ISOLATION OF POLIOMYELITIS VIRUS FROM THE NASOPHARYNX*

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The number of times that the virus of poliomyelitis has been isolated from the nasopharynx of individuals either ill or in contact with this disease is small.[1] It is so small, in fact, that it is questionable whether the epidemiology of poliomyelitis will be readily elucidated through this approach unless it is found that the infrequent detection of the virus in the nasopharynx is the result of inadequate methods. In an effort to improve these methods, therefore, the investigations described in this paper have been carried out, the opportunity having been presented by the epidemic of poliomyelitis which occurred in California during the summer of 1934. As a result of this study another case of poliomyelitis which harbored the virus in the throat was found. The circumstances under which the virus was isolated will be described.

Methods

Clinical Material.—Nasopharyngeal washings were obtained from patients in various stages of suspected or diagnosed cases of poliomyelitis. A few determina-

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[1] The virus of poliomyelitis has been isolated, according to our estimation, thirteen times from the throat or tonsils of living human beings, and eleven (or perhaps thirteen) times from the nasopharynx or tonsils of fatal human cases. Eight positive results have been obtained from patients in, or prior to the acute stage of the disease (2–5); two from convalescents, one early (17 days) (6) and the other late (5 months) (7); and three from what appear to be healthy contacts (8–10).

tions were also made on contacts. Choosing and classifying cases for study proved difficult owing to the fact that the great majority of diagnosed cases in the epidemic were mild. According to Kessel, Hoyt, and Fisk (1), the mortality rates and per cent of cases with residual paralysis or pleocytosis of spinal fluid were exceptionally low, while the number of ailments which were diagnosed poliomyelitis among adults and hospital attendants was extraordinarily high.

Our cases have been classified on the basis of the following diagnostic terminology: (1) paralytic, *i.e.* cases with paralysis developing in association with the usual signs of the disease, and positive spinal fluid findings, such as a pleocytosis (more than 12 cells) or an increase of globulin; (2) abortive, *i.e.* cases in which paralysis did not develop but which presented positive spinal fluid findings in association with characteristic symptoms and signs; (3) suspected abortive, *i.e.* cases with characteristic symptoms or signs similar to those in the abortive group in which lumbar punctures were either not done, or in which the results were negative.

Preparation of the Inoculum.—Sterile physiological saline solution was used as the irrigating medium in the majority of instances. The washings were prepared for inoculation by (*a*) phenolization, (*b*) filtration, and (*c*) glycerinization.

(*a*) *Phenolization.*—Samples of freshly obtained washings generally amounting to from 10 to 20 cc. were employed. Within 1 to 2 hours of the time of their collection 5 per cent phenol was added in an amount sufficient to make a final concentration of 0.5 per cent. The material was then allowed to stand at room temperature for at least 30 minutes before being inoculated into the monkey.

(*b*) *Filtration.*—Samples of freshly obtained nasopharyngeal washings generally amounting to about 10 to 15 cc. were centrifuged at about 1,600 R.P.M. for 10 to 20 minutes. The supernatant fluid, still turbid, was filtered under the following conditions: Seitz filters with disc 2.3 cm. in diameter and a chamber of 15 cc. capacity, measuring 1.5 x 10.5 cm., were used. 10 to 15 cc. of beef infusion broth, pH 7.8, was first passed through the filter at 15 pounds pressure. This procedure required not more than 5 minutes. The test material was then filtered at 30 pounds pressure. The lapse of time for this process varied from 5 to not more than 30 minutes. The filtrate was immediately used for inoculating purposes.

(*c*) *Glycerinization.*—Samples of nasopharyngeal washings, generally amounting to about 10 to 15 cc., were centrifuged as stated above. The supernatant fluid was removed and usually set aside for filtration (method given above). The sediment consisting of small flakes of mucopurulent exudate, cells, and bacteria, was transferred with a capillary pipette either immediately to 2 or 3 cc. of a solution of 50 per cent glycerine in saline, or it was left in the ice box for from 1 to 4 days and was subsequently transferred to the glycerine solution.

Before inoculating the sediment it was washed once in 10 to 15 cc. of saline solution. The washed sediment, in a volume from 0.2 to 0.5 cc., was inoculated intracerebrally, and the remaining supernatant saline solution (9 to 14 cc.) was usually

inoculated intraperitoneally. All material eventually tested by this method had remained in glycerine for more than 12 days.

Inoculations.—Small *rhesus* monkeys weighing on an average from 4 to 5 pounds were used. Inoculations with phenolized and filtered material were performed within 3 to 4 hours after collection and were made under ether anesthesia by the intracerebral (0.2 to 1 cc.) and intraperitoneal routes (2 to 30 cc.). Inoculated monkeys were observed daily, and daily temperature records were taken over a period of from 3 to 4 weeks. Twenty animals which failed to show symptoms after 3 weeks were used again. Furthermore, in ten instances the same animal was inoculated at from 2 to 5 day intervals with similarly treated material from two or three different patients who represented similar types of cases. Thus, one monkey would receive, at 2 or 3 day intervals, inoculations of filtered material from contacts; another, inoculations of phenolized material from suspected abortive cases.

Single inoculations were employed with phenolized and filtered material. When glycerine had been used as a preservative for the inoculum, the initial inoculation was often reinforced by a second intracerebral and intraperitoneal inoculation given 7 to 8 days later.

If any of the inoculated animals died within 3 weeks of the last inoculation from some cause other than poliomyelitis, the experiment was considered unsatisfactory and was discarded. All animals which showed symptoms of poliomyelitis, or remotely suggesting poliomyelitis, were sacrificed at what appeared to be an appropriate time, and material from the mid-brain, medulla, and cervical, thoracic, and lumbar regions of the spinal cord was preserved in 50 per cent glycerine, 10 per cent formalin, and 95 per cent alcohol. Histological sections stained with hematoxylin and eosin were made from the material preserved in formalin, and in those in which lesions of poliomyelitis or lesions resembling poliomyelitis were found, sections stained in toluidine blue were prepared from the material in alcohol. Criteria for the establishment of the diagnosis of poliomyelitis employed in these experiments were that: (1) after an incubation period of from 4 to 12 days the animal showed acute fever with subsequent defervescence, tremor, ataxia, or paralyses (they were sacrificed shortly afterwards inasmuch as none of the animals died which contracted the experimental disease on the first passage); (2) lesions typical of poliomyelitis were found in the cord; and (3) the strain was brought at least through its second passage. Criteria for a negative result were that the animal did not die from some cause other than poliomyelitis during a period of 3 weeks from the last inoculation, and that it failed to show the first two criteria requisite for a positive result.

Passage Experiments.—Samples of medulla and upper cervical cord, usually weighing from 0.7 to 1.5 gm., were ground in a mortar with sand or alundum in saline solution to make a 10 per cent emulsion. 1 cc. was inoculated intracerebrally and the remainder of the emulsion inoculated intraperitoneally. If the animal failed to show symptoms within 6 days, a second inoculation (reinforce-

ment) was made on the 6th, 7th, or 8th day from a freshly prepared suspension of medulla and cord.[2] Animals thus inoculated were observed and sacrificed in the same manner as those which received human material.

RESULTS

These may be summarized by the brief statement that the virus of poliomyelitis was isolated from the nasopharynx of a single individual with an illness which conformed in its symptomatology to a case of suspected abortive poliomyelitis. It was not detected in the small number of frank (abortive) cases tested, or in healthy contacts. The type of clinical material studied and the methods employed in each appear in Table I. Here the cases have been listed according to the day of illness on which washings were obtained. Experiments which were considered unsatisfactory because of the premature death of the monkey have not been included in this list.

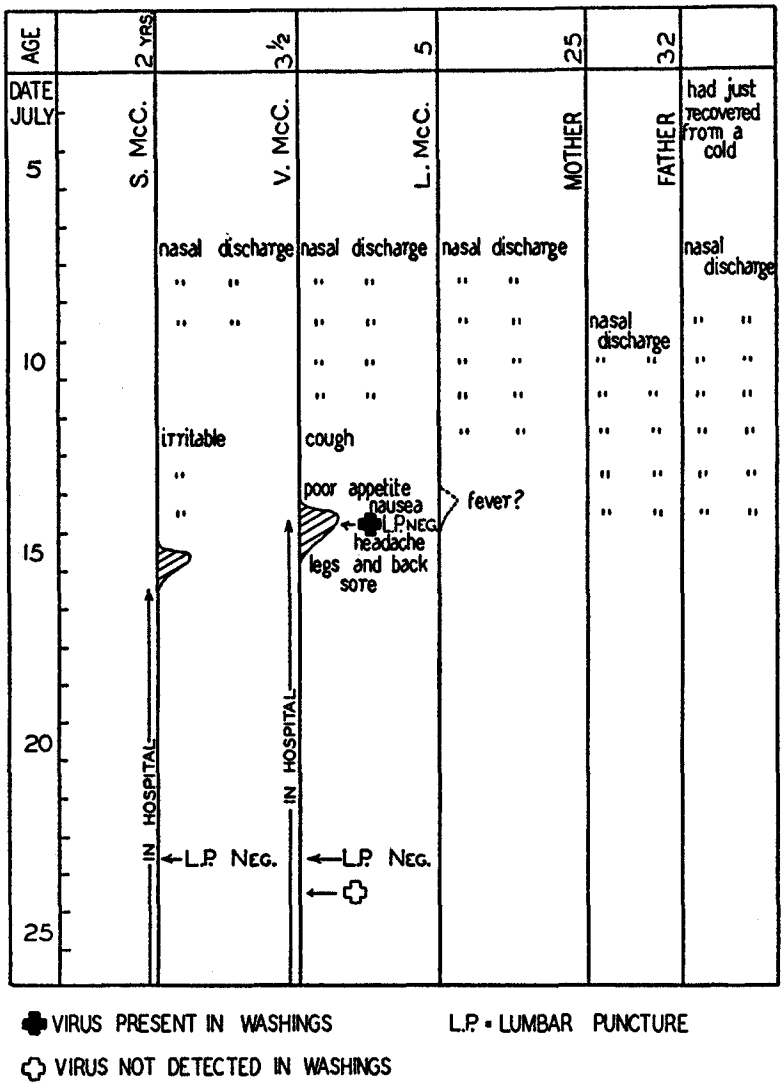
Description of Case Which Yielded the Virus.—The virus was obtained from the nasopharynx on the 1st day of a brief illness. Details of this illness can perhaps be best presented in terms of the family history (Text-fig. 1).

Family History.—V. McC., aged 3½ years, was one of three children. The family resided in Los Angeles when poliomyelitis became epidemic in that city; *i.e.*, during the late spring and summer months of 1934. Its members had all been well during May and June with the exception of the father who had contracted a "cold" in the last week of June. Between July 7 and 9 all of the family members, including the father, suffered from "colds" characterized chiefly by nasal discharge. This symptom lasted from 3 to 7 days. On July 14, V. developed fever, headache, anorexia, nausea, and pains in the legs and back. She was admitted to the Los Angeles County General Hospital on the same day, with the diagnosis of suspected poliomyelitis. On admission the child presented the usual signs of an acute, mild febrile (temperature 101.4°) illness. The tonsils were large and red and in addition her neck was slightly stiff. However, her spine was flexible and there were no abnormal reflexes or muscle weaknesses. Nasopharyngeal washings taken at this time contained an excess of mucopurulent material. A lumbar puncture performed at this time, and also a second one performed 9 days later, were negative.

On July 15 the child's younger brother S., aged 2 years, also developed fever

[2] It has been our experience that in early (second through fourth) passages of strains isolated from human cases, if a single inoculation of a 10 to 20 per cent cord emulsion is used, positive results have occurred in but 45 per cent of the animals inoculated. If double inoculations of a similar dose are employed, positive results have occurred in 90 per cent.

and symptoms similar to those of his sister. He was admitted to the hospital on July 16. A lumbar puncture, performed on July 23, was negative. In both children the illness did not last more than 48 hours.



TEXT-FIG. 1. Schematic diagram of illnesses which occurred in the McC. family. The vertical lines represent individual members of the family; their respective ages appear at the top. The shaded areas indicate roughly the time of onset and duration of fever in the two youngest children.

A second nasopharyngeal washing was obtained from V. on the 10th day after the onset.

Experimental Details.—The volume of the saline washings obtained on July 14 from V. McC. totalled 18 cc. They were divided into two parts, A and B. To part A (6 cc.) 0.5 per cent phenol was added and (after standing for 45 minutes) 1 cc. was inoculated intracerebrally and 5 cc. intraperitoneally into one monkey (No. C-2-9). The remaining 12 cc. of the washings (part B) was centrifuged, the supernatant fluid was removed for filtration, and the sediment designated as part C. The supernatant fluid (part B) was filtered through a Seitz filter (see under Methods) and of this filtrate 1 cc. was immediately inoculated intracerebrally and 7 cc. intraperitoneally into one monkey (No. C-3-1). Both of these animals (Nos. C-2-9 and C-3-1) had been inoculated within a week previously with material which was subsequently shown not to contain the virus. Part C, the sediment from 12 cc. of the original washings in saline, was placed in the ice box and after 3 days was transferred to 50 per cent glycerine. On July 27 (13 days after its collection), 0.5 cc. of the washed sediment was inoculated into each of two monkeys (Nos. C-4 and C-1-4). All of the animals (Nos. C-2-9, C-3-1, C-4, and C-1-4) developed experimental poliomyelitis.

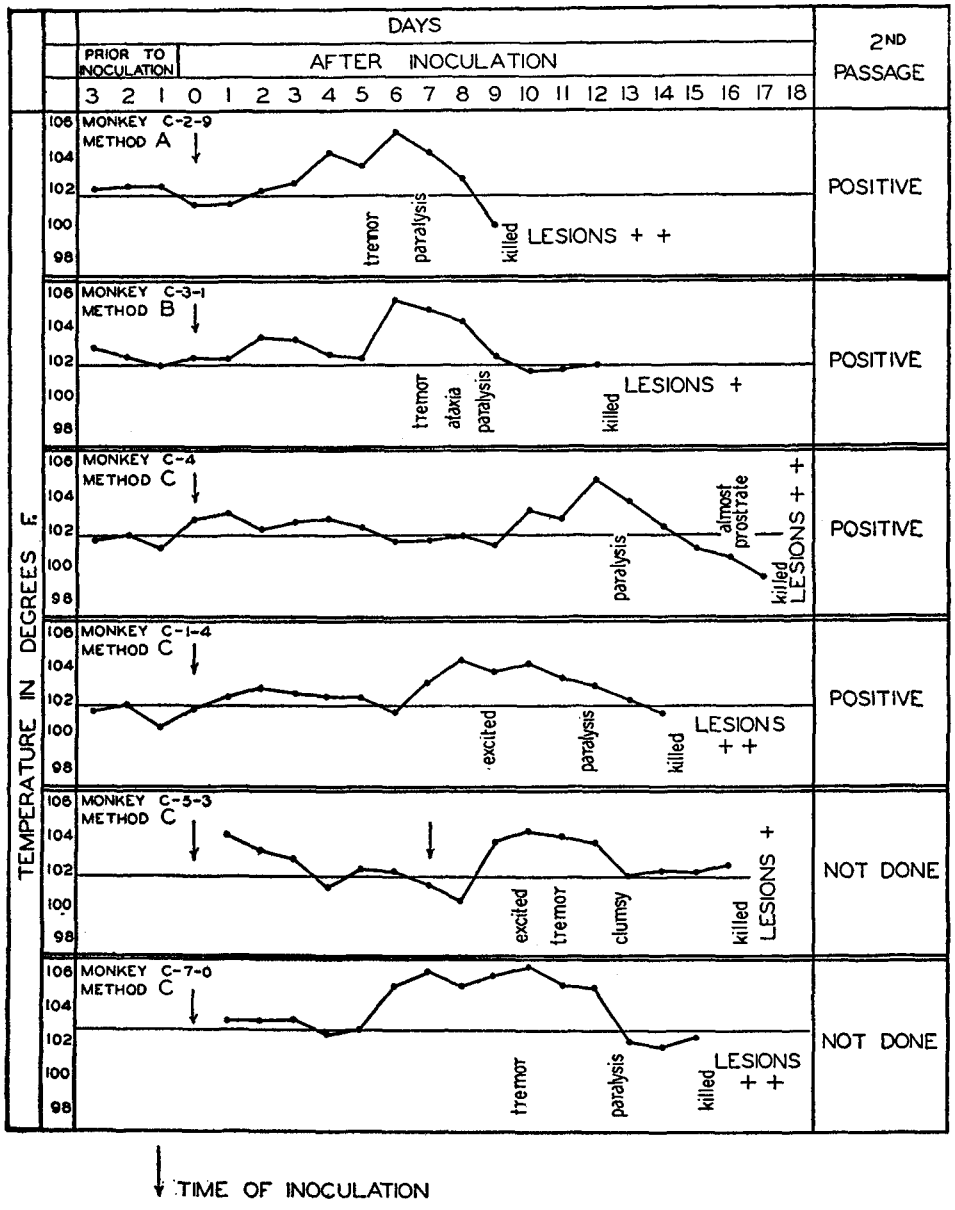
Subsequently (after 39 days), 0.3 cc. of the washed sediment from glycerine was inoculated intracerebrally and 12.0 cc. of the supernatant intraperitoneally into one monkey (No. C-5-3) with subsequent reinforcement, after a weekly interval. Again after 101 days a single inoculation was made in another monkey (No. C-7-0). Both of these animals developed experimental poliomyelitis.

Samples of the washings taken on the 11th day of the illness (July 24) were divided into two parts, A and B. A was treated with 0.5 per cent phenol and inoculated into one monkey; B was filtered and inoculated into one monkey. The former animal died on the 3rd day and the latter failed to develop the experimental disease.

Evidences of the Experimental Disease in Monkeys Inoculated with Material from Case of V. McC.—Details of the course of the experimental disease exhibited by the six monkeys inoculated with material from the first washings from V. McC. appear in Text-fig. 2. The incubation period to the onset of fever ranged from 4 to 10 days. All of the animals developed either tremor, ataxia, or paralysis in varying degree. All probably would have recovered had they not been sacrificed as soon as signs of recovery were apparent. In all of these six animals characteristic lesions were present in the spinal cord and medulla. The strains from four of the animals (Nos. C-2-9, C-3-1, C-4, and C-1-4) were brought through the second passage. Two of these strains were subsequently brought to the fourth and seventh passages.

A small quantity of the original glycerinized sediment from the nasopharyngeal washings from this case, and 10 per cent emulsions of spinal cord representing early passages of this strain of virus were instilled into the nose of three monkeys. The results were negative.

Small quantities of the original glycerinized sediment were also inoculated



TEXT-FIG. 2. Temperature charts from six monkeys inoculated with material from the first washings from V. McC., all of which developed experimental poliomyelitis.

TABLE I

Clinical and Experimental Data on Nasopharyngeal Washings

	Case	Initials	Age	Type of poliomyelitis or clinical diagnosis	Day of illness	Evidences of upper respiratory inflammation *(a)	Patient's temperature when irrigated	Lumbar puncture when irrigated *(b)	Irrigating fluid	Method *(c)	Amount inoculated: Intracerebrally	Amount inoculated: Intraperitoneally	Monkey No.	Results in monkey: Fever *(d)	Results in monkey: Symptoms *(e)	Results in monkey: Lesions *(f)
			yrs.				*°F.*				*cc.*	*cc.*				
Tested individually	1	V. McC.	3½	Suspected abortive	1	+	101.4	Negative	Saline	A	1.0	5.0	C-2-9	+4	+	++
										B	1.0	7.0	C-3-1	+6	+	+
										C-13	0.5	0	C-4	+10	+	++
										C-13	0.5	0	C-1-4	+7	+	++
										C-39	0.3 + 0.2	12.0 + 11.0	C-5-3	+9	+	+
										C-101	0.4	10.0	C-7-0	+6	+	++
	2	E. R.	9	" "	1	+	103.4	"	"	A	0.8	10.0	C-1-9	−	−	0
										C-121	0.4 + 0.4	10.0 + 10.0	C-7-2	−	−	0†
	3	J. Hn.	2	" "	1	+	103.4	Not done	"	A	1.0	6.0	C-4-6	−	−	0
										B	1.0	5.0	C-4-5	±7	−	−
										C-30	0.4 + 0.3	12.0 + 11.0	C-5-6	±6	−	0†
	4	J. S.	3	Abortive	1	+	101.0	38 W. B. C. Glob. +	Water	A	1.0	13.0	C-3-8	−	−	−
										B	1.0	10.0	C-3-7	−	−	0
										C-76	0.2 + 0.4	9.0 + 9.0	C-5-4	−	−	0
	5	A. G.	10	Suspected abortive (familial exposure)	1	−	103.0	Not done	Saline	B	1.0	7.0	C-3-9	−	−	0
										C-75	0.3 + 0.4	9.0 + 9.0	C-6-4	−	−	0
	6	P. G.	7	Abortive	1	?	100.0	39 W. B. C. Glob. +	Broth	B	1.0	2.0	C-3-7	−	−	0
										C-70	0.4 + 0.4	9.0 + 9.0	C-6-3	−	−	0

Tested individually—*Continued*

7	Z. D.	15	Abortive	1	+	102.4	450 W. B. C. Glob. +	Saline	B C–66	1.0 0.3 + 0.5	2.0 9.0 + 9.0	C-3-7 C-6-2	– –	– –	0 0
8	J. C.	11	Suspected abortive (acute tonsillitis ?)	1	++	103.0	Not done	"	C–130	0.4 + 0.4	10.0 + 10.0	C-7-3	–	–	0†
9	J. Hy.	1	Suspected abortive	2	?	99.2	" "	"	B C–32	1.0 0.5 + 0.3	4.0 12.0 + 11.0	C-4-2 C-5-4	+8 –	± –	– 0
10	G. S.	1½	" "	2	?	102.0	Negative	Water	B	1.0	3.0	C-4-0	–	–	0
11	D. B.	7	" "	2	++	99.4	Not done	"	C–140	0.3 + 0.3	10.0 + 10.0	C-7-7	–	–	0

* (*a*) Clinical appearance of throat:
++ Red throat, exudate present.
\+ Red throat.
± Questionable red throat.
– Normal throat.
? Throat not seen.

* (*b*) Lumbar punctures were considered negative when: (1) the cell count was below 12 white blood cells per c. mm. and (2) the Pandy test was negative.

* (*c*) Method of preparing inoculum:
A, 0.5 per cent phenol added.
B, Seitz filtration (concentration *in vacuo* in a few of the pooled samples).
C–15, sediment preserved in 50 per cent glycerine,—inoculated 15 days after collection of washings.
D, untreated washings.

* (*d*) Type of fever in monkey:
+5 Fever lasting more than 2 days with onset 5 days after inoculation.
± Fever lasting 1 or 2 days.
– No fever.

* (*e*) Symtomatology of monkey:
\+ Paralyses or ataxia.
± Nervous, agitated.
– No symptoms suggestive of experimental poliomyelitis.

* (*f*) Lesions in the central nervous system:
++ Extensive lesions in medulla and spinal cord.
\+ Few lesions.
± Very scanty lesions.
– No lesions.
0 Animal not sacrificed.
0† Monkey subsequently infected with a strain of poliomyelitis virus isolated from the 1934 California epidemic.

TABLE I—*Concluded*

	Case	Initials	Age	Type of poliomyelitis or clinical diagnosis	Day of illness	Evidences of upper respiratory inflammation *(a)	Patient's temperature when irrigated	Lumbar puncture when irrigated *(b)	Irrigating fluid	Method *(c)	Amount inoculated		Monkey No.	Results in monkey		
											Intracerebrally	Intraperitoneally		Fever *(d)	Symptoms *(e)	Lesions *(f)
			yrs.				°F.				*cc.*	*cc.*				
Tested individually—*Concluded*	12	P. C.	4	Abortive	2	+	102.0	109 W. B. C. Glob. −	Water	C–140	0.5 + 0.4	10.0 + 10.0	C-7-5	−	−	0
	13	R. B.	9	"	3	+	100.0	79 W. B. C. Glob. +	Broth	B	0.9	1.0	C-7	−	−	−
	14	N. K.	19	"	3	+	101.6	210 W. B. C. Glob. +	"	C–96	0.3 + 0.4	9.0 + 9.0	C-6-5	−	−	−
	15	G. M.	17	Suspected abortive	4	+	101.2	Negative	Saline	B	1.4	2.4	C-6	−	−	−
	16	L. N.	3	Abortive	4	++	101.0	38 W. B. C. Glob. +	Water	C–140	0.4 + 0.3	10.0 + 12.0	C-6-4	−	−	0
	17	D. R.	20	Suspected abortive	4	±	99.0	Not done	"	C–147	0.4 + 0.4	10.0 + 10.0	C-7-3	−	−	0†
	18	A. W.	5	" "	4	+	103.6	" "	"	C–140	0.3 + 0.5	10.0 + 10.0	C-7-6	−	−	0
	19	E. M.	12	" "	5	+	102.4	Negative	Saline	B	0.9		C-5	−	−	0
	1 Repeat	V. McC.	3½	" "	11	−	98.4	"	"	B	1.0	3.0	C-4-7	−	−	0
		Jo. S.	½	Contact		?			"	C–41	0.4 + 0.3	12.0 + 11.0	C-5-7	−	−	−

Pooled	20	J. B.	4½	Suspected abortive	2	±	99.2	Not done	Saline	A	0.8	20.0	C-3-4	+16	–	–
										B	0.8	10.0	C-3-3	±19	–	–
	21	L. B.	5½	" "	1	–	101.8	" "	"	C–42	0.5 + 0.3	12.0 + 11.0	C-5-5	–	–	0†
Pooled	22	R. S.	9	Abortive	3	+	101.0	49 W. B. C. Glob. +	Broth	B	1.0	3.0	C-9	–	–	–
	14	N. K.		As stated above—see Case 14						D	0.5	5.0	C-8	±5	–	±
Pooled	11	D. B.		As stated above—see Case 11						B and D	0.8	10.0 (intra-tracheally)	C-1-0	+22	±	±
	12	P. C.		" " " " " 12												
	16	L. N.		" " " " " 16						B	0.8	1.5	C-1-1	–	–	0
	17	D. R.		" " " " " 17												
	18	A. W.		" " " " " 18						C–163	0.5 + 0.6	10.0 + 10.0	C-6-9	+6	±	–
Pooled	23	E. C.	36	Suspected abortive	2	±	?	Negative	Water							
	24	A. F.	21	" "	8	+	97.6	Not done	"	B and D	0.8	7.0 (intra-tracheally)	C-1-8	–	–	0
	25	R. So.	13	" "	2	++	101.5	Negative	"							
	26	H. W.	24	" "	8	+	100.0	Not done	"	B	0.8	0.5	C-1-9	–	–	0
	27	S. T.	19	Abortive	7	+	100.2	156 W. B. C. Glob. +	"							
Pooled		L. F.	3½	Contact					Saline	A	0.8	30.0	C-3-6	–	–	–
										B	1.0	11.0	C-3-5	+15	–	–
		C. F.	10	"					"	C–41	0.4 + 0.3	12.0 + 11.0	C-5-0	±26	–	0†
Pooled				5 suspected contacts					Broth	B	1.0	13.0	C-2-2	–	–	0
Pooled				12 contacts					Broth	B	1.0 + 1.0	6.0 + 8.0	C-2-7	+23	–	–
										B	1.0 + 1.0	3.0 + 4.0	C-2-8	–	–	–
Pooled				3 normal					Water	B	1.0	5.0	C-1	–	–	0

intracerebrally, intraperitoneally, and intranasally into many white mice. It was also inoculated into the cornea of the rabbit's eye. The results were uniformly negative.

Questionable Results.—A few results of this type occurred in these experiments and are listed under Results in monkey in Table I. None of them fulfilled all three criteria necessary to establish the diagnosis; namely: (1) that the animal should show signs compatible with the experimental disease, such as fever, tremor, ataxia, or paralysis; (2) that lesions of poliomyelitis should be demonstrated in the spinal cord; and (3) that the strain should be brought to the second passage. Of the several questionable results obtained, only one series of tests deserves attention, namely, that with pooled material from Cases 11, 12, 16, 17, and 18 (see Table I). Material (preserved in glycerine for 140 to 147 days) from each of these individual cases was subsequently tested, with negative results. This experiment has not been considered significant except that it illustrates some of the difficulties and pitfalls in this type of investigation.

DISCUSSION

The experience described in this report confirms the view that the virus of poliomyelitis cannot readily be obtained from the nasal passages of living persons. It cannot yet be decided, however, whether the difficulty is due to absence of virus or to unsatisfactory technique. The facilities for this investigation were excellent but the epidemic was mild. A search for the poliomyelitis virus in the living still needs to be made during a severe epidemic, by means of an experimental method the sensitivity of which can be determined.

SUMMARY

A single example of mild illness diagnosed as suspected abortive poliomyelitis is described in which the virus of poliomyelitis was recovered from the nasopharynx by three different methods. Failure to recover virus from a total of twenty-six cases diagnosed as suspected or abortive poliomyelitis and fourteen contacts is also reported.

The original material from the nasopharynx of the positive case proved unusually infective for the monkey, apparently even more so than are the majority of suspensions of spinal cords from fatal human cases of poliomyelitis. An explanation of this fact is not clear.

The method of isolating human virus from the throat, by means of preserving the sediment of washings from this site in glycerine, has been shown to be efficient in one case for a period of 101 days.

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