complete physical examination, and routine laboratory tests, including hematocrit, total and differential white blood cell counts, urinalysis, blood urea-nitrogen tests, tests of liver function, chest X-ray, and electrocardiograms were carried out. Stools collected before administration of the inocula were free of known enteric pathogens.

Care and observation of volunteers. Volunteers were placed in an isolated hospital ward within the prison or in an isolated room at the Clinical Center, for 1–2 days before and for 10 days after challenge. They were examined daily by three physicians during the entire course of the study. They received no specific therapy except minor analgesics upon request. As prophylaxis against possible hepatitis caused by an infectious agent requiring short periods of incubation, which might have conceivably been present in the inocula, each volunteer received intramuscularly 2 ml of human gamma globulin five days after the oral administration of fecal filtrate [18]. Precautions were taken for prevention of contamination of volunteers by needles and stools for eight weeks after oral administration of the inocula. Daily measurement of weight, oral temperatures recorded every 4 hr, complete blood-cell counts, liver function tests, analyses of amylase, electrolytes, and urea-nitrogen in sera, were determined during acute

illness. All stools passed by volunteers were collected and examined. Tests of liver function were done 3, 5, and 8 weeks after administration of the inoculum.

Method of administration. Five minutes before inoculation, each volunteer received two g of $NaHCO_3$ orally; it was thought that this would circumvent possible acid lability of an infectious agent. Ten ml of the inoculum, diluted with 30 ml of water, was then swallowed by the volunteer.

Results

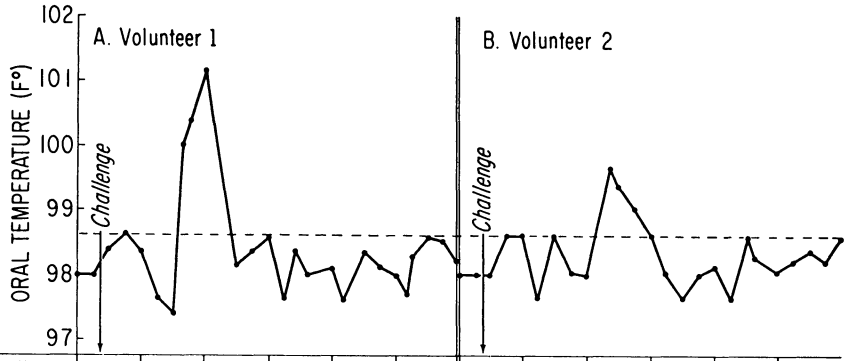
Two inocula derived from the outbreak in Norwalk, two from the epidemic in New Britain, one from the outbreak in Bethesda, and one from the outbreak on board the *Shenandoah* were each administered to groups of 3–4 volunteers. Only one inoculum, that from the Norwalk outbreak, produced illness. The response of volunteers to the Norwalk material is summarized in table 1. Two of the three volunteers given one of the Norwalk inocula (in table 1, A) developed illness 48 hr after administration. Manifestations of disease lasted 48 hr, and consisted of mild loose diarrhea (4–6 stools) that lasted 24 hr, low-grade fever lasting 8–12 hr, anorexia, moderate abdominal cramps, malaise, headache, and nausea without vomiting. Neither volunteer was in acute distress, and both had recovered spontaneously without treatment by 96 hr after challenge. No sequelae were noted. Laboratory tests, including hematocrit, total and differential white blood cell counts, liver function tests, and analyses of electrolytes, amylase, and blood urea-nitrogen were within normal limits. The third volunteer who received the inoculum remained asymptomatic throughout the period of observation.

A second passage of the Norwalk material was performed by administration of a filtrate of diarrheal stool (table 1, B) from one of the volunteers who developed illness after the administration of the original Norwalk rectal swab specimen. Seven of nine volunteers who received the inoculum developed clinical illness lasting an average of 33 hr, with a mean incubation period of 37 hr (table 1). Two volunteers vomited but did not have diarrhea; one of these individuals vomited approximately 20 times within a 24-hr period and required parenteral administration of fluid (figure 1, **A**). Two volunteers passed loose to watery stools but

Table 1. Response of volunteers to filtrates of fecal suspensions derived from patients studied in an outbreak of gastroenteritis in Norwalk, Ohio

Inoculum	No. of volunteers	No. with gastrointestinal illness	No. with indicated symptoms						Duration-Interval, feeding to onset	
			Fever*	Diarrhea† (mean no. of stools)	Vomiting	Abdominal cramps	Malaise	Headache	Incubation	Signs and symptoms
A. Rectal swab diluted 1:100 and filtered	3	2	2 (100.5, 100.8)	2 (4)	0	2	2	2	48 hr	48 hr
B. Filtered, 2% suspension of stool from volunteer who received A	9	7	4 (99.6, 100.0, 100.4, 101.2)	5 (5)	5	6	7	7	18–48 hr (mean, 37 hr)	24–48 hr (mean, 33 hr)
C. Fluid from third passage in human fetal intestinal organ culture, passage series initiated with A‡	4	1	1 (99.8)	1 (3)	0	1	1	1	48 hr	24 hr

* Numbers in parentheses are the highest oral temperatures in degrees F.
† Diarrhea did not last more than 24 hr.
‡ Prior to 1:100 dilution.



Days after challenge	0	1	2	3	4	5	0	1	2	3	4	5
Diarrhea	0	0	0	0	0	0	0	0	8 stools		0	0
Vomiting	0	20X		0	0	0	0	0	0	0	0	0
Abdominal cramps	0			0	0	0	0	0			0	0
Nausea	0			0	0	0	0	0		0	0	0
Malaise	0			0	0	0	0				0	0
Headache	0			0	0	0	0	0			0	0
Myalgia	0			0	0	0	0	0		0	0	0
Anorexia	0				0	0	0				0	0
WBC	8,400	18,450	16,600	14,100	11,200	–	8,200	8,100	8,700	8,200	8,300	–

Figure 1. Response of two volunteers to oral administration of stool filtrate derived from volunteer who received original Norwalk rectal-swab specimen. The height of the curve is directly proportional to the severity of the sign or symptom. Volunteer 1 had severe vomiting without diarrhea while volunteer 2 had diarrhea without vomiting, although both received the same inoculum.

did not vomit; clinical data on one of the latter individuals are given in figure 1, **B**. The remaining three subjects both vomited and had diarrhea. Four men had low-grade fever lasting 8–12 hr. Each of the seven sick men complained of headache and malaise. All the volunteers recovered spontaneously without sequelae. Laboratory findings were within normal limits except for a transient elevation of the white blood cell count in one man (figure 1, **A**). Two of the nine volunteers who received this inoculum remained well throughout the period of observation.

Fluid harvested from the third serial passage of the original specimen from Norwalk in human fetal intestinal organ culture was given to four men in an attempt to determine whether the agent had propagated in vitro (table 1, C). One of four men who received this inoculum developed clinical illness, consisting of mild diarrhea and low-grade fever, similar to that experienced by volunteers who received the original filtrate of the rectal swab (table 1, A) and the first passage of the stool filtrate (table 1, B). The other three men who received the inoculum remained well.

The volunteer who provided the first-passage stool for inoculum B (table 1) was challenged with his own stool filtrate eight weeks after onset of his illness. No evidence of disease was noted.

Discussion

These experiments confirm the previous observations that acute infectious nonbacterial gastroenteritis can be induced experimentally by oral administration of bacteria-free filtrates of stool from patients with the syndrome [6–8]. It appears likely that the causative agent was of an infectious nature, since serial transmission was achieved through two groups of volunteers, beginning with a dilute specimen of a rectal swab from a patient with the disease. The resulting dilution of the original rectal swab material makes it improbable that a non-replicating substance, such as a toxin, served as the causative agent, although a toxin that is still active at such dilution has not been completely ruled out. The epidemiologic pattern of acute infectious nonbacterial gastroenteritis, as well as its incubation period, support the concept of an infectious etiology. Further evidence supporting this view was provided by the occurrence of two ter-

tiary cases, with the characteristic incubation period and clinical course, in two physicians who processed second-passage diarrheal stool in the laboratory.

The induction of disease by the administration of fluid from a third-passage intestinal organ culture suggests that in-vitro replication of an agent may have taken place. However, since the dilution of the original material through three passages was only $10^{-4.9}$, disease could have been caused by persistence of an agent from a rectal-swab specimen with a high titer of infectious agent. Experiments in volunteers, with higher passage organ-culture material, are planned to resolve this question.

Experimentally produced acute infectious nonbacterial gastroenteritis was similar in its incubation period, duration, and clinical course to the naturally occurring illness. The incubation period was somewhat shorter during the second passage of the Norwalk agent in volunteers, but this may have been a result of the administration of a more concentrated inoculum. During the second passage of the Norwalk agent in volunteers, a spectrum of clinical responses was observed, ranging from a predominantly diarrheal disease without vomiting to an illness characterized by repeated vomiting without accompanying diarrhea. In the outbreak in Norwalk, which involved mainly children from elementary schools, vomiting was the major manifestation of disease and diarrhea was less common [13]. Since the original Norwalk specimen came from an adult (who, on the basis of age, would not be expected to harbor a second unrelated enteric virus) [19], and since serial transmission in volunteers was accomplished with stool from a single individual, it is unlikely that more than one agent was responsible for the diversity of disease patterns observed. The basis for this variability of clinical expression of disease is not understood at this time. However, this finding suggests that at least under certain circumstances, such clinically diverse syndromes as epidemic vomiting [2], epidemic collapse [3], and viral diarrhea [20] may be caused by the same agent.

The Norwalk agent was notable for the high frequency with which it produced disease in volunteers. Two of three men who received the filtered diluted portions of the original Norwalk rectal-swab specimen developed acute infectious nonbacterial gastroenteritis; similarly, a stool filtrate derived from one of these sick volunteers induced disease in seven of nine men. This suggests that immunity to the Norwalk agent is not commonly present in the general population or possibly that the agent has the capacity to overcome readily the immunologic defenses of the host.

The inocula that produced disease in man failed to induce a similar effect in rhesus monkeys or in a number of common laboratory animals; this finding suggests that the Norwalk agent has a relatively restricted host range in terms of production of disease.

The reasons for the failure of five of the six inocula that were administered to induce disease in volunteers remain unclear. These inocula were derived from secondary cases in which disease developed during typical outbreaks of acute infectious nonbacterial gastroenteritis. This suggests that the agents responsible for these outbreaks may not have been filterable, or if they were filterable, that widespread immunity existed to them. On the other hand, it may have been that the specimens did not contain the agent in sufficient titer because of inappropriate collection or processing.

These studies in volunteers provide the necessary infectious material for the continued experimental study of acute infectious nonbacterial gastroenteritis. Laboratory investigation of the etiologic agent of acute infectious nonbacterial gastroenteritis can now be done with use of stool filtrates in which the disease-producing agent is known to be present. In parallel studies, it should be possible to define certain important biologic and biophysical properties of the Norwalk agent, with human volunteers as the experimental host.

Summary

Acute gastroenteritis was induced in two of three volunteers by the oral administration of a bacteria-free stool filtrate derived from a patient with naturally occurring acute infectious nonbacterial gastroenteritis. Subsequently, oral administration of a stool filtrate from one of the sick volunteers induced gastroenteritis in seven of nine additional men. The original rectal swab specimen from the naturally occurring case was passaged three times in human fetal intestinal organ culture; material from the third passage induced gastroenteritis in one of four volunteers. A spectrum of clinical responses developed, ranging from a predominantly diarrheal disease without vomiting to

an illness characterized by repeated vomiting without accompanying diarrhea.

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