II. POLIOMYELITIC VIRUS IN URBAN SEWAGE*

By JOHN R. PAUL, M.D., JAMES D. TRASK, M.D., and SVEN GARD, M.D.

(From the Departments of Medicine and Pediatrics, Yale University School of Medicine, New Haven)

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In the preceding paper (1) attention has been called to the fact that poliomyelitic virus can be readily isolated from the stools of some patients with this disease. Our own experiments, and those of many others, now testify to the ease with which this can be accomplished; not only with paralytic cases but also with the more common abortive types; and not only during active stages of the disease but also during convalescence. Obviously, therefore, when an epidemic of poliomyelitis occurs within a city there must be ample opportunity for the virus to enter the local sewage system. And, considering the frequency of mild and unrecognized forms of poliomyelitis, and the length of time which such cases may be potentially infectious, it seems possible that the concentration of virus in urban sewage may become appreciable. Prior to 1939, however, poliomyelitic virus had never been actually demonstrated in sewage, although it is questionable how vigorously it had been looked for; at least there are no published records of such attempts.[1]

During the summer of 1939 several opportunities arose to examine this situation under what would seem to be favorable circumstances, for, at least three large urban epidemics of poliomyelitis occurred in eastern sections of this country within this period, namely, in Charleston, S. C., in Detroit, Mich., and in Buffalo, N. Y. In each of them we were fortunate in being allowed to examine the local sewage.[2] The first few tests in this

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[1] In the Philadelphia epidemic of 1932 we made our first attempts of this type, using samples from sewer outlets in the Schuylkill and Delaware Rivers. We tried again with material from New Haven sources in 1937. The methods were crude in both series of attempts and the results, which were all either unsatisfactory or negative, were not reported.

[2] For the privilege of making these studies we are indebted to Dr. Leon Banov, Health

series, which were made in Charleston, have already been described in a brief preliminary note (2). The present report deals with these earlier tests in more detail, as well as the later ones from the other two epidemics. The whole series of individual tests is listed in Table I.

Experimental Methods

Preparation of Sewage.—In general, the preparation of sewage samples for inoculation into monkeys has been based upon methods for the isolation of poliomyelitic virus from stools (1). The actual steps used in the adaptation of the stool method for use with sewage may be seen in Table I. This indicates that changes were made from time to time, but the usual procedure was as follows:—

Specimens of sewage were collected in the morning (between 7.00 and 10.00 o'clock) either by one of us, or by a member of the local Department of Health. These specimens (usually 4 liters in volume) were collected in sterile containers, and under as sterile conditions as are feasible within the confines of a manhole or well. First they were allowed to stand in the cold for about 2 hours. During this time settling took place. The supernatant fluid amounting to at least four-fifths of the original volume was then discarded and the sediment, amounting to about 750 cc., was then transferred to a tightly stoppered container and to this 10 to 15 per cent ether by volume was added for bactericidal purposes. These etherized specimens were immediately transported (usually by airplane) to our laboratory in New Haven and on the day following their collection some of the material from each specimen was inoculated intra-abdominally into one or more monkeys. (The time element, that is, the shortness of the interval between the collection of the specimen and its inoculation, seems important, for the infectivity of poliomyelitic virus seems to diminish fairly quickly on standing in sewage.) The amount of the inoculum varied. At first we used large quantities (up to 200 cc.) but the mortality from acute peritonitis was such that it was soon found better to reduce the size of the inoculum to 20 cc. This seems to be a fairly safe dose; for out of 29 monkeys inoculated with plain (etherized) sewage in amounts of 20 cc. or less, only one monkey died of acute peritonitis; whereas of 15 monkeys inoculated with amounts of similar material ranging from 21 to 200 cc., the mortality from peritonitis (chemical or bacteriological) was 60 per cent.

From the onset of these experiments attempts were made to increase the delicacy of the method, and to improve it in so far as the preparation of the inoculum was concerned. Concentration of the volume of the specimens, either by evaporation at room temperature with an electric fan (designated "aeration" in Table I), or at low temperature with a vacuum pump was attempted a few times without particular success. Precipitation of the virus from sewage by half saturation with ammonium sulfate was tried more extensively because this procedure had potentially the double virtue of concentrating the virus in the precipitate, and of the elimination of unprecipitated substances, some of which were possibly toxic. Accordingly early in the course of these experiments,

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a method was devised by one of us along these lines. Some of the tests in which this precipitation method was used appear in Table I, but the actual procedures are described in the third paper in this series (3).

Monkey Inoculations.—For the inoculation of sewage specimens, fresh monkeys were employed in about 80 per cent of the tests. Those monkeys which had been used more than once are so designated in Table I. *Macacus rhesus* monkeys were employed in 64 tests recorded in this paper, *Macacus mordax* in 2 tests. Daily temperature and exercise records were kept on all inoculated monkeys for a period of 4 weeks, unless it was found necessary to kill them earlier, and daily exercise records of all monkeys were made before and after the experiments, or as long as the animals were in our possession. If an animal died during the 4 weeks' observation period from some cause other than poliomyelitis, the test was considered unsatisfactory. This occurred 13 times (about 20 per cent) in the 66 tests listed in this series of experiments. If an animal showed signs suggesting experimental poliomyelitis, it was killed at what seemed an appropriate time; histologic sections were examined from the medulla, cervical, thoracic, and lumbar regions of the spinal cord, and, if it seemed indicated, an attempt was made immediately to pass the virus to another monkey, using multiple intracerebral inoculations (acceleration) at intervals of 5 to 7 days, if necessary.

Identification of the Virus.—One of the first questions which invariably arises when claims are made that poliomyelitic virus has been isolated from any unusual source is: Are you sure that it is poliomyelitic virus? Criteria on which the answer to this question is based will probably change from year to year but at present we know of no reason to alter those which we have used in previous experiments on the isolation of poliomyelitic virus from nasopharyngeal washings (4) and from human stools (5, 1). They include three, and in many instances four standards which each strain must fulfill: (*a*) The production of a "clinical picture" in the inoculated monkey which is compatible with that of experimental poliomyelitis, *viz.* after an appropriate incubation period there is the development of a train of characteristic symptoms, usually exemplified by fever, excitement, tremor, ataxia, weakness, and paralysis, the latter being generally associated with a fall in temperature. (*b*) When the animal is killed, lesions typical of experimental poliomyelitis must be found in the region of the anterior horns of the spinal cord, in lumbar as well as in cervical levels. These lesions should be "unequivocal," and besides presenting evidence of neuronophagia there must be perivascular infiltrations with mononuclear cells. (*c*) Passage of the strain to a second monkey must be successfully accomplished in the course of which, criteria (*a*) and (*b*) must again be fulfilled. The last criterion, which has been used in most instances is: (*c*) that the suspected material, or strain, when inoculated into other laboratory animals, such as, rabbits, guinea pigs, and Swiss mice,[3] does not produce an encephalomyelitis in these animals. It has been

[3] For testing monkey passage material each mouse was inoculated intracerebrally and intra-abdominally; guinea pigs were inoculated both intracerebrally and subcutaneously; rabbits intracerebrally, subcutaneously, and the virus was also instilled into the scarified cornea of one eye. For testing straight sewage, mice were inoculated as above; guinea pigs were inoculated intra-abdominally and by ocular instillation; rabbits by ocular instillation alone. An occasional mouse, and 3 of the guinea pigs (see Table I) died within a few days of the inoculation. No evidence of an encephalitis or a myelitis was found in histological sections from the brains and spinal cords of these animals. Attempts to "pass" material from these animals were not made.

TABLE I

Sewage Tests in Three Epidemic Areas

	Site of collection	Specimen	Date of collection	Preparation of etherized specimen (a)	Inoculation: Time since collection	Inoculation: Monkeys: Monkey (b)	Inoculation: Monkeys: Dose (c)	Inoculation: Monkeys: Route (d)	Result of monkey inoculation: Fever (e)	Paralysis (f)	Lesions in spinal cord (g)	Remarks (h)	Passage: 2nd generation: Monkey or rodents	Result (h)
					days		*cc.*							
Charleston														
Exp. 1	Pumping Station	A	July 7	Aeration	6 & 7	12-29	36 & 22	ip.				K. 6th day, peritonitis		
	Harbor	B	" 7	None	7	12-31	24	"			−	D. 19th day		
	Pumping Station	C	" 11	"	1 & 2	12-27	75 & 45	"	+10	+11	+		12-48	Polio.
	" "	"	" "	"	3	12-32	125	"	+8	+9	+			
	" "	"	" "	"	15	12-50	19	"	−	−	−			
	" "	"	" "	$(NH_4)_2SO_4$	18	12-55	5 (100)	"	+12	+15	+	.....	13-00	"
													13-14**	"
	Hospital	D	" 14	None	5	12-39	75 & 125	ip. & sc.				D. 3rd day, cellulitis		
	"	"	" "	$(NH_4)_2SO_4$	6	12-40	25 & 36	" " "			−	K. 5th day, tetanus		
	"	"	" "	None	12	12-49	25	ip.	+6	−	−			
	"	"	" "	$(NH_4)_2SO_4$	13	12-52	22 (100)	"	?11	−	−			
Exp. 2	Pumping Station	C-1	Aug. 4	None	7	12-20*	20	"	?13	−	−			
	Harbor	B-1	" 8	"	1	12-76	87	"				D. 2nd day, peritonitis		
	"	"	" "	"	3	12-22*	20	"	−	−	n.s.			
	Pumping Station	C-2	" "	"	1	12-75	90	"			−	D. 6th day, cellulitis		
	" "	"	" "	"	3	12-21*	15	"	−	−	n.s.			
	" "	"	" "	Filtered (Seitz)	15	12-50*	1	ic.	−	−	−			
	Hospital	D-1	" "	None	1	12-73	30	ip.				D. 2nd day, peritonitis		
	"	"	" "	"	7	12-00*	50	"	−	−	n.s.			
	Cal. St.	E	" "	"	1	12-74	80	"				D. 3rd day, peritonitis		
Exp. 3	Pumping Station	C-3	Oct. 19	"	1	13-63(*M*)	20	"	−	−	"			
	" "	"	" "	"	1	13-25*	20	"	−	−	"			
	" "	"	" "	$(NH_4)_2SO_4$	2	13-64(*M*)	35 (200)	"	+8	−	−			
	" "	"	" "	"	2	13-26*	38 (200)	"	?27	−	n.s.			

Detroit Exp. 1	Frankenmuth	F	Aug. 9	None	2	12-78	20	"	+10	–	–			
	"	"	" "	"	3	12-83	30	"	–	–	n.s			
	Windsor, Canada	G	" "	"	2	12-23*	20	"	–	–	"			
	" "	"	" "	"	3	12-85	33	"	?13	–	–			
	No. Hamtramck	H	" 10	"	1	12-24*	20	"	–	–	n.s.			
	" "	"	" "	"	2	12-84	30	"				D. 2nd day, peritonitis		
	So. "	I	" "	"	1	12-28*	20	"	–	–	–		1 g. pig	D. 4th day
	Hospital J	J	" "	"	1	12-26*	20	"	–	–	n.s.		1 rabbit	Neg.
	" K	K	" "	"	1	12-25*	20	"	+11	+12	+		6 mice	"
	" "	"	" "	"	12	12-98	19	"	–	–	n.s.		12-97	Polio.
													14-03	"
Exp. 2	" "	K-1	" 16	"	3	12-34*	17	"	+9	+10	+		12-57*	"
	" "	"	" "	"	14	13-04	20	"	–	–	n.s.		13-38	"
	" "	"	" "	$(NH_4)_2SO_4$	14	13-06	17 (200)	"	+15	+16	+		6 mice	Neg.
	" "	"	" "	"	14	13-07	5 (200)	"			–	D. 6th day, dysentery		
	" "	"	" "	Vac. distil.	7	12-99	20 (500)	"	–	–	n.s.			
													14-04	Polio.
													13-41	"
Exp. 3	" "	K-2	Sept. 7	None	1	13-15‡	20	"	+12	+13	+		1 rabbit	Neg.
	" "	"	" "	"	1	13-17	2	"	–	–	n.s.		6 mice	"
	" "	"	" "	"	1	13-18	0.5	ic.				D. 2nd day, brain abscess	2 g. pigs	D. 8th & 9th days
	" "	"	" "	"	2	13-19	20	ip.	?15	–	"			
	" "	"	" "	Filtered (Seitz)	2	13-20	1 & 19	ic. & ip.	–	–	–			
Exp. 4	" "	K-3	Oct. 19	None	1	13-59	20	ip.	–	–	n.s.			
	" "	"	" "	$(NH_4)_2SO_4$	2	13-17*	43 (200)	"	–	–	"			
	No. Hamtramck	H-2	Nov. 7	None	1	13-75	20	"	–	–	"			
	So. "	I-2	" "	"	1	13-76	20	"	–	–	"			
	No.&So. Hamtramck	H-2 & I-2	" "	$(NH_4)_2SO_4$	2	13-78	45 (200)	"	–	–	"			
	" " " "	" "	" "	Vac. distil.	2	13-77	55 (100)	"	±15	–	"			
Exp. 5	Hospital K	K-4	" 23	None	1	13-83	20	"	?14	–	–			
	" "	"	" "	$(NH_4)_2SO_4$	2	13-84	15 (200)	"	?19	–	–			
	" "	"	" "	Vac. distil.	2	13-85	27 (100)	"	?19	–	–			

(*a*) $(NH_4)_2SO_4$ = specimen precipitated with ammonium sulfate and dissolved precipitate used for inoculum.

(*b*) *Monkey previously used but thought to be non-immune to poliomyelitis. (*M*) = *Macacus mordax*.

(*c*) 5 (100) = the inoculum of 5 cc. represented the concentrate from 100 cc. of raw sewage.

(*d*) ip. = intra-abdominal inoculation, sc. = subcutaneous, ic. = intracerebral.

(*e*) +10 = fever developed 10 days after inoculation. (*f*) +11 = paralysis developed 11 days after inoculation. (*g*) n.s. = the monkey was not sacrificed.

(*h*) K. = killed; D. = died.

** Passage of strain from 13-14 in 3rd generation: Monkey 13-36, poliomyelitis, not sacrificed. 4 mice, 3 guinea pigs, 2 rabbits, negative.

‡ Inoculation of specimen K-2 into rodents: 4 mice, 2 guinea pigs, 1 rabbit, negative.

TABLE I—*Concluded*

	Site of collection	Specimen	Date of collection	Preparation of etherized specimen (*a*)	Inoculation: Time since collection	Inoculation: Monkeys: Monkey (*b*)	Inoculation: Monkeys: Dose (*c*)	Inoculation: Monkeys: Route (*d*)	Result of monkey inoculation: Fever (*e*)	Result of monkey inoculation: Paralysis (*f*)	Result of monkey inoculation: Lesions in spinal cord (*g*)	Result of monkey inoculation: Remarks (*h*)	Passage: 2nd generation: Monkey or rodents	Passage: 2nd generation: Result (*h*)
					days		*cc.*							
Buffalo													13-55	Neg.
Exp. 1	Island community	L	Sept. 16	None	1	13-28	20	i. p.	±21	−	n.s.		13-12*	"
	" "	"	" "	$(NH_4)_2SO_4$	3	13-35	20 (200)	"	+16	+18	+		13-20*	"
	Main sewer	M	" "	None	1	13-29	20	"	±26	−	−		12-80(*M*)	"
	" "	"	" "	$(NH_4)_2SO_4$	3	13-33	32 (200)	"	+8	−	−		13-80	"
	Hospital N	N	" "	None	1	13-30	20	"	+10	−	−		6 mice	"
	" "	"	" "	$(NH_4)_2SO_4$	3	13-34	26 (200)	"	+8	−	−			
	Disp. Pl.—Sludge	P	" "	Diluted	1	13-32	20	"				D. 4th day, peritonitis		
	Hospital O	O	" 26	None	1	13-45	20	"	+14	−	−	Tuberculosis		
	" "	"	" "	$(NH_4)_2SO_4$	2	13-46	35 (200)	"	−	−	−			
Exp. 2	Disp. Pl.—Sludge	P-1	Oct. 9	$(NH_4)_2SO_4$	5	13-58	45 (200)	"				D. 2nd day, peritonitis		
	Island community	L-1	" "	None	1	13-56	20	"	−	−	n.s.			
	" "	"	" "	$(NH_4)_2SO_4$	2	13-62	45 (200)	"	−	−	"			
	Main sewer	M-1	" "	None	1	13-60	20	"	−	−	"			
	" "	"	" "	$(NH_4)_2SO_4$	2	13-61	55 (200)	"	−	−	"			

our practice to observe these smaller animals for a period of 4 weeks; to take daily temperatures on the rabbits and guinea pigs during this period; to sacrifice all the animals at the end of the 4 week period and to study the mid-brain and three levels of the cord histologically. Such tests should be helpful in differentiating the virus of poliomyelitis from that of lymphocytic choriomeningitis, equine encephalomyelitis, and other viruses capable of producing encephalitis in these species. The extent to which these four criteria have been met appears in Table I (specimen C, monkey 12-55; and specimens K, K-1, and K-2, monkeys 12-25, 13-06, and 13-15).

We have not resorted to cross neutralization experiments as a diagnostic procedure with the sewage strains because of the expense of this procedure and of the difficulty of interpreting the results, particularly in view of the immunologic differences which exist between different strains of poliomyelitic virus. The value of neutralization and of reinoculation methods, and some of the difficulties in their use as a means of identification of a recently isolated strain have been described in a recent note in which a series of such tests are recorded on a single strain of poliomyelitic virus isolated by two of us from a human stool in 1937 (6).

RESULTS

Charleston.—The first city in which sewage was tested was Charleston, where poliomyelitis was epidemic from April through July, 1939. About 80 cases were reported within the municipal area during this period (see Fig. 1, right) with an equal number from surrounding districts, almost all of which were hospitalized in Charleston. Clinically the disease was fairly typical although unusual features of this epidemic were: its occurrence so far south, and the low age group of those attacked, for more than half of the cases were aged 3 years or under.

In the collection of the first sewage specimens an effort was made to locate sewers draining areas where the cases were, or had been most concentrated, such as the vicinity of one of the local Isolation Hospitals and also those areas within the city where the spot map indicated a high prevalence of the disease. One of the diagrams (Fig. 1, left) indicates the general relationships between three sites of this type. It shows the sewer from the hospital joining that from the infected district E. This junction occurs a short distance above a pumping station where our first positive tests were obtained. A sample of sewage from this pumping station obtained on July 11th (specimen C in Table I) was inoculated into 2 monkeys (Nos. 12-27 and 12-32) in amounts of 120 and 125 cc., respectively. Both of these animals promptly developed poliomyelitis, both were prostrate on the 3rd day of their disease, and one of them died on this day. It is probable that these 2 monkeys would have been infected even if doses smaller than 120 had been used, for 18 days later the precipitate from 100 cc. of the same specimen was also shown to be infective (see monkey 12-55). But large

as all three of these doses may seem to be, they are but drops in the bucket compared to the total volume of virus-containing sewage, pouring through this station at this time, estimated at about 470 gallons per minute. Theo-

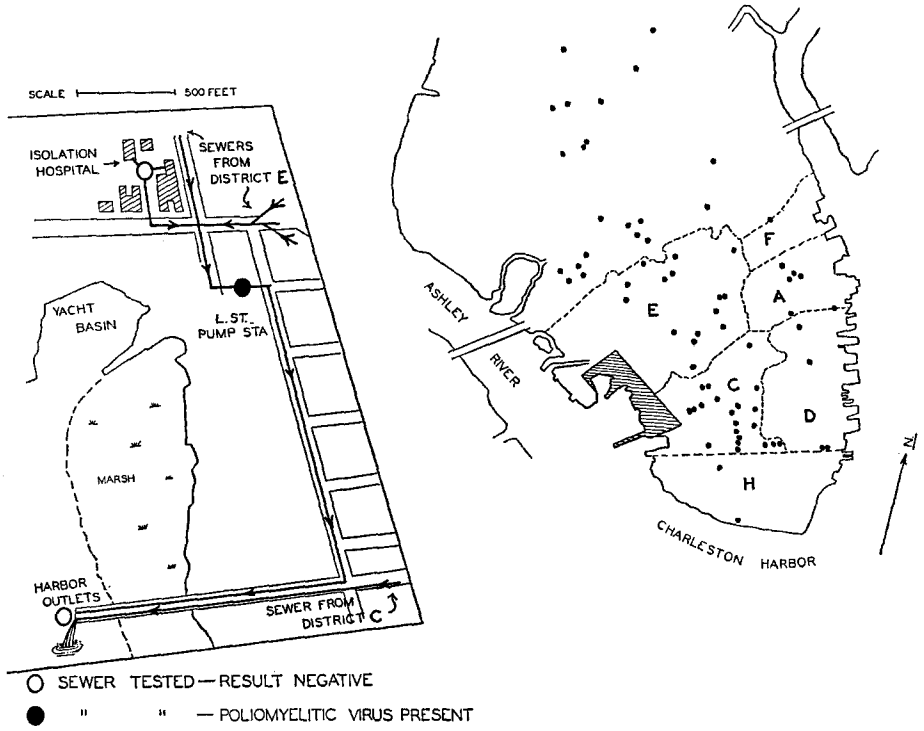

FIG. 1

Right.—An outline map of the City of Charleston on which have been dotted the home sites of poliomyelitis cases occurring in this city between Apr. 1 and Aug. 1, 1939. In the lower half of this city map, six sewer districts (A, C, D, etc.) are shown, each district being drained by one or more main sewers. The shaded area on the southwestern shore of the city has been enlarged in the left hand diagram and represents the area from which all our specimens were taken.

Left.—Three sites where tests were made are shown. Heavy lines indicate sewers which lead from the Isolation Hospital (upper left) and join with sewers from district E (upper right). Subsequently they pass through the L St. Pumping Station where the first positive tests for poliomyelitic virus were obtained on July 11. The two other sites from which negative tests were obtained contemporaneously, are also shown.

retically then, one could calculate that more than 18,000 doses of poliomyelitic virus infective for the monkey, were passing through this site each minute of the hour in which the sewage specimen was collected.

Of equal importance to the amount of virus in this large volume of sewage

is the question of the source and transportation of the virus. If we assume that this virus was of human origin, then it must follow that it came either from patients in the Isolation Hospital, or from patients or "carriers" in district E, or from both. We have no way of answering this question at present. 4 tests (2 unsatisfactory and 2 negative) were made on sewage specimens obtained on the hospital grounds (specimen D), that is, from a site more proximal to the hospital than the pumping station; but the material was old when tested, and the results cannot be regarded as being particularly significant. One conclusion that can be drawn, however, from the positive finding is that whether the virus came from the hospital or not, it survived in an urban sewer long enough to be transported for a distance of at least one-sixteenth of a mile.

Subsequent tests were made from the pumping station and the other sites in early August, at a time when the epidemic was practically over, and again in October. All of them were negative.

Detroit.—The second city in which sewage tests were made was Detroit, where a large epidemic totalling more than 500 cases, occurred between July 1st and Nov. 1st, 1939. Early in this epidemic (Aug. 10) 2 specimens were collected within the city (sample H, north; sample I, south of the Hamtramck district). A concentration of cases seemed to exist in these two large areas but the actual numbers of cases there were relatively small at this time. A similar specimen was obtained (Aug. 9) in the city of Windsor, Canada, and another in a more rural setting in the town of Frankenmuth, Mich. All of the tests of the sewage from these four sites proved negative for poliomyelitic virus.

At about the same time (Aug. 10) the sewer outlets from two Detroit Isolation Hospitals were tested. In one of these hospitals, samples were secured from a trap in the basement where the sewer passed out of the Isolation building. These samples appeared as moderately cloudy or soapy water containing a sediment of dark particles. No particles recognizable in the gross as fecal material could be seen and yet in spite of this rather unfavorable looking material, poliomyelitic virus was recovered from the sample of Aug. 10, and from 2 other samples obtained on Aug. 16 and Sept. 7 respectively; subsequent samples obtained on Oct. 19 and Nov. 23 were negative.[4] The relation of these positive findings to the number of cases of poliomyelitis in the Isolation wards of this hospital appears in Table II. Certainly for a period of a month, virus was being discharged by this sewer.

[4] We are particularly indebted to Dr. J. G. Molner of the Detroit Department of Health for the collection of 3 of these samples.

It is unfortunate, perhaps, that we did not carry out more tests on sewage samples collected within the City of Detroit during the course of this large epidemic. However, after the epidemic was practically over (Nov. 9) we again tested samples from sites H and I. The tests were again negative.

Buffalo.—The third, and relatively the most severe epidemic of poliomyelitis in our series, was in Buffalo, where about 330 cases were reported

TABLE II

Results of Tests on Sewage from Hospital K, Showing the Number of Patients with Poliomyelitis at the Time of Each Test

Date	Poliomyelitic virus in hospital sewer	No. of hospitalized patients with poliomyelitis
Aug. 10	+	37
Aug. 16	+	55
Sept. 7	+	33
Oct. 19	−	9
Nov. 23	−	3

TABLE III

Number of Satisfactory Sewage Tests and Per Cent Which Were Positive

	During the epidemic				After the epidemic				Total satisfactory tests
	Fresh sewage*		Old sewage		Fresh sewage		Old sewage		
	Plain	Ppted.	Plain	Ppted.	Plain	Ppted.	Plain	Ppted.	
No. of tests	19	4	5	3	12	6	3	1	53
No. positive	5	0	0	2	0	0	0	0	7
Positive, *per cent*	26	0	0	66†	0	0	0	0	13

* Fresh sewage is sewage inoculated within 3 days after its collection. Old sewage has been allowed to stand for more than 3 days.

† Two of these specimens were selected for the precipitation method because virus had already been demonstrated in the fresh specimens; the 66 per cent positive figure is, therefore, not significant.

between Aug. 1 and Nov. 1, 1939. On Sept. 16 and 26, samples from sewers leading from two hospitals were tested; one at a point about 50 yards from Hospital N, and another distant by about half a mile from Hospital O. Both of these tests were negative. Specimens were also obtained from several other points (M and M-1, which represented the main city sewer influent as it entered the Disposal Plant; and P and P-1 which were specimens of sludge, representing material which had been through the plant). All of these tests were either negative or unsatisfactory. The sludge mate-

rial proved unusually toxic in that both of the monkeys inoculated with relatively small doses of it, promptly died.

A fourth site in Buffalo was chosen because it represented a more or less circumscribed "island" community with a population of about 750 people. 3 cases of poliomyelitis had been reported from this community. Samples from four sewers draining the island area were pooled (sample L) and 2 monkeys were inoculated. One of the monkeys (13-35) receiving precipitated material from this site developed, after an incubation period of 16 days, the clinical picture of poliomyelitis with fever and paralysis. Extensive histological lesions were also present in the spinal cord, particularly in the medulla. Repeated attempts to pass this disease to subsequent monkeys failed and our inability to fulfill the third and fourth criteria marks this a negative result.[5]

A subsequent sample of pooled sewage from this site taken Nov. 9 was also negative.

A summary of all the satisfactory tests made in these 3 epidemics appears in Table III. The results thus arranged suggest, (*a*) that it has been easier to isolate poliomyelitic virus from urban sewers during than after epidemics, and (*b*) that the precipitation method is the superior if old sewage is to be used.

DISCUSSION

From the experience in these 3 epidemics, several general conclusions can be drawn. These are: (*a*) There seems to be little doubt that poliomyelitic virus can be occasionally isolated from urban sewage during the course of epidemics. (*b*) The 22 negative, post-epidemic results (see Tables II and III) are of some importance in establishing the fact that poliomyelitic virus cannot be readily demonstrated in urban sewers at all times. (*c*) It appears to have been easier to isolate the virus in the vicinity of Isolation Hospitals than elsewhere. (*d*) There are indications that the total virus content of sewage may occasionally be large. (*e*) Virus may be transported by flowing sewage for at least one-sixteenth of a mile.

Further than this we cannot go; for it is evident that methods for the detection of poliomyelitic virus in sewage have not been perfected and the

[5] Once before we had an experience similar to this one, namely we were unable to carry a strain beyond its first passage. In the epidemic of poliomyelitis which occurred in New Haven in 1935 a monkey (D-4), infected by nasopharyngeal washings, gave a picture of experimental poliomyelitis typical in most respects except that the lesions which were predominant in the cervical cord and brain stem, were minimal in the lumbar cord. Repeated attempts to reproduce this disease in other monkeys failed.

performance of many more tests of greater delicacy than those used here are necessary before one can say more about the circumstances under which this virus can be found outside the human host.

From the technical side of this work we have also learned something about the properties of poliomyelitic virus, namely that although it is sufficiently resistant to survive in urban sewage at some distance from its probable source, it appears somewhat unstable in this medium, for it has been difficult, though not impossible, to demonstrate virus after the collected sample has been allowed to stand for 3 days (see Table III) whereas in some stools it has kept for weeks (1, 5).

Of the many questions which arise in connection with this whole subject, however, one of the first is: Why should poliomyelitic virus have been singled out, as it were, by our procedures from all the other possible "infectious agents" which urban sewage must contain; and indeed this same query applies to the virus in human stools also? The number of pathogenic bacteria in sewage is, of course, greatly reduced by the first step in our method, *viz.*, the addition of ether, but the inoculum is generally not rendered completely bacteria-free by this procedure. What action ether might have upon viruses, other than poliomyelitic virus, which might be present there, is unknown, although it seems reasonable to regard poliomyelitic virus as one of the fairly stable viruses, in so far as its resistance to germicidal agents is concerned. It may be recalled in this connection that poliomyelitic virus can withstand 1 per cent phenol for a period of weeks (7), and that aqueous stool suspensions containing poliomyelitic virus and 15 per cent ether, have been shown to be infective after standing for $2\frac{1}{2}$ months at ice box temperature.

But another feature in the "selectiveness" of the method is the fact that apart from daily temperature records, only the neuromuscular system of the animals was examined systematically, and the central nervous system tissue alone was used for the passage of the strains. Undoubtedly the inocula, in spite of their treatment by ether, contained bacteria, and if these were present in adequate amounts the inoculated monkeys contracted an infection which was fatal to 10 monkeys in this series. Undoubtedly, also several monkeys became ill as a result of being injected with some of the many toxic agents (either bacterial, viral, or chemical) which sewage must contain. To this the fairly frequent presence of unexplained fever during the course of the 4 week post-inoculation period may testify. But unless our inoculated monkeys also developed symptoms pointing to involvement of the central nervous system, fever was not taken seriously. In other words our methods were designed essentially for the detection of neurotropic viruses.

But perhaps the most important question of all is, what does the finding of the virus mean in so far as the spread of poliomyelitis is concerned? In answer one could say that it is not evident from this work whether or not the presence of poliomyelitic virus in sewage is a direct or even an indirect link in the chain which usually or even occasionally leads this infectious agent from one patient to another in this disease. Our report merely calls attention to the fact that during urban epidemics of this disease the local sewage may contain this virus.

SUMMARY

In two out of three large urban epidemics of poliomyelitis the virus of this disease has been detected in samples of sewage. From one of the sites it was found repeatedly. Both positive sites were located in the vicinity of isolation hospitals, and we believe that the findings indicate that this virus can be transported, for short distances at least, through the medium of flowing sewage.

BIBLIOGRAPHY

1. Trask, J. D., Paul, J. R., and Vignec, A. J., *J. Exp. Med.*, 1940, **71**, 751.
2. Paul, J. R., Trask, J. D., and Culotta, C. S., *Science*, 1939, **90**, 258.
3. Gard, S., *J. Exp. Med.*, 1940, **71**, 779.
4. (*a*) Paul, J. R., and Trask, J. D., *J. Exp. Med.*, 1932, **56**, 319. (*b*) Paul, J. R., Trask, J. D., and Webster, L. T., *J. Exp. Med.*, 1935, **62**, 245.
5. Trask, J. D., Vignec, A. J., and Paul, J. R., *J. Am. Med. Assn.*, 1938, **111**, 6.
6. Trask, J. D., Paul, J. R., and Vignec, A. J., *Proc. Soc. Exp. Biol. and Med.*, 1939, **41**, 241.
7. Literature dealing with the stability of poliomyelitic virus is summarized (up to 1932) in Poliomyelitis, International Committee for the Study of Infantile Paralysis, Baltimore, The Williams & Wilkins Co., 1932, Chapter II, 52–57.