

PERIODIC EXAMINATION OF SEWAGE FOR THE VIRUS OF POLIO-MYELITIS*

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During epidemics of poliomyelitis in 1939, the virus was detected in sewage collected in Charleston, South Carolina (1-3) and Detroit, Michigan (2). All told in North America 6 positive tests were obtained with 4 samples of sewage. These were among a total of 30 specimens examined which, in addition to representing the cities mentioned above, included samples from Windsor, Ontario; Buffalo, New York; and from a rural area in Michigan. The series included 22 tests on 11 post-epidemic specimens from 4 large cities and the results of these tests were negative (2). In Sweden during an epidemic in Stockholm a positive result was obtained by Kling in October, 1939 (4). These positive findings probably have epidemiological significance but as yet they throw no crucial light on the pathogenesis of poliomyelitis and in order to understand them better it is still a matter of importance to know more about normal conditions in respect to virus in sewage. Accordingly, data on sewage collected routinely over a number of months is presented in the following report. It describes periodic sampling in New Haven, Connecticut, and in New York, New York, largely during non-epidemic periods.

Methods

Samples of raw, unchlorinated sewage were collected monthly from New Haven, Connecticut, and from New York, New York, from April, 1940, to June, 1941. The sites of collection in New Haven were: (a) a trap in the Isolation Pavilion, a 60-bed unit of the New Haven Hospital, and (b) a comminuting chamber of the City Point Sewage Disposal Plant. In New York the collections were made at the Manhattan Grit Chamber at 110th Street and East River Drive.¹ There is an average daily

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¹ For the privilege of examining samples of sewage from New York and for access to the daily analyses of it we are indebted to Mr. W. Donaldson, Director, Bureau of Sewage Disposal. For the privilege of examining the sewage of the City Point Disposal Plant, New Haven, Connecticut, we are indebted to Mr. W. Vincent Barry, Acting Director of Public Works and Dr. J. I. Linde, Health Officer, New Haven, Connecticut.

flow of about 14 million gallons of sewage through the New Haven City Point Plant which serves a population of 119,800 and an area of 5500 acres in New Haven and its suburb Hamden. The Manhattan Grit Chamber has a daily average flow of 88 million gallons and it serves a population of 623,000 and an area of 3,253 acres in Manhattan.

The technique was based on that used successfully in Charleston and Detroit (1-3). 4 liters of sewage were collected between 8 and 10 a.m., placed in the refrigerator to sediment for 3 to 6 hours when 700 cc. of the middle layer were siphoned into a 1 quart bottle, treated with 105 cc. of ether, corked, and refrigerated overnight. The next morning 20 cc. of etherized sewage were inoculated intraabdominally into a rhesus monkey (*M. mulatta*) and 200 to 400 cc. were concentrated to a volume of 30 to 50 cc. with $(\text{NH}_4)_2\text{SO}_4$ by Gard's method (3) and on the next day the concentrate was inoculated intraabdominally into another *rhesus* monkey. After 12 months of sampling, inoculations of unconcentrated sewage were discontinued and tests were limited to the use of concentrated inocula. Criteria for the identification of the virus of poliomyelitis were the same as those used previously (2) and consisted in: (a) the reproduction of infectious myelitis in the monkey with (b) characteristic histological lesions in upper and lower levels of the spinal cord and (c) passage to another monkey. Also tests in small animals were used. The criteria for a negative test were that the monkey survived for 4 weeks of observation either remaining well, or if an irregular temperature curve or other signs appeared, that the animal revealed no histological lesions in the parenchyma of its brain stem or cord on autopsy. Among these animals special attention was given to the presence or absence of histological evidence of choroidal or meningeal reaction.

If the monkey inoculated with the concentrate was lost before the expiration of 4 weeks the test was considered incomplete and is recorded in Table I with a zero sign unless a repeated specimen was tested satisfactorily that month.

RESULTS

The results are presented in Table I which reveals that of 45 monthly tests for the virus of poliomyelitis 36 were completed and of these 35 were negative and 1 was positive.²

The details of the positive test were as follows:

September 19th, the specimen was collected at the Manhattan Grit Chamber; September 21st, the inoculum of 30 cc. (the concentrate from 400 cc. of etherized sewage) was injected intraabdominally into monkey 16-24 which remained well until September 30th, when it had fever. October 1st, it was tremulous and both legs were paralyzed; October 2nd, it was prostrate and was killed with ether. Passage with glycerolated spinal cord was successful in *M. mulatta* and the histological lesions were typical. Some further tests were made and on intracutaneous inoculation the

² This positive test was mentioned recently in a footnote; Paul, J. R., and Trask, J. D., *J. Am. Med. Assn.*, 1941, **116**, 493.

strain was non-infective in 1 *M. mulatta*. On intracerebral inoculation in small animals with the strain, 2 guinea pigs remained well and 1 of 6 Swiss mice died. The mouse died on the 14th day but had no histological signs in brain, cord, or meninges; 1 of the other mice had slight microscopic evidence of a low grade encephalitis which seemed to be non-acute. There was no meningitis, encephalitis, or myelitis in the guinea pigs. Serial passage in small animals was not tried.

TABLE I

Tests for the Virus of Poliomyelitis in Sewage in Relation to Cases Reported in New York, N. Y., and in New Haven, Conn.,—April, 1940 to June, 1941

Month	New York, N. Y.			New Haven, Conn.			
	Cases of poliomyelitis reported		Tests for virus at grit chamber	Cases of poliomyelitis reported		Tests for virus in sewage	
	In city	On sewer line		In city	In hospital	City Point disposal plant	Hospital
<i>1940</i>							
Apr.	1	None	—	None	None	0	—
May	None	“	0	“	“	—	—*
June	“	“	0	“	“	—	0
July	7	4	—	“	“	—	—
Aug.	16	3	—	“	3	—	—
Sept.	25	4	+	“	2	0	0
Oct.	14	None	—	“	2	—	—
Nov.	3	“	—	“	1	—	0
Dec.	1	“	—	“	1	—	—
<i>1941</i>							
Jan.	None	“	—*	“	None	0	—
Feb.	2	“	—	“	“	—	—
Mar.	None	“	—	“	“	—	—
Apr.	“	“	—	“	“	—*	—
May	1	“	—	“	“	—	0
June	None	“	—	1	1	—	—

+, positive for poliomyelitis virus; —, negative; 0, not completed.

* Mononuclear meningitis without lesions in cord or brain stem.

The other monkey (No. 16-23) used in the routine test of the sample of September 19, from New York received only 20 cc. of unconcentrated sewage and remained well during its period of observation. At autopsy no lesions were seen in its cord on microscopic examination. This furnishes corroborative evidence that Gard's (3) method of concentration is valuable.

Previously the stability of the virus in sewage kept in the refrigerator had been slight (3). In order to make another test of this property the process of concentration was repeated October 7, on 400 cc. of the original specimen which had been kept in the refrigerator since September 19. The result was negative.

Tests with subsequent samplings from the Manhattan Grit Chamber, of which

there were 2 in October, 1940, and which up to the present writing are being continued at monthly intervals, have been negative.³

Among the 35 tests which appear in Table I as negative for poliomyelitis there were 3 (1940, May; 1941, January and April) in which microscopic examination at the close of the experiments revealed a mild meningitis or chorooiditis without lesions in parenchyma of brain stem or cord. The 3 monkeys had fever and agitation during the 2nd and 3rd weeks but no weakness or other signs of myelitis were observed. Passage of these 3 cords to monkeys or small animals has not been tried.

It seems pertinent to state that the inocula used for 2 of these 3 animals had been prepared by Gard's method and that in the single instance (January, 1941) when a meningeal reaction was observed in a monkey following the injection of unconcentrated sewage its mate which received the concentrate was lost on the 2nd day. A subsequent sample that month evoked no reaction, with or without concentration.

The results for September and October, 1940, and for January and May, 1941, represent repetitive sampling, generally because of the premature loss of the inoculated monkeys. In the entire series 87 monkeys were used. Of these, 11 were lost intercurrently: 8 following concentrated and 3 following unconcentrated inocula; 3 died in the first 3 days with intestinal distension and fatty liver as chief findings, 1 died on the 4th day with purulent peritonitis, and 7 died on the 7th to 25th day also with purulent peritonitis.

Besides the results of the tests, it may also be seen from Table I that there were remarkably few cases of poliomyelitis reported during the period of 15 months covered by the experiments. Actually there was only one reported from New Haven although a few patients from the nearby cities of Waterbury, Cheshire, and Naugatuck were in the Isolation Pavilion of the New Haven Hospital at various times from August to December. Accordingly, in respect to New Haven, the period under study may be considered essentially as inter-

³ The serial monthly examinations have now been carried through October, 1941, and only one further significant result has been obtained. This was with sewage collected from the Manhattan Grit Chamber, October 14. The monkey inoculated intraperitoneally with the $(\text{NH}_4)_2\text{SO}_4$ concentrate from 400 cc. of etherized sewage, remained well until the 10th day, then it developed fever. On the 12th day, tremor and ataxia were observed and on the 15th paralysis of the legs. On histological examination the lesions were characteristic and well marked throughout the spinal cord. Thus the picture was typical of experimental poliomyelitis but successful passages are necessary before the criteria of a positive result have been met. Attempts at these passages are now in progress. So far guinea pigs and Swiss mice inoculated intracerebrally with the infected cord have remained well.

During the summer and fall of 1941 there was a distinct increase in cases of poliomyelitis reported from Manhattan in the area served by the Grit Chamber.

epidemic. In New York City there was a slight but progressive increase in cases reported⁴ in 1940 during July and August, to reach a peak in September with a gradual decline subsequently to none for January, 1941. Some of the cases recorded for July, August, and September (4, 3, and 4 respectively) were from the region served by the sewer being sampled. Nine of these 11 cases were from a relatively small area between 160th and 180th Streets near Audubon Avenue and they appear as a small focus in Fig. 1.

An incidental finding, concerning the detection of tubercle bacilli in sewage, was made during the study. Certain monkeys, inoculated intraabdominally with the routine dose of 20 cc. of etherized unconcentrated sewage, contracted an unusual form of peritoneal tuberculosis whereby, 6 to 12 weeks after inoculation, the omentum and visceral peritoneum were found studded with tubercles of fairly uniform size while lungs, pulmonary nodes, liver, spleen, and kidney

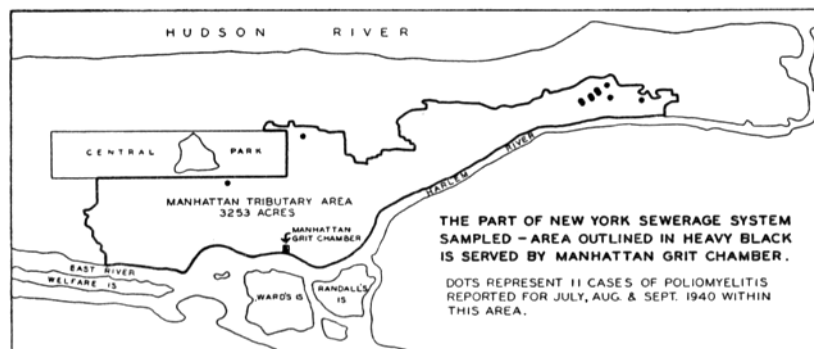


FIG. 1

were relatively free. This reaction was not encountered after the sewage had been subjected to concentration but after the inoculation of 20 cc. unconcentrated Manhattan sewage this form of tuberculous peritonitis was common. In fact, in monkeys observed for 6 weeks or more, it was seen in all of 7 monkeys so inoculated. From one of them a strain of tubercle bacillus was recovered which was identified as of human type by its growth in the presence of glycerol and its pathogenicity for guinea pigs and relative lack of pathogenicity for rabbits.

DISCUSSION AND SUMMARY

This communication records an additional example of the detection of the virus of poliomyelitis in urban sewage. If we assume that the virus detected had a direct human origin and that this is represented, even remotely in Fig. 1,

⁴ For help at the inception of the study and for subsequent epidemiological data we are indebted to Dr. John L. Rice, Commissioner of Health, and to Dr. Samuel Frant, Director, Bureau of Preventable Disease, New York City.

then either the heaviness of the contamination of the sewage, or the amount of dilution which the virus tolerated, are truly amazing.

The positive result and its relation to cases of poliomyelitis reported add little to our knowledge of the *pathogenesis* of poliomyelitis, but at least the data are of some special epidemiological significance. The nature of this is not yet obvious, chiefly, perhaps, because it is not yet known whether the increase of cases precedes or follows an increase in the hypothetical basal level of the virus in sewage. However, as far as the data go they do not suggest that the virus is more or less evenly distributed throughout the year and that epidemics of paralysis are conditioned chiefly by variations in susceptibility which is postulated by Aycock's (5) hypothesis of autarcesis in poliomyelitis. However, it seems likely that the solution of this problem must await the development of better methods for it must be admitted that our present ones are crude.

Incidentally, the positive result recorded herein helps to confirm the value of Gard's method of concentration (3) which not only lead to the detection of the virus but also decreased the hazard of tuberculous peritonitis in the experimental animals in the present series. It is also suggested that the monkey may be a useful animal for the detection of tubercle bacilli in sewage. This raises the issue of how it was possible to inoculate monkeys with as much sewage as was done without detecting more varieties of familiar pathogenic agents. There are some obvious reasons for this. One is that by neglecting to try passage we may have missed a "meningitic virus" in the 3 monkeys which had fever and histological evidence of mononuclear meningitis. Another reason is that our methods were highly selective because systematic observations were limited to body temperature, to general features, to the function of motion, and to the histology of the cord and brain stem. Thus pathogenic bacteria, measles, mumps, etc., could have infected our monkeys without our knowing it.

CONCLUSIONS

From a series of tests covering 15 months, another instance is recorded of the detection of the virus of poliomyelitis in urban sewage.

It has not yet been possible to detect the virus of poliomyelitis in sewage during non-epidemic periods.

It is likely that the periodic sampling of sewage for pathogenic viruses or bacteria may be a method of epidemiological value.

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