TRANSMISSION OF EPIDEMIC GASTROENTERITIS TO HUMAN VOLUNTEERS BY ORAL ADMINISTRATION OF FECAL FILTRATES

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Numerous outbreaks of gastroenteritis occurred in New York State institutions during the fall and winter of 1946–47. The disease was characterized by sudden onset, with profuse diarrhea, usually accompanied by vomiting, and by a lack of fever. Symptoms ordinarily persisted for about 3 days. Bacteriologic examinations failed to reveal agents known to cause enteric disease and postmortem investigations also failed to indicate the nature of the etiologic agent. A search was therefore undertaken for a non-bacterial pathogen. Since preliminary animal experiments were unsuccessful, human volunteers were utilized. The illness was reproduced and transmitted in series in volunteers and the responsible agent was shown to be filtrable. Oral administration of stool suspensions or throat washings induced the disease, but inhalation of nebulized throat washings did not. Volunteers who were fed material from the third embryonated egg passage likewise remained asymptomatic. The present report describes these experiments.

Epidemiologic and Clinical Features of the Disease

The first of the series of outbreaks with which this report deals was noted in the summer and fall of 1946 in New York City hospital populations. Later the disease occurred in several large mental hospitals near the metropolitan area, and during the course of the winter, there were sizable outbreaks in at least thirteen of the twenty-four New York State mental institutions and in two prisons. Similar episodes are known to have affected a number of mental hospitals in Massachusetts (1).

The specific epidemic from which material was obtained for transmission experiments occurred at the Marcy State Hospital, near Utica, New York. The epidemic was typical, and thus can serve to describe the outbreaks in all the institutions. The disease appeared on December 16, 1946, and was at first largely confined to one building, where in a 2 week period two hundred sixty-one of the five hundred eighty-nine patients suffered from the clinical disease. It gradually spread to each of the other buildings and by January 31, 1947, when the last case was noted, five hundred eighty-nine cases with seven deaths had

occurred among the two thousand, six hundred twenty-three inmates and one hundred fifteen cases among the three hundred fifty-four employees. The deaths occurred in aged or deteriorated mental patients. Within the limitation of the institution population there seemed to be no age or sex selection in the attack rate. The pattern of spread bore no evident relationship to the distribution of milk, food, or water, and sanitary inspection failed to reveal faults that could have accounted for the outbreak.

Thus, epidemiologic investigation indicated that these outbreaks were not from a common source but spread through direct person-to-person contact. Outbreaks occurred during the period from August, 1946, through June, 1947, with some tendency for grouping during December and January. The concentration of the disease in institution populations may be more apparent than real. There is less precise evidence that the general population of upstate New York was also affected. Field studies did not indicate whether the spread occurred through the gastrointestinal or the respiratory routes.

Clinically the patients had a rapid, sometimes dramatic onset of nausea, vomiting, or diarrhea. These happenings, almost always accompanied by anorexia, occurred singly or in combination. Some patients never developed diarrhea. The vomiting was frequently unexpected, sudden, and violent. Abdominal cramps, borborygmi, headache, dizziness, and malaise were common symptoms. Fever was usually absent, or, if present, slight. Recovery ordinarily ensued in 48 to 72 hours, but at times required a week. Respiratory symptoms were not evident. Leucocyte counts were within normal limits except in dehydrated patients. The stools were copious and watery, often justifying description as "pea soup." Blood was rarely seen. Stool specimens from a total of thirty-four patients were examined for bacterial pathogens by the methods given below; none were found.[1]

Methods of Study

Subjects.—Twenty-eight men 21 to 25 years of age and six between 16 and 21 years of age volunteered to participate in the study. Only the adult volunteers were inoculated with presumably infective material; the minors received autoclaved inoculum. Thorough studies were carried out on each adult subject before he was accepted. These included physical examination, chest roentgenogram, blood count, urine examination, sedimentation rate, pharyngeal culture, and multiple stool cultures. Each volunteer was free of recognized infectious or organic disease. None had had diarrhea, nausea, or vomiting during the previous year. It was impossible to restrict selection to individuals who had not been exposed to epidemic diarrhea, since a sharp outbreak of the disease had occurred 2 months previously in an institution

[1] Identical results were obtained by Dr. George C. Bower, who examined by rectal swab on *Shigella-Salmonella*-agar plates stools from five hundred eighty nine patients at Marcy State Hospital, and by Dr. W. R. Strutton who examined stools from over six hundred patients at Rockland State Hospital. Both of these hospitals had recently done rectal swab surveys designed to discover all carriers of *Shigella* organisms.

from which many of the volunteers were drawn. Men who had suffered even trivial symptoms, however, were not accepted.

Precautions for Isolation.—The isolation quarters consisted of two rows of eleven cubicles separated by a central corridor. The corridor was isolated from the rest of the institution by a draft-proof door. Each cubicle had an outside window and a draft-proof door to the corridor; there were no other openings. The volunteers, quartered one to a cubicle, remained in them for the duration of each experiment.

Each cubicle had sink and toilet facilities and each volunteer had his own set of dishes, which he washed himself. Food was dispensed from a cart without touching the dishes. Only one door was opened at a time. The precautions of a contagious pavilion were observed. Only the personnel conducting the experiment entered the quarters.

Collection and Preparation of Inocula.—Three types of inoculum were used: fecal suspensions, throat washings, and chick embryo tissues and fluids. All were frozen within 5 to 15 minutes after collection and stored in a dry-ice chest at approximately −70°C. Specimens were thawed at 37°C. when required for inoculation.

All stool specimens used as inoculum were watery and further dilution was unnecessary. They were centrifuged for a total of 75 minutes at 3000 R.P.M. in a refrigerated horizontal centrifuge (radius 15.5 cm.) which was halted once to remove the supernatant fraction. The final supernatant, which constituted the inoculum, was clear.

Throat washings were collected by having the donors gargle beef-infusion broth containing 10 per cent horse serum. Subsequent clarification by centrifugation was identical with that of stool specimens.

The tissues and fluids harvested from embryonated eggs were chorioallantoic membrane, amniotic membrane, yolk sac, amniotic fluid, and allantoic fluid. Not all of these materials were harvested from each egg. Tissues were ground in a mortar with alundum and made into a 10 per cent suspension (by weight) with allantoic fluid or a mixture of amniotic and allantoic fluids. After light horizontal centrifugation (500–1000 R.P.M.) for 10 minutes, the supernatant was employed as inoculum.

Inocula were kept on cracked ice if they were to be used within several hours after preparation; for longer periods they were refrozen, stored in the dry-ice chest, and thawed immediately before use.

Filtration.—Material to be filtered was drawn by light suction through Corning sintered glass "UF" filters known to hold back bacteria (streptococci, staphylococci, *Bacterium coli*, *Bacterium enterocoliticum*). The filter surfaces were prepared with broth before filtration. Duplicate thioglycollate, aerobic and anaerobic sterility broth, and blood-agar cultures of the filtrates were incubated at 37°C. for 1 month. All were sterile.

Methods of Inoculation.—Two routes of inoculation, oral and respiratory, were used. Inoculum administered by the oral route was fed in double gelatin capsules or drunk from a paper cup. A penicillin nebulizer[2] was employed to inoculate by the respiratory route. The system was a closed one; the volunteers' lips were shut around the opening of the nebulizer and they exhaled into a rebreathing bag. Compressed air or compressed nitrogen was used as a source of pressure to nebulize the inoculum. Inoculations by nebulizer were conducted in an area distant from the isolation quarters, either in the open air or in an entry-way for shelter against the weather.

Observations on Experimental Subjects.—The volunteers were seen at least once each day and symptoms and signs of illness were recorded in a uniform manner. White blood counts, differential counts, specific gravity determinations of the blood and plasma (2), and sedimentation rates (3) were done, and chest roentgenograms were prepared if the subject had been

[2] Distributed by the Oxygen Equipment Company, New York City.

inoculated by inhalation. Medication was limited to the use of barbiturates and codeine. Specimens of serum were collected before inoculation and at intervals thereafter. They were examined for antibodies to influenza viruses A and B (4) and for cold agglutinins (5) since an epidemic of influenza and other respiratory disease occurred in New York State during the course of the experiments. No rises in antibody levels were detected.

Stool Cultures.[3]—Stool cultures from patients with diarrhea were made with fresh or glycerolated material. Endo, eosin-methylene blue, bismuth sulfite, and *Shigella-Salmonella* (bile salt) agar plates were streaked with each specimen, whether from a case of natural or experimental infection, and in some instances tetrathionate broth was also inoculated.

Other Technical Procedures.—Technical procedures not specifically mentioned were those of the Division of Laboratories and Research (6).

Experiments on Transmission

Two experiments were done. The first, a preliminary study, was intended to determine the relative activity of stools and throat washings. The disease was reproduced in three volunteers who swallowed unfiltered stool suspension, but not in three who inhaled throat washings. The second experiment was designed to demonstrate whether the agent was filtrable; to extend the investigations on the infectivity of throat washings; and to test material from embryonated eggs previously inoculated with infective stool. Twenty-two volunteers were employed in these studies, in the course of which the opportunity was presented of reinoculating recovered subjects, thus obtaining limited data on active immunity. Finally, a group of six boys were fed autoclaved stool as a control observation.

Donors.—Two patients at Marcy State Hospital who had characteristic disease were selected as donors. Case reports follow.

J. H., a 35 year old female, had been in good physical health until early in the afternoon of January 7, 1947, when she experienced nausea and abdominal discomfort, quickly followed by vomiting and diarrhea. During the night she vomited six times and had ten watery stools. She complained of anorexia, dizziness, headache, weakness, and feverishness, was slightly dehydrated, had a temperature of 100.2°F., and a leucocyte count of 13,850. Physical examination was negative. Treatment was symptomatic. On the next day her symptoms abated, her temperature was 99.4, and the leucocyte count 10,000. She was completely recovered by the end of the 3rd day. Slight albuminuria, the only abnormal urinary finding, was noted during the period of dehydration and persisted for a few days after recovery. Stool specimens and throat washings were collected approximately 5 hours after onset.

C. W. was a 46 year old male also in good physical health, whose illness began suddenly on January 5, 1947, with diarrhea followed by nausea, vomiting, giddiness, weakness, and abdominal cramps. These symptoms diminished somewhat on the following day but during the 2nd day of illness he vomited once, had five stools, his temperature was 99.6, and the leucocyte count was 7,850. Physical examination showed slight edema of the uvula and hyperemia of the pharynx. Anterior cervical and axillary lymph nodes were palpable but not tender. He was completely recovered on the 3rd day after onset. Stool specimens and throat washings were collected approximately 48 hours after onset.

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[3] The stool cultures were examined by Miss Marion B. Coleman.

Neither of these patients had evidence of acute respiratory infection. Repeated throat cultures revealed none of the commonly recognized pathogens. Roentgenograms of the chest were normal. Bacteriologic and parasitologic examination of multiple stool specimens was negative for known pathogens. Blood cultures, taken at the time the stools were collected, were sterile.

First Experiment

Clinical observation of six volunteers was begun 3 days before each of three was fed 3.6 ml. of a pool of equal parts of unfiltered stool suspension from J. H. and C. W. All three developed characteristic gastroenteritis; case 1 had an incubation period of 1½ days; case 2, 2½ days; case 3, 5 days. Their illnesses closely resembled those of the donors but symptoms were considerably milder in case 3.

The other three volunteers each inhaled approximately 1 ml. of a nebulized mixture of equal parts of throat washings from J. H. and C. W. 3 days after inoculation of the first group. No illness resulted.

During the experiment one of the attendants had a mild episode of diarrhea, unaccompanied by other symptoms, and lasting for a single day. This occurred when cases 1 and 2 first showed signs of illness. His contact with the volunteers during this episode lasted only for 3 hours, and then was broken, but there was a history of exposure to a family epidemic of gastroenteritis 2 days previously. It was felt that the attendant's illness was coincidental and unrelated to the illnesses of the volunteers. All six subjects had equivalent contact with the attendant, whereas only those inoculated with stool became ill. The findings in the second experiment supported this opinion.

Bacteriologic examination of the diarrheal stools of the three subjects revealed *Bacterium enterocoliticum* in one specimen from case 2. This organism, which has been isolated under circumstances which incriminate it as a pathogen (7, 8), is also known to occur in the absence of disease (8). Its lack of etiologic significance in this study was proved by the results of the later experiments with filtered stool from the same patient.

Second Experiment

Because of the importance of detecting cross-infection, emphasized by the occurrence of diarrhea in the attendant, the inoculation schedule of the second experiment was staggered so that at all times there was a group of well, susceptible men in the isolation quarters. Of the various inocula employed, each stool filtrate induced typical illness and one pool of unfiltered throat washings caused gastroenteritis when given by mouth; the others failed to cause disease. The first three men to be inoculated were fed stool filtrate on the day following the quarantine of sixteen volunteers (Fig. 1). Six days later, after all three had become ill, nine more subjects were inoculated orally; three with the same stool filtrate, the other six with material from previously inoculated embryonated eggs. Nine days after isolation began four men inhaled unfiltered throat washings, and a group of six, who were newly arrived, were put into isolation. On the following day, three of these were fed diluted

stool filtrate and three drank unfiltered throat washings. Reinoculations with the various materials were also done, as described below. The time distribution of the illnesses and their strict relation to specific inocula indicate that cross-infection did not occur (Figs. 1 and 2).

Experiments with Stool Filtrates.—Stool suspensions taken in the first 3 days of illness from cases 1 and 2 of the first experiment were filtered and equal

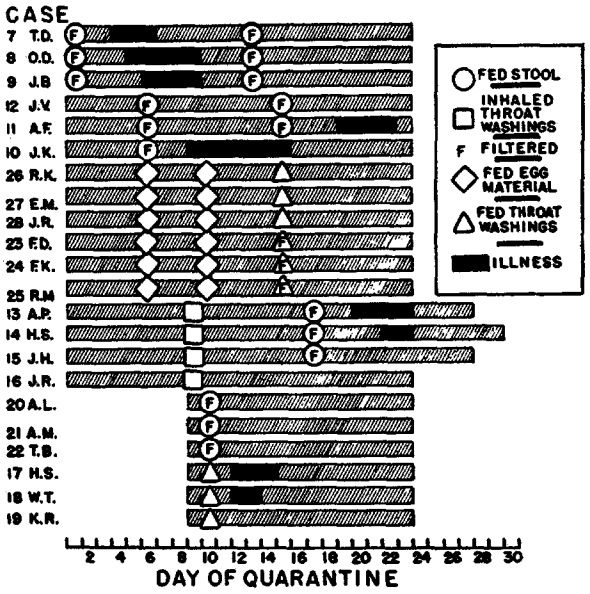

FIG. 1. Duration of quarantine and results of inoculations of each volunteer in the second experiment. Occurrence of gastroenteritis is shown in black.

portions of filtrate pooled. Capsules containing a total of 3.5 to 4.0 ml. of the pool were fed to each of six volunteers; three additional subjects drank 0.05 ml. of the same inoculum diluted in 100 ml. of tap water. The nine men were inoculated on three different days.

Four of the six men who took the larger dose developed typical gastroenteritis, but none of the three receiving 0.05 ml. became ill (Fig. 2). The following report illustrates a moderately severe case:

Case 10.—A 24 year old white male was fed eight capsules containing a total of 4.0 ml. of pooled stool filtrate from cases 1 and 2. Approximately 48 hours later he experienced abdominal cramps and borborygmi. Within the next 12 hours the characteristic symptoms of anorexia, nausea, vomiting, and diarrhea developed (Fig. 3). Although symptoms were moderately severe, there was no abdominal tenderness or spasm. The leucocyte count was 7,000. These symptoms continued undiminished for another day during which he became somewhat dehydrated and had a fever (temperature 100.8°F.). Because of the indications in published reports that changes in the central nervous system might be associated with this (9, 10) or

similar (11) disease entities, and that symptoms of vomiting and giddiness might be accounted for on this basis, permission was obtained to perform a spinal tap at this time. The fluid was under normal pressure and there was one mononuclear cell per c. mm., a protein content of 34 mg. per 100 ml., and a normal colloidal gold curve.[4] On the 3rd day of illness the patient was markedly improved, and his symptoms shortly afterward disappeared except for diarrhea; loose stools continued for another 4 days.

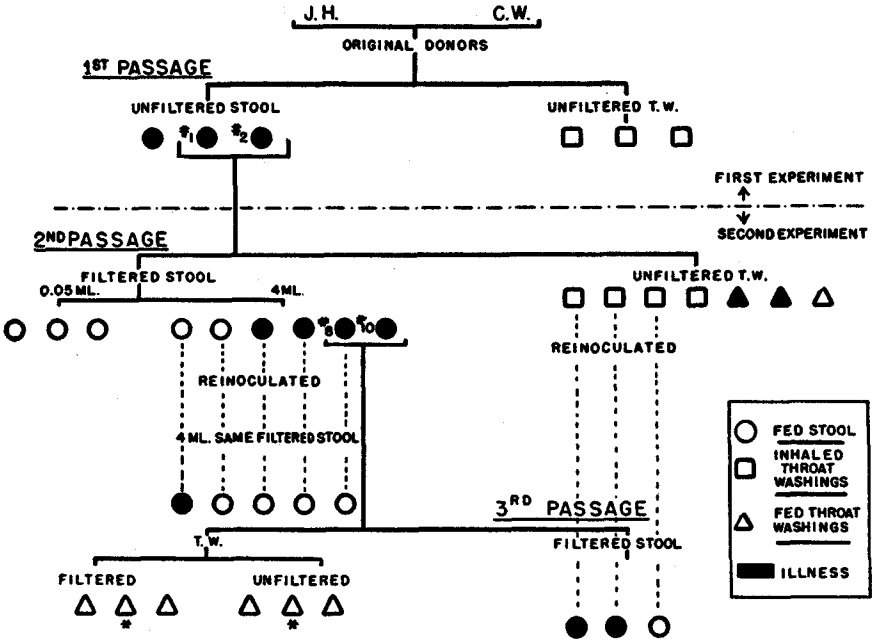

FIG. 2. Passage scheme of the first and second experiments. Occurrence of gastroenteritis is shown in black. Groups of volunteers marked with an asterisk had previously been inoculated with egg material and had remained asymptomatic.

It was previously mentioned that some spontaneous cases of gastroenteritis never exhibited diarrhea. One experimental case was similar.

Case 7.—A 21 year old white male, also fed capsules containing 4.0 ml. of pooled stool filtrate from cases 1 and 2, had as his first symptom precipitate vomiting 3 days later followed by moderate nausea and anorexia (Fig. 4). His appetite was never completely lost, however, nor was his nausea continuous. He vomited ten times on the 1st day, six on the 2nd, three on the 3rd, and recovered on the 4th day after onset. During this time he was constipated. Temperature was normal and abdominal examination negative. Reinoculated with a second equal dose of the same inoculum 10 days after onset, he remained well.

To demonstrate whether the gastroenteritis caused by unheated filtrate was due to a heat-resistant component of the stool or perhaps to the ingestion of the

[4] The spinal fluid examination was performed by Dr. Carl Lange.

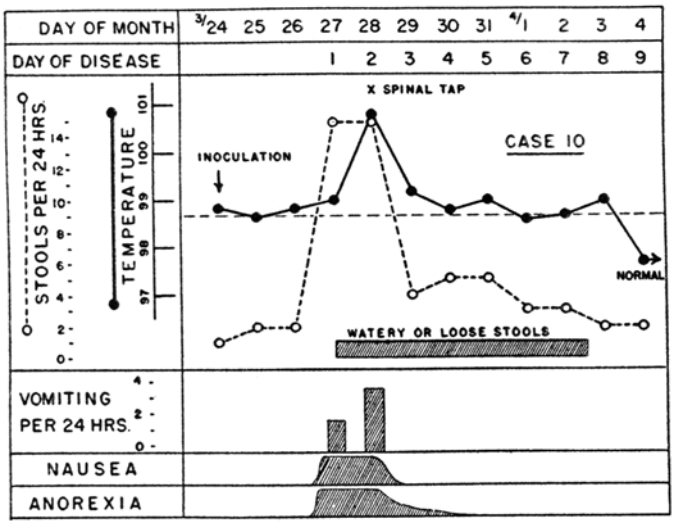

FIG. 3. Case 10. Clinical chart of patient with experimentally induced gastroenteritis.

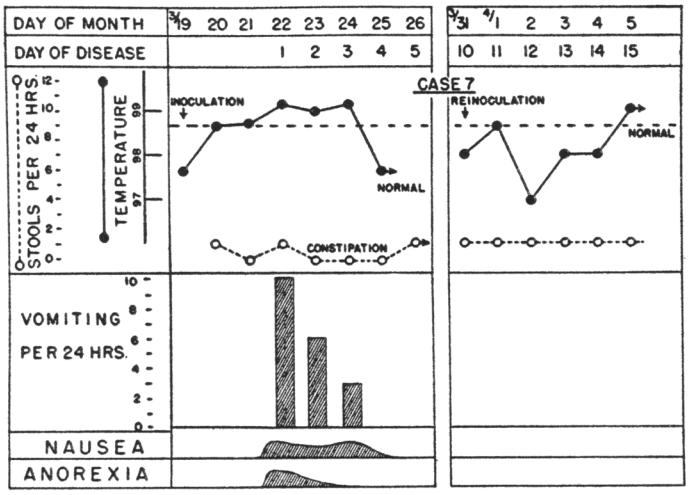

FIG. 4. Case 7. Clinical chart of patient with experimentally induced gastroenteritis; no diarrhea.

capsules as such, a specimen of the pooled stool filtrate from cases 1 and 2 was autoclaved for 20 minutes at 15 pounds' pressure and capsules containing 3.5 ml. of it were fed to each of six boys aged 16 to 21. None subsequently became ill.

The experiments with fecal filtrate showed that the agent was non-bacterial, and that the effective dose probably lay between 0.05 and 4.0 ml. of pooled fil-

trate from cases 1 and 2. There was no certainty that the symptoms were not caused by a filtrable toxin produced by bacteria and a second passage was done to settle this point.

Since dilution of the original 4.0 ml. of inoculum in the voluminous watery stools of the recipients was far greater than the 80-fold difference between the 4.0 ml. effective dose and the 0.05 ml. ineffective dose, a toxin would be diluted beyond the point of causing symptoms. Pooled fecal filtrate from cases 8 and 10, whose illnesses were induced by 4.0 ml. of pooled filtrate from cases 1 and 2, was therefore fed in 6.0 ml. amounts to each of three men[5] (Fig. 2). Two (cases 8 and 10) developed typical gastroenteritis, indicating that the agent had multiplied and was not a toxin ingested by the original cases.

TABLE I

Results of First and Second Inoculations with Pooled Fecal Filtrate from Cases 1 and 2

Case No.	Results of inoculations		Time	
	First	Second	Between inoculations	Between onset and second inoculation
			days	*days*
7	Gastroenteritis	No illness	12	10
8	"	" "	12	9
9	"	" "	12	8
11	No illness	Gastroenteritis	9	0
12	" "	No illness	9	0

It was possible to reinoculate five of the six men who were fed 4 ml. of stool filtrate from cases 1 and 2, thus securing limited data concerning the development of active immunity. Since circumstances did not permit delay, the volunteers were reinoculated 9 to 12 days after the first inoculation (Table I). The second dose was 4 to 5 ml. of the same filtrate. Typical gastroenteritis followed in one of the two subjects who had failed to contract the disease the first time. The others remained asymptomatic. A single loose stool was reported by one volunteer but since other symptoms were absent, it was considered to be of little significance. The findings suggest that active immunity may develop within 10 days after onset of the disease (Table I), but results of immunity tests in such a small group with an inoculum of unknown degree of infectivity must be interpreted with great caution.

Incubation Period and Symptomatology.—For analysis of the range and average duration of the incubation periods and several of the symptoms, data from the three cases induced by unfiltered stool in the first experiment have been added to those obtained from the seven cases induced by filtered stool in the second experiment. The mean incubation period of the ten

[5] These subjects had previously inhaled pooled nebulized throat washings from cases 1 and 2 and had remained well. Further details are given subsequently.

cases was 3 days; it ranged from 1 to 5 days. Vomiting occurred in four of the ten cases, and lasted for no more than 3 days. In contrast, seven of nine cases still had loose stools 4 days after onset, and in one instance this symptom persisted for a week. Borborygmi and abdominal cramps were a constant accompaniment of diarrhea. Leucocyte counts, sedimentation rates, and blood and plasma specific gravities were normal.

Experiments with Throat Washings.—The failure of nebulized throat washings to induce gastroenteritis when inhaled by three subjects during the first experiment could have been due either to lack of activity of the inoculum or to non-transmissibility of the disease by the respiratory route. To investigate these points, throat washings were administered orally in the second experiment as well as by inhalation.

Garglings from cases 1 and 2, taken in the first 3 days of illness, *i.e.* during the same period as the stool inocula were obtained, were centrifuged and the pooled supernatant set aside as inoculum. Cultures revealed the following organisms to be present in it: alpha and gamma streptococci, *Staphylococcus aureus*, Gram-negative cocci, and diphtheroids. The latter organisms were not further identified. Four subjects each inhaled a total of 1.5 to 2.0 ml. of nebulized inoculum (Fig. 2). Three others each drank 8 ml. and swallowed capsules containing 2 ml., a total dose of 10 ml.

The four volunteers who inhaled the inoculum remained well and had normal roentgenograms of the chest; and as mentioned previously, when three of them were subsequently fed fecal filtrate, two proved to be susceptible. Of the three men who swallowed 10 ml. of washings, two developed gastroenteritis, the incubation period being 2 days in each instance. These patients had very brief and mild illnesses. One had only diarrhea, the other had anorexia, nausea, and vomiting as well. Both reported "blood" in their stools, but as the reliability of their observations was questionable and as no blood was seen by the personnel conducting the experiments, this point remains in doubt.

Six other volunteers were fed throat washings. The men had been previously inoculated with embryonated egg material, as reported below, and had remained well. Since the pools of washings from cases 1 and 2 were exhausted, a pool from cases 7 and 10 was utilized. Three received 2.0 ml. of unfiltered washings in capsules, the other three were similarly given 3.5 to 4.0 ml. of the corresponding filtrate. None became ill.

Experiments with Inocula from Embryonated Hens' Eggs[6].—A series of blind passages in embryonated hens' eggs (White Leghorn) originally inoculated with stool suspensions by various methods were not productive of unequivocal signs of infection. Irregular deaths of embryos from the 1st to the 9th day after inoculation, a few hemorrhages into various tissues, and edema and ulceration of some chorioallantoic membranes were noted. These phenomena were not reproducible in series and are known to occur in uninfected eggs (12). A virus (*e.g.*, mumps (13)) may grow in eggs in the absence of lesions, however, and since immunologic methods of testing were unavailable, a second series of passages were carried out for the specific purpose of inoculating volunteers with pooled tissue extracts and extraembryonic fluids from selected eggs.

[6] Dr. Lisbeth M. Kraft aided in this phase of the work.

All eggs were incubated at 35-37°C. both before and after inoculation. The inoculum was unfiltered stool from donor J. H., which had been fed to volunteers in the first experiment. That each of these three developed the disease suggested that the inoculum might contain enough of the etiologic agent to inhibit growth through an autointerference effect (14). Therefore, duplicate passage series by each route of inoculation were initiated with both undiluted and 10^{-1} broth dilution of the unfiltered stool suspension. Three serial passages were carried out by each of three routes: yolk sac, amniotic sac, and chorioallantoic membrane, using embryos of differing ages (Table II). Yolk fluid and sac were harvested from eggs inoculated into the yolk sac after 6 to 7 days of incubation. Those injected by the other two routes were harvested at intervals ranging from 3 to 7 days after inoculation. The amniotic membrane,

TABLE II

Methods Employed in Passaging Embryonated Eggs

Route of inoculation	Age of embryos	Original inoculum		Harvest		
		Dilution	Dose	Day	Tissue	Fluid
	days		*ml.*			
Yolk sac	6–7	Undiluted	0.5	6–7	Yolk sac	Yolk
		10^{-1}	0.5	6–7	" "	"
Amniotic sac	11–12	Undiluted	0.2	3–6	Amniotic membrane and embryo	Amniotic
		10^{-1}	0.2	3–6	" "	"
Chorioallantoic membrane	11–12	Undiluted	0.2	4–7	Chorioallantoic membrane	Allantoic
		10^{-1}	0.2	4–7	" "	"

embryo, and amniotic fluid were taken from amniotically inoculated eggs, but only the chorioallantoic membrane and allantoic fluid were taken from those inoculated on the membrane. Ten to 20 per cent (by weight) suspensions of tissues, made up in the respective fluids harvested, were utilized for passage inocula. Penicillin and streptomycin, 100 units of each per egg, were mixed with the original stool suspension and all passage inocula, cultures of which yielded no growth in sterility broth and thioglycollate medium.

In general the distribution of deaths and the minor anatomical changes observed in this group of eggs resembled those found in the previous group. There was no discernible pattern of deaths or lesions. Tissues and fluids from dead eggs or those which showed lesions, however, were included in each inoculum.

The harvest of the third serial passage was employed to inoculate volunteers. A pool of the yolk sac and amniotic sac harvests was made and each of three volunteers given 4 to 5 ml. of the mixture to drink. Three others each drank an equal amount of the harvest from the chorioallantoic membrane eggs. All six remained symptom-free, and reinoculation with the same pools 4 days later likewise had no effect.

DISCUSSION

In the great majority of outbreaks of gastroenteritis which have been investigated by the New York State Department of Health in the last two dec-

ades, the recognized methods of bacteriologic investigation have failed to reveal the etiologic agent (15). This is particularly true of those epidemics in which afebrile attacks are the rule.

Reimann, Price, and Hodges (16) have reported experiments in human volunteers which suggested that the incitant of a clinically similar disease was present in filtered throat washings and fecal suspensions of patients and could be transmitted by the respiratory but not by the alimentary route. Their investigations were carried out without precautions for isolation during the course of a natural epidemic (17). Within a few hours after collection of specimens of stool or throat washing, Mandler filtrates were inoculated by the respiratory or alimentary routes. The inoculations were done on 6 different days so that presumably the inoculum came from at least six different donors. The effects of inocula from each donor were not specifically given, but over half of fifty-three men who inhaled nebulized filtered stool or throat washings developed gastroenteritis. Incubation periods varied between 1 and 21 days. There was no illness among fifteen subjects who swallowed capsules containing a total of 3 ml. of filtered washings or the four who similarly took an equal dose of stool filtrates.

It appears not unlikely that different etiologic agents were responsible for the disease studied by Reimann and his colleagues, and that described in this paper. The variations in epidemiologic and clinical pattern of outbreaks investigated by this Department suggest that gastroenteritis may be caused by more than one unrecognized agent. This might explain the discrepancies between the respective results. Technical factors, however, may be responsible.

Hodges (18) found that penicillin and streptomycin inhibited bacterial growth in embryonated eggs inoculated amniotically with centrifuged unfiltered stool specimens. The present studies confirm this observation; in addition, they demonstrate that it is also true of eggs inoculated by the yolk sac or the chorioallantoic membrane. The etiologic agent of epidemic gastroenteritis apparently did not multiply in eggs inoculated by these methods, since human volunteers fed tissue extracts and extraembryonic fluids from third passage eggs remained asymptomatic. Possibly the penicillin or streptomycin inactivated the agent. Penicillin inhibits the growth of psittacosis virus in chick embryos (19) for example, although some viruses propagate well in the presence of both antibiotics.

The demonstration that the causative agent of the form of epidemic gastroenteritis here described is present in fecal filtrates and that the disease is readily reproduced in volunteers by oral inoculation should be helpful in orienting further epidemiologic studies and in attempts to establish the agent in experimental animals. Full identification of the agent awaits the successful completion of such studies.

SUMMARY

Epidemic gastroenteritis was transmitted to human volunteers by the oral administration of fecal filtrates. The original inocula were obtained from

patients in a natural outbreak which occurred at Marcy State Hospital in the winter of 1946–47. The experimental disease closely resembled that of the donors. The incubation period ranged from 1 to 5 days, with a mean of 3 days. The disease was carried through three generations, in the last two by means of fecal filtrates.

Oral administration of unfiltered throat washings from experimental cases of the disease likewise induced gastroenteritis but subjects who inhaled a portion of the same throat washings remained asymptomatic. Volunteers who inhaled throat washings taken from patients in the epidemic at Marcy State Hospital also failed to develop the disease.

Five volunteers who had previously been inoculated with fecal filtrates were reinoculated with the same material. Gastroenteritis followed in one of the two subjects who had failed to contract the disease the first time. The others remained well.

Embryonated hens' eggs were inoculated with one of the two unfiltered stool suspensions used in the pool which had induced gastroenteritis in each of the three volunteers to whom it was fed. Three sets of eggs were inoculated: one on the chorioallantoic membrane, another into the yolk sac, and a third into the amniotic sac. Three serial passages were carried out by each method at varying time intervals. Penicillin and streptomycin were employed as antibacterial agents. Tissue and extraembryonic fluids from the third passage were non-infective for volunteers.

These experiments were done at the New York State Vocational Institution, West Coxsackie, New York. We wish to express our gratitude to J. A. Lyons, Commissioner of Correction; to D. D. Scarborough, Superintendent, J. P. Conboy, Assistant Superintendent, and Dr. A. J. Flood, Senior Physician, of the Vocational Institution, and to their respective staffs, for the complete cooperation and support which made these studies possible.

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BIBLIOGRAPHY

1. Feemster, R. F., verbal communication.
2. Phillips, R. A., and others, New York, Josiah Macy, Jr., Foundation, 1945, photoprint.
3. Wintrobe, M. M., Clinical Hematology, Philadelphia, Lea and Febiger, 2nd edition, 1946, 230.
4. Hirst, G. K., *J. Exp. Med.*, 1942, **75**, 49.
5. Commission on Acute Respiratory Diseases, *Am. J. Med. Sc.*, 1944, **208**, 742.
6. Wadsworth, A. B., Standard Methods of the Division of Laboratories and Research of the New York State Department of Health, Baltimore, The Williams and Wilkins Company, 3rd edition, 1947.
7. Schleifstein, J. I., and Coleman, M. B., *New York State J. Med.*, 1939, **39**, 1749.

8. Schleifstein, J., and Coleman, M. B., *Bacterium enterocoliticum*, in New York State Department of Health. Division of Laboratories and Research, Annual Report, 1943, 56.
9. Gray, J. D., *Brit. Med. J.*, 1939, **1,** 209.
10. Bradley, W. H., *Brit. Med. J.*, 1943, **1,** 309.
11. Cristensen, E., and Biering-Sørensen, K., *Acta Path. et Microbiol. Scand.*, 1946, **23,** 395.
12. Beveridge, W. I. B., and Burnet, F. M., *Great Britain Med. Research Council, Special Rep. Series, No. 256*, 1946, 92.
13. Habel, K., *Pub. Health Rep., U. S. P. H. S.*, 1945, **60,** 201.
14. Henle, W., and Henle, G., *Am. J. Med. Sc.*, 1944, **207,** 705.
15. New York State Department of Health, Division of Communicable Diseases, Annual Reports, 1926–1946.
16. Reimann, H. A., Price, A. H., and Hodges, J. H., *Proc. Soc. Exp. Biol. and Med.*, 1945, **59,** 8.
17. Reimann, H. A., Hodges, J. H., and Price, A. H., *J. Am. Med. Assn.*, 1945, **127,** 1.
18. Hodges, J. H., *Science*, 1946, **104,** 460.
19. Meiklejohn, G., Wagner, J. C., and Beveridge, G. W., *J. Immunol.*, 1946, **54,** 1.