

A STUDY OF ILLNESS IN A GROUP OF CLEVELAND FAMILIES

VII. TRANSMISSION OF ACUTE NON-BACTERIAL GASTROENTERITIS TO VOLUNTEERS: EVIDENCE FOR TWO DIFFERENT ETIOLOGIC AGENTS*

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Acute infectious non-bacterial gastroenteritis not only occurs as an epidemic disease in institutionalized and civilian populations (1-4) but also persists as an endemic disease responsible for sporadic cases or localized family outbreaks (4). The results of transmission studies in volunteers (2, 5) and variations in patterns of outbreaks in New York State (6) prompted the suggestion that such gastroenteritis may be caused by more than one agent (2). This hypothesis has recently been supported by a study of cases of non-bacterial gastroenteritis occurring in a population of Cleveland families (4), for clinical and epidemiologic data imply that at least two types of infectious gastroenteritis, afebrile and febrile, occur in the families.

The purpose of the present investigation was to characterize further the types of gastroenteritis observed in the Family Study just mentioned and to compare them with the disease caused by the previously described Marcy strain, a filterable agent obtained from an institutional outbreak of gastroenteritis in New York State (2). The Marcy strain, when fed as bacteria-free supernate of watery stool specimens, has caused a syndrome characterized chiefly by watery diarrhea in approximately two-thirds of volunteers inoculated. The patients were usually afebrile, a low grade fever developing only when dehydration occurred. Incubation periods ranged from 1 to 5 days, with an average of 3 days. One attack of the disease conferred a short immunity (2, 7, 8).

Since efforts to establish the incitants in laboratory hosts had failed, the

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experiments were carried out with the aid of volunteers. The febrile type of gastroenteritis was reproduced by the feeding of supernate prepared from a stool obtained from an individual in the Family Study, and the agent responsible was carried through two subsequent passages by feeding similar supernates. The illnesses produced differed from the afebrile ones induced by the Marcy agent; and while an attack of the latter disease continued without exception to stimulate resistance to homologous reinoculation, cross-immunity was not demonstrated. Two attempts to transmit disease with respiratory tract secretions obtained from individuals with gastroenteritis were unsuccessful.

Methods of Study

Collection and Preparation of Inocula.—Two types of inocula were used: throat washings and fecal suspensions. Throat washings were collected by having the donor gargle with sterile skim milk. These specimens were stored at -70°C . without further treatment, and thawed at 37°C . prior to use.

Marcy inoculum, obtained from cases of the afebrile type of gastroenteritis, consisted of undiluted, watery stool supernate prepared as previously described (2). It was free from recognized bacteria, fungi, or protozoa. The experiments to be reported represent the seventh serial passage in volunteers of the Marcy strain (8).

FS inocula, obtained from cases of both afebrile and febrile types of gastroenteritis, were prepared by two different methods. Gastroenteritis was induced by only one of the supernates prepared from stools obtained from individuals in the Family Study (FS). The volunteers developed febrile illnesses, and "FS" has subsequently been used only in reference to the agent of febrile gastroenteritis. The inocula used in the first three experiments were processed from stools obtained from individuals in the Family Study and from stools of volunteers with induced febrile gastroenteritis by a method adopted from Howitt and Barnett (9) and Shaw, Melnick, and Curnen (10). The specimens were collected in cardboard cartons and stored at -70°C . for from 2 to 3 months prior to processing. Cultures of the stools on Endo's medium and desoxycholate agar and in selenite F broth revealed no recognizable enteric pathogens. The stools were ground with alundum and sterile distilled water added to make a 20 per cent suspension. This was centrifuged horizontally at 2000 R.P.M. for 20 minutes at approximately 4°C . The supernate was then centrifuged at 12,000 R.P.M. for 30 minutes either in the high speed angle head of an International refrigerated centrifuge or in the Sorval SS-1 centrifuge. Penicillin and streptomycin were added to give final concentrations of 2,000 units and 10 mg. per ml., respectively. No growth resulted when the treated supernate was inoculated on blood agar or added to tryptose phosphate or thioglycollate broth.

The inoculum used in the fourth experiment was prepared from stools obtained from an individual in the Family Study. Two loose stools were pooled, diluted with an equal volume of distilled water, ground with alundum, and the supernates centrifuged three times at 3000 R.P.M. for a total of 80 minutes. Further treatment was identical with that of other FS inocula.

None of the inocula caused disease when injected intracerebrally into *rhesus* monkeys and both intracerebrally and subcutaneously into suckling mice.

The stool supernates were either thawed immediately or stored for 2 to 3 hours on cracked ice before use.

The source of the individual inocula will be described with the various experiments.

Method of Inoculation.—The stool supernates were fed to volunteers after individual inocula of from 2 to 10 ml. had been mixed with from 15 to 75 ml. of chocolate milk in clean paper cups or in sterile glass jars.

The throat washings were given intranasally by slowly running 0.5 ml. from a 1.0 ml. pipette into each nostril of the recumbent subject. The subject was asked to "sniff" the material as it trickled up his nose; some of the inoculum was undoubtedly swallowed.

Subjects.—Twenty-nine subjects, both male and female, were medical students or members of the professional or technical staff of the Department of Preventive Medicine. These persons continued with their daily routines. Each was provided with a thermometer and a symptom check sheet. If illness developed, additional details were recorded by the observer. All temperatures were obtained orally. Gastroenteritis was not prevalent at the time the experiments were done.

Sixty-two subjects were young adult males selected, isolated, and observed as previously described (2).

Experiments on Oral Transmission

Five experiments were done. The first indicated that an agent (FS) different from the Marcy strain of gastroenteritis was transmitted. The second and third experiments were designed to examine the relationship of these two agents and to test the further transmissibility of the FS strain. The fourth was an unsuccessful attempt to transmit another Cleveland gastroenteritis agent. The fifth experiment indicated that control inocula failed to induce illness.

First Experiment.—To explore experimentally the possibility that two types of gastroenteritis, afebrile and febrile, do occur in families, an attempt was made to reproduce each type in volunteers by feeding specimens from cases.

Afebrile Case.—(Family Study illness 7576) A 32 year old female awoke at 3:00 a.m. February 13, 1951, with malaise, nausea, and abdominal pain. She passed three loose stools. Anorexia, nausea, and malaise persisted without feverishness. An exacerbation of abdominal pain on February 15 was associated with the passage of two more loose stools, one of which was the specimen processed. 3 days before the mother's onset, her 5 year old daughter vomited three times but developed no other symptoms.

Febrile Case.—(Family Study illness 7549—Fig. 1.) A 35 year old female, during the afternoon of January 15, 1951, suddenly developed nausea, malaise, and abdominal cramps. She vomited twice, felt chilly and then feverish, and noted headache and somnolence. At 9 p.m. her temperature was 101°F. She felt better the next day, although nausea, anorexia, and abdominal "grumbling" were still present. She took a cathartic the evening of the day of onset; she had no diarrhea. Examination about 18 hours after onset revealed no abnormal findings. A formed stool passed on January 17, about forty-eight hours after onset, was the source of the inoculum. No cases occurred in the other four members of the family.

Seven young adult males ingested 2 ml. of inoculum from the afebrile case. Only one developed symptoms. 56 hours after inoculation he noted headache, malaise, and nausea, and vomited once. He had no fever, abdominal pain, or diarrhea. No further studies were made of material from the afebrile case.

Eight young adult males ingested 2 ml. of inoculum from the febrile case. Four (Fig. 2) developed anorexia, headache, abdominal cramps, and loose stools after from 26 to 30 hours. Three of the four also experienced vomiting and fever. The illness in one of these, E. E., who served as the source of other inocula, is illustrative of the group.

Case E. E.—Initial symptoms, beginning at 5 p.m. about 28 hours after inoculation, were nausea and epigastric discomfort soon followed by vomiting. The vomitus contained bile, and the patient continued to retch after his stomach had been emptied. He passed two loose stools between 8 p.m. and midnight. Shortly after midnight, he had an explosive, watery stool,

small in amount. He spent a restless night, later passing another loose stool. The maximum recorded temperature was 99.8°F. When seen in his quarters in the morning, he was complaining of headache and considerable malaise and fatigue. These symptoms subsided and he felt well within 24 hours after onset. The specimens which were processed as inoculum for the second experiment consisted of the first two stools and the last one. A throat washing obtained 16 hours after onset was used in the sixth experiment.

Second Experiment.—The fecal suspension from the febrile case (termed FS inoculum) induced illnesses which differed from those induced by the Marcy agent. This experiment was designed to compare the Marcy and FS strains. Two groups of volunteers in individual isolation were fed Marcy and FS inocula,

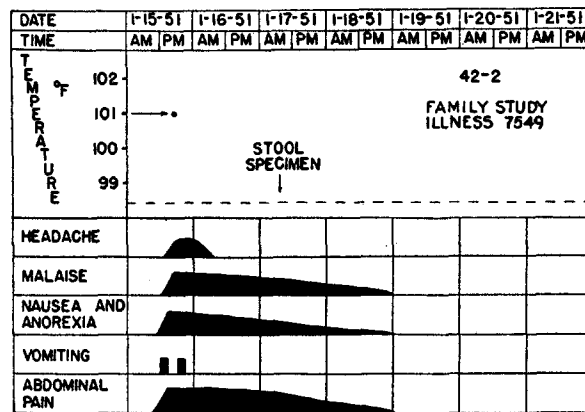


FIG. 1. Chart of findings in an attack of febrile gastroenteritis experienced by an individual in Family Study. A Stool specimen from this case was processed to procure the initial FS inoculum (Experiment 1).

respectively. Each group was reinoculated twice at intervals of approximately 2 weeks, first with the homologous, then with the heterologous material (Fig. 3).

Marcy inoculum consisted of unfiltered diarrheal feces collected July 3, 1950, from T. H., subject 61, representing the sixth human passage. It was processed April 12, 1951. Storage was at from -50° to -70°C . *FS inoculum* was obtained from case E. E. during the first human passage. The specimens were collected May 16, 1951, and processed August 27, 1951. Storage was at -70°C .

Volunteers were first inoculated on December 17, 1951. Six of the seven men given 7 ml. of *Marcy inoculum* developed typical disease within 24 to 120 hours. All had abdominal cramps and watery diarrhea. Two had vomiting. Two (N. B. and S. S.) had temperatures of 100° and 101°F . with dehydration (Fig. 4). One (R. G.) of the eight men given 2 ml. of *FS inoculum* developed a temperature of 101.8°F ., nausea, anorexia, abdominal pain, and headache in 24 hours (Fig. 5). Five volunteers noted mild abdominal symptoms, such as epigastric pain, "heartburn," "growling," and one loose stool after from 20 to 30 hours.

16 days later the men ingested doses of the same inocula, but the amount of *FS inoculum* was increased to 10 ml. No illnesses occurred in the Marcy group. In the FS group, one man

(H. A., Fig. 5) developed anorexia, nausea, and abdominal cramps after 30 hours. For 3 days he had an urge to defecate but was unable to do so. Single stools on the next two days were loose. Although he complained of headache and considerable malaise, the maximum temperature recorded was 99.4°F. Two other men had mild abdominal cramps at 28 and 31 hours.

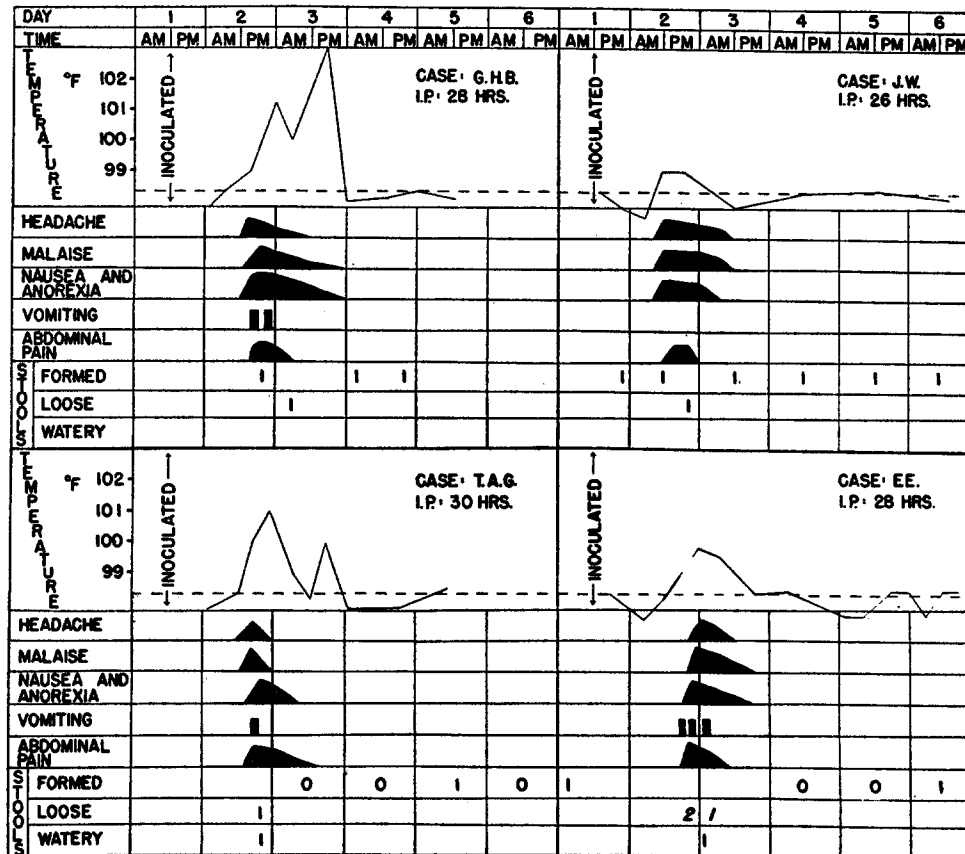


FIG. 2. Graphic representation of the illnesses induced in 4 of 8 volunteers by feeding 2 ml. of stool supernate from a patient with febrile gastroenteritis (Experiment 1).

13 days later cross-immunity was tested. *Marcy inoculum* induced diarrheal disease within 24 to 76 hours in seven of eight men, including the two previously sick following the FS inoculum. One of these (R. G.) exhibited a febrile response with a temperature rise to 102°F. similar to that previously induced in him by FS inoculum. *FS inoculum* induced febrile (100 to 101°F.) disease within 21 to 30 hours in three of seven men, all previously sick following *Marcy inoculum*. Two of these volunteers (N. B. and H. W.) complained bitterly of headache and stated that the anorexia, nausea, and abdominal cramps provoked by FS inoculum were worse than those associated with the watery diarrhea of the *Marcy* disease. The complaint of urgency but inability to defecate was again voiced. These men stated that, if given a choice, they would prefer to have the *Marcy* type rather than the FS type of illness.

One of the three men (S. S.) given FS inoculum at this time developed fever and gastrointestinal symptoms. His subsequent course was complicated by the appearance of prostatitis 10 days after inoculation.

Previous studies had revealed no significant changes in the values of a number of laboratory tests performed on specimens from patients with illness induced by the Marcy strain (8), and these examinations were not repeated in the present experiment. The total and differential leucocyte counts and the sedimentation rates of the three men in the final FS group were normal.

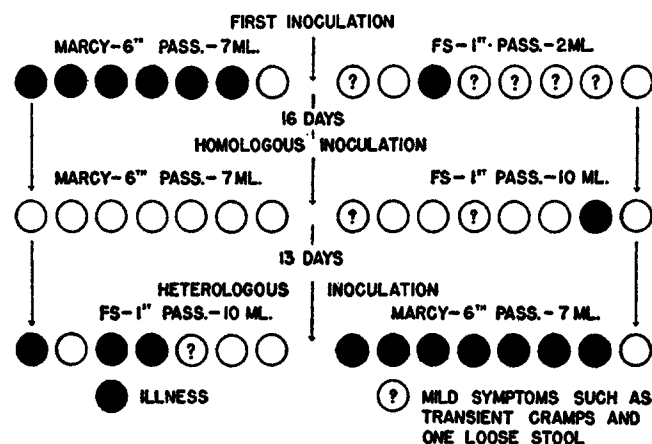


FIG. 3. The results of inoculation of homologous and heterologous stool supernates from cases of afebrile (Marcy) and febrile (FS) gastroenteritis (Experiment 2). Each circle represents a volunteer; the sequence of events for each man is indicated by a single vertical column of circles.

Third Experiment.—This experiment was done to determine whether the FS agent could be carried through another human passage. It also furnished an additional measure of susceptibility to reinfection with heterologous inoculum.

FS inoculum came from Case H. W. of the second experiment representing the second serial passage of FS. The specimen consisted of a formed stool passed January 15, 1952, about 38 hours after the onset of symptoms. Stored at -50° to -70°C ., it was processed on April 29, 1952, but was not rendered free of bacteria by centrifugation at 3,000 R.P.M. On May 8, 1952, centrifugation at 12,000 R.P.M. produced a bacteria-free supernate. The Marcy inoculum was the same as that used in the second experiment.

On June 2, 1952, eight young adult males in individual isolation were fed 10 ml. of second passage FS inoculum in chocolate milk. One man (E. H.) developed a definite illness (Fig. 5). At 3 p.m., 27 hours after inoculation, he complained of "butterflies" in his stomach. This progressed to manifest nausea associated with dizziness. At 4 p.m., he began to vomit and noted cramping lower abdominal pains. He then had a bowel movement, the first portion of stool being formed, the second watery. He vomited a total of five times and was observed to be pale, sweaty, and in acute discomfort. He complained of chilliness and feverishness but the maximum temperature recorded was only 99.6°F .

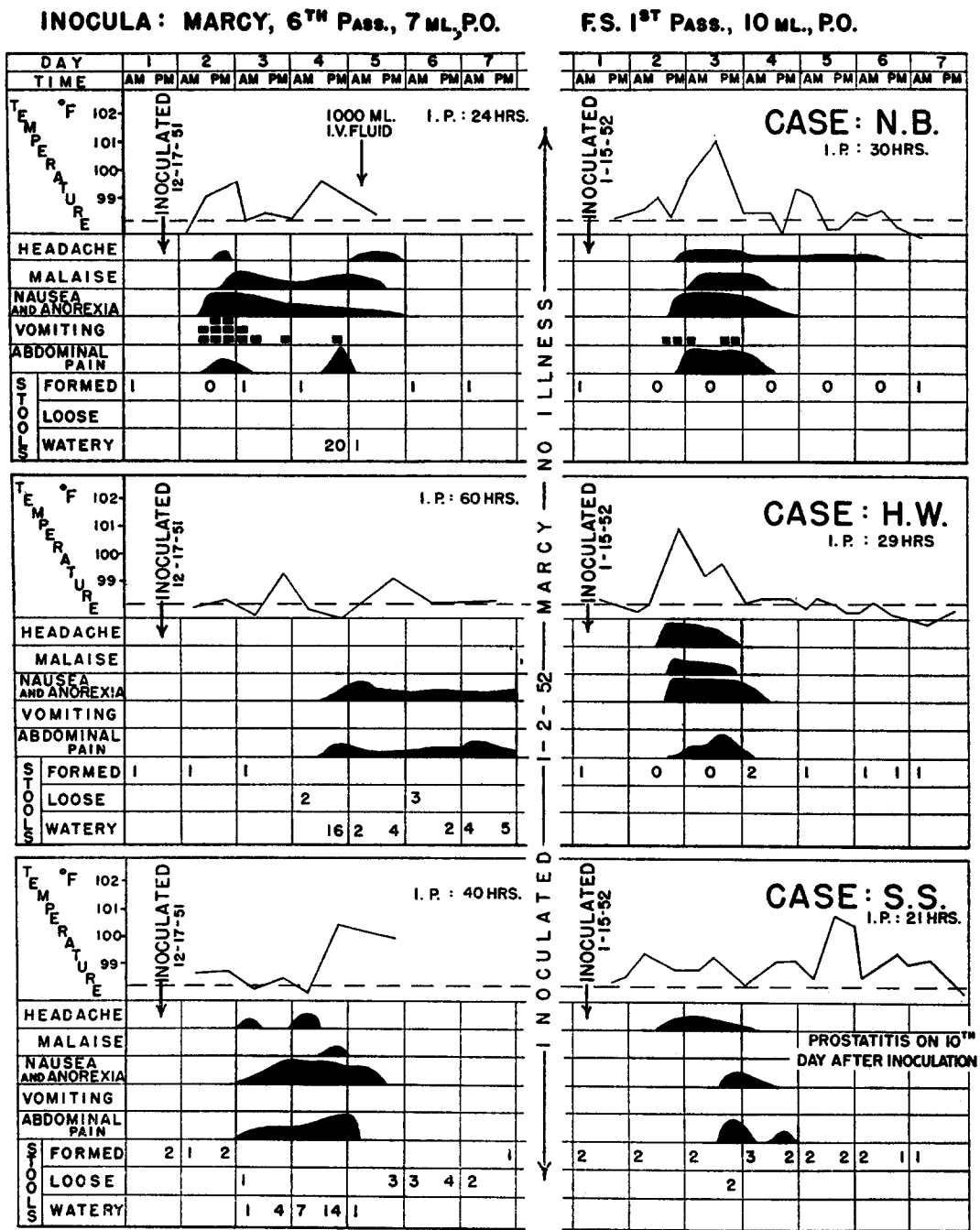


FIG. 4. Graphic representation of the responses of the same individual to two different gastroenteritis inocula. Volunteers who initially responded to Marcy inoculum had no symptoms when fed the same type of inoculum 16 days later. They did become ill following the ingestion of FS inoculum.

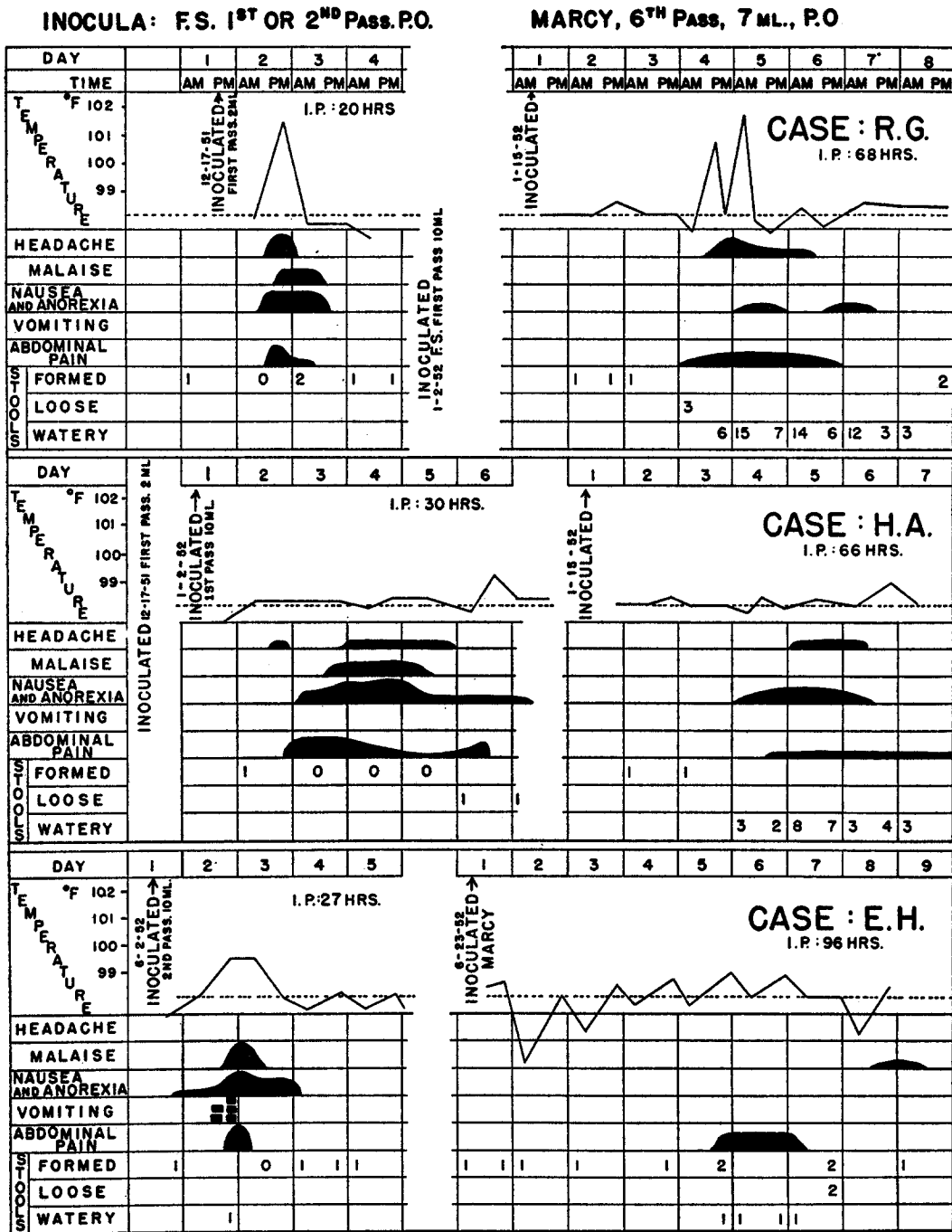


FIG. 5. Graphic representation of the responses of the same individuals to two different gastroenteritis inocula. Volunteer R. G. developed symptoms after ingesting 2 ml. of first passage FS inoculum, but remained well when fed 10 ml. of similar material 16 days later. Volunteer H. A. had indefinite symptoms after ingesting 2 ml. of this inoculum, but had a definite illness when fed 10 ml. The symptoms in volunteer E. H. were induced by second passage FS inoculum. These volunteers were later shown to be susceptible to the Marcy agent.

Another volunteer developed a possible illness after 32 hours. He complained of anorexia, vomited once, passed one loose and one watery stool, and had a temperature of 99.6°F. It was thought that this man may have exaggerated his symptoms, and because doubt existed as to his reliability as a witness, his experience was not classified as a definite illness. Four other men noted one or more symptoms such as nausea, abdominal cramps, headache, and loose stool after from 26 to 36 hours, but these symptoms were mild and transient.

3 weeks later volunteer E. H. ingested 7 ml. of Marcy inoculum. After 96 hours, he developed abdominal cramps and watery diarrhea, experiencing a typical, although comparatively mild, attack of Marcy illness.

TABLE I
Characteristics of Illnesses Induced by Two Different Gastroenteritis Inocula

	Inoculum	
	Marcy	FS
Incubation period:		
Range, <i>hrs.</i>	24-120	20-30
Average, <i>hrs.</i>	60	27
Duration: Average, <i>hrs.</i> ...	96	24
Fever.....	Usually absent; late with dehydration, below 101°F.	Usually present; often relatively high
Constitutional symptoms..	Usually mild	Usually marked
Headache.....	Mild or absent	Moderate or severe
Nausea and anorexia.....	Common; moderate or severe	Common; moderate or severe
Abdominal pain.....	Hyperactive peristalsis; cramps of moderate intensity preceding diarrheal stools	Persistent pain and cramps, often severe, associated with desire to vomit or move bowels
Vomiting.....	Often occurs in bouts early in illness	Often occurs in bouts early in illness
Stools.....	Frequent; watery	Infrequent; normal or loose

Comparison of Marcy and FS Illnesses

Differences in the clinical syndromes resulting from the two different inocula—sixth passage Marcy and first and second passage FS—were readily apparent (Table I) and are illustrated by the consecutive responses of the same individuals (Figs. 4 and 5). In general, the Marcy illness was characterized by profuse watery diarrhea, with fever only following dehydration, while the FS illness was characterized by fever, abdominal pain, and constitutional symptoms. There were variations; patients with FS disease did not always exhibit fever, and Marcy inoculum did induce a prompt febrile response similar to that seen with FS (case R. G., Fig. 5).

The incubation periods of the ten cases of illness resulting from FS inoculation were clearly shorter than those of cases of Marcy disease (Fig. 6). In each case onset occurred between 20 and 30 hours after ingestion of the inoculum.

The average was 27 hours. In contrast, the incubation periods of the fourteen cases of Marcy disease observed during the second and third experiments ranged from 24 to 120 hours, with an average of 60 hours. Including these fourteen cases, records of 46 volunteers who developed experimental Marcy

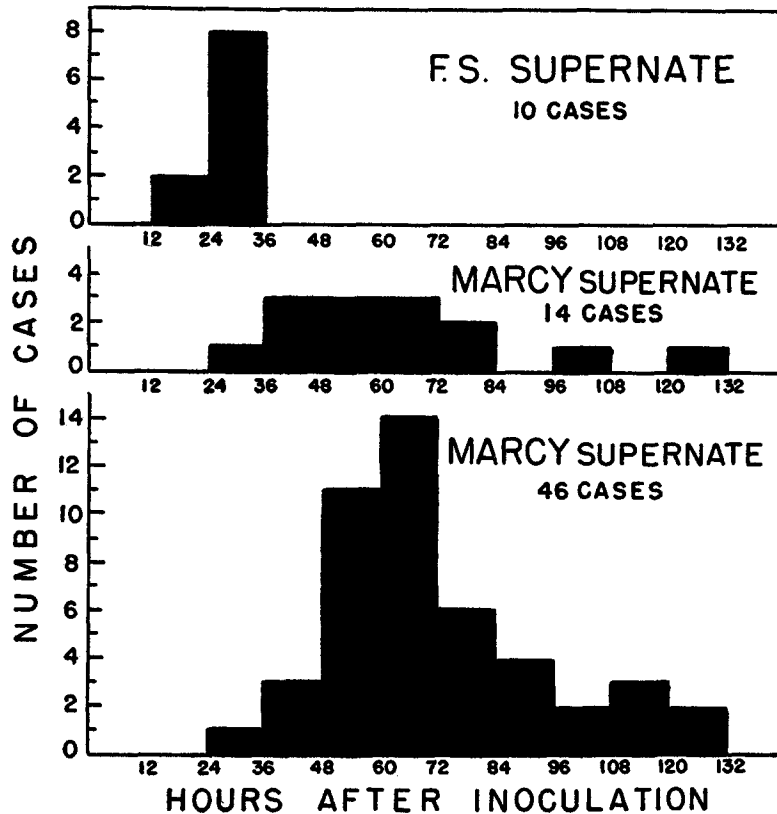


FIG. 6. The charted incubation periods for FS supernate are those observed in the first, second, and third experiments of the present work. The small block illustrating Marcy incubation periods includes those of the second and third experiments. The large block includes the total experience to date in volunteers fed different Marcy inocula.

infection are available (2, 7, 8, 11). Only one had an incubation period of less than 36 hours, while in no case was the incubation period of FS illness greater than 30 hours.

The duration of the two types of illness also varied. Men sick with the Marcy illness continued to have diarrheal stools, in decreasing numbers, for as long as a week. The more intense FS symptoms were of short duration, complete anorexia on one day being followed by return of appetite the next. Yet, as

mentioned previously, the men preferred the watery diarrhea of the afebrile illness (Marcy) to the headache and abdominal pain of the febrile illness (FS).

Fourth Experiment.—The occurrence in a family of an outbreak of vomiting, feverishness, and abdominal pain, with diarrhea in one of the parents, prompted transmission studies in volunteers to compare this disease with the Marcy and FS prototypes. Fifteen young adult males, divided into three groups, were inoculated. Eight subjects had recently recovered from experimentally induced Marcy illness and were presumably resistant to this agent (2, 7). Three men had been fed fecal supernate from a patient with the afebrile type of gastroenteritis but had remained well. Four had never been previously inoculated.

Source of Inoculum.—(Family Study illness 12084) A 37 year old male awoke at 6:00 a.m. on April 8, 1952, with abdominal pain. He vomited three times and passed 12 to 14 watery stools during the next 8 hours. For the next 2 days he noted feverishness, anorexia, and malaise. His temperature was not recorded. Two loose stools passed at 4:00 and 7:00 p.m., about 12 hours after onset, served as the source of inoculum. 26 to 30 hours before the father's onset, his two sons, aged 9 and 2, had become ill. The youngest boy vomited 5 times and was described as listless and feverish; anorexia, with the passage of loose stools, persisted for 4 days. The other boy complained of abdominal pain and had a temperature of 102°F. He became asymptomatic by the 3rd day, but again became anorexic and vomited once on the 6th and twice on the 7th day after onset. The mother and an older daughter had no illness. The volunteers ingested 10 ml. of stool supernate. All remained well.

Fifth Experiment.—The purpose of this experiment was to determine the clinical effect of ingestion of non-infectious stool supernate, since toxic or psychosomatic factors might conceivably have played a part in evoking the symptoms and signs of gastroenteritis. One group of volunteers was fed autoclaved supernate prepared from pooled normal stools; each individual in a second group was fed unheated supernate from his own normal stool.

Autoclaved Inoculum.—Stool specimens from 5 normal young adult males were collected August 27, 1952, and stored at -50°C . to -70°C . The donors remained well for the next 3 months. On November 24, 1952, the specimens were pooled and processed as if they were FS inoculum. The pool was autoclaved for 40 minutes at 121°C .

Several hours after it was autoclaved, 10 ml. of the unrefrigerated inoculum was fed in chocolate milk to each of 18 volunteers in individual isolation. The 5 donors were unaware of the fact that they were among the recipients, all of whom were handled in the same manner as in the previous experiments.

None developed fever. 6 of the 18 remained asymptomatic. There were indefinite symptoms in 12, including 3 of the 5 donors, such as mild headache of short duration, slight cramps, and "growling," and, in 4 cases, a loose stool. The onset of such minor complaints, which resembled in some respects those deemed suggestive of questionable illness in the second experiment (Fig. 3), were scattered between 4 hours and 1 week after inoculation.

The autogenous stool supernates were rendered bacteria-free by the same technic used to process FS inocula, except that in 3 instances a second centrifugation at 12,000 r.p.m. was required. Observation and laboratory studies prior to collection of the stool specimens showed

that the subjects were in good health. 8 subjects were fed 10 ml. of autogenous stool supernate; a 9th was fed 5 ml. All remained well.

The results of these tests imply that the definite illnesses following ingestion of FS and Marcy inocula cannot be attributed to the fecal content of the inocula.

Experiments on Respiratory Transmission

The epidemiologic behavior of acute non-bacterial gastroenteritis is consistent with intimate contact spread (4), and transmission of the disease by the inhalation (5) of throat washings has been reported. For this reason, two attempts were made to transmit gastroenteritis by the respiratory route.

Sixth Experiment.—A 32 year old female at 9:00 p.m. on April 22, 1951, suddenly developed nausea, anorexia, and abdominal pain (Family Study illness 7956). She vomited once and had watery diarrhea (16 stools) throughout the night. The next morning, 15 hours after onset, a well gargled throat washing was obtained. Her temperature at this time was 100.4°F. (R); she did not feel feverish. Two of 4 other members of the family experienced diarrheal disease. On April 21, a daughter vomited 4 times, passed 4 watery stools, and had a temperature of 102°F. (R); on April 23, another daughter had 5 watery stools. A neighboring family with 3 boys of comparable ages had just recovered from an episode of similar illnesses. 7 volunteers were given 1 ml. of undiluted throat washing intranasally on May 15, 1951. No illness developed.

Seventh Experiment.—Case E. E. of the first experiment provided a well-gargled throat washing 16 hours after the onset of symptoms. Supernate from his stools proved infectious when fed in the second experiment of those here described. 7 volunteers were given 1 ml. of undiluted throat washing intranasally on April 22, 1952. No illness developed.

DISCUSSION

The present experiments confirm and extend the earlier observations concerning the Marcy agent (2, 7, 8), and suggest that one of the types of gastroenteritis observed in the Cleveland Family Study, the febrile type, differs from the Marcy type in etiology. The evidence in support of this suggestion is the lack of cross-immunity, disparity in incubation periods, and the different clinical pictures produced.

The Marcy agent, isolated in the state of New York, is filterable, induces disease when fed as stool supernate, and produces temporary immunity. An agent with similar characteristics has been studied in Japan (3). Afebrile gastroenteritis with watery diarrhea of the Marcy type is more frequently observed in epidemics (1-4, 12, 13) than is the type of illness induced by the FS agent, although a description of one outbreak (14) indicates that many of the illnesses resembled the latter. Similarly, the Marcy type of gastroenteritis is more common in the Cleveland families than the FS type. In retrospect, the afebrile case reported in the first experiment was not an ideal source of inoculum. The results of the fourth experiment, like earlier studies (7), indicate

that there is considerable variation in the infectivity of fecal supernate from patients with gastroenteritis.

The naturally occurring febrile disease has a lower secondary attack rate than the afebrile type (4). In producing FS inocula it was necessary to start with formed or loose stools rather than with watery fluid essentially free from fecal material. To obtain bacteria-free supernates, therefore, the FS stools were centrifuged at speeds used in work with the poliomyelitis and Coxsackie viruses, which are among the smallest. No filtration experiments have been done with the FS agent. Its size being unknown, many infectious particles may have been lost during centrifugation. This may account in part for the fact that the attack rate of FS in volunteers has not been so high as that with unfiltered Marcy material. The inoculum dosage in the second experiment was 5-fold greater than in the first. No conclusions can be drawn regarding active immunity.

The results of the fifth experiment tend to rule out reaction to normal stool components as the cause of FS illness. The supernates used resembled those from the febrile cases as regards odor and intensity of color, yet this material induced no symptoms after ingestion. The volunteer who reacted violently to 2 ml. of FS supernate had no symptoms when given 10 ml. of the same material. These facts, together with the serial transmission of the febrile illness, imply that it is an infectious disease.

In poliomyelitis, infectious hepatitis, Coxsackie virus infection, and gastroenteritis the causative filterable agents are found in the stool. As with poliomyelitis and infectious hepatitis, doubt exists as to the natural mode of spread of gastroenteritis. Epidemiologic studies have suggested that respiratory as well as enteric spread may be important (4, 12) or have sufficed only for the conclusion that close personal contact is important (4, 13). The observation of Reimann, Price, and Hodges (5) that the inhalation of nebulized, filtered throat washings transmitted gastroenteritis has not been confirmed. In a previous study, none of 7 subjects who inhaled throat washings developed disease, while of 10 who drank throat washings only 2 had questionable symptoms (2, 8). Kuhns and Wetherbee (14) administered 5 ml. of pooled filtered throat washings from 2 patients to 4 volunteers. The inoculum was gargled and then swallowed; no illnesses resulted. Kojima *et al.* (3) were also unable to infect volunteers by feeding throat washings, but as had Reimann, Price, and Hodges (5), they did induce disease by nebulizing watery stools.

Experiments six and seven add to previous findings two more instances of failure to transmit the disease by the inhalation of throat washings. The washing used in the sixth experiment was collected during the acute illness (afebrile type) of a case observed during a family (and neighborhood) outbreak. Although the disease was communicable under natural circumstances, the infection could not be induced by throat washings which had been stored at -70°C . for 23 days. The washing used in experiment seven was collected from a case whose

stool was shown to be infectious; the washing was not infectious when tested after 11 months' storage at -70°C . As noted previously, however, the infectivity of the FS inocula was low, either because of processing or host resistance, or both. This low attack rate could conceivably account for failure to pass the agent by the nasal route, for if nasal infectivity were one-half that of the oral route, it would be difficult to demonstrate any passage. Experiment seven, therefore, does not exclude the possibility of respiratory transmission of the FS agent. The bulk of evidence, however, indicates that the fecal-oral route rather than the respiratory is of importance in the spread of non-bacterial gastroenteritis.

The demonstration that non-bacterial gastroenteritis may be caused by more than one agent is in keeping with the epidemiologic observations previously mentioned (4, 6). Like the respiratory tract, the gastrointestinal tract probably has a limited capacity to respond to noxious stimuli. At least two non-bacterial agents, causing acute gastroenteritis, can be demonstrated in stool supernates, and it is conceivable that still others exist. The analogy with respiratory disease can be carried further for, as with the viruses responsible for the common respiratory diseases, the agents responsible for non-bacterial gastroenteritis have not been established in experimental animals (2, 15-17). A suggestive result with Marcy material (8) has encouraged further attempts to propagate the agents in tissue cultures. All such studies are handicapped by the necessity to use volunteers to test the infectivity of passage material. Solution of the many problems related to the etiology, epidemiology, and control of acute non-bacterial gastroenteritis will be possible only when some ready means of identifying the causative agents is available. Until such identification has permitted the classification of the various agents capable of producing non-bacterial gastroenteritis, proper nomenclature and strain designation are impossible. The two types of gastroenteritis described have been separated as clinical and epidemiologic entities, and it is proposed that the terms "afebrile" and "febrile" infectious non-bacterial gastroenteritis be adopted as interim nomenclature.

SUMMARY

Feeding of stool supernates to volunteers has demonstrated that there are at least two different types—afebrile and febrile—of acute infectious non-bacterial gastroenteritis.

The afebrile illness, produced by the Marcy agent previously described, has an average incubation period of 60 hours and is characterized by watery diarrhea.

The agent (FS) responsible for the febrile illness has been carried through 3 human passages. The febrile disease has an average incubation period of 27 hours and is characterized by constitutional symptoms.

It is believed that the two agents are not the same because of the differences in incubation period, the clinical picture, and the absence of cross-immunity.

Intranasal instillation of throat washings obtained from 2 persons with gastroenteritis failed to produce illness in 2 groups of volunteers.

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