

DETECTION OF THE VIRUS OF POLIOMYELITIS IN THE
NOSE AND THROAT AND GASTRO-INTESTINAL
TRACT OF HUMAN BEINGS AND MONKEYS*

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The epidemiology of poliomyelitis strongly suggests the contact mode of infection. Experimental evidence furthermore points more specifically to the olfactory portion of the nasal mucous membrane as the portal of entry of the virus into the body. This concept predicates the presence of the virus in the nasal and oral secretions of patients ill with the disease and perhaps also of healthy carriers and contacts. The experimental evidence in this regard, when subjected to rigid criteria, remains sparse; yet in view of the acknowledged difficulties and uncertainties attending the detection of the virus in human material other than that from the central nervous system, we believe that the virus has been recovered with sufficient frequency to serve as strong confirmatory evidence in support of the contact mode of infection.

A review of the literature in search of experiments in which the virus of poliomyelitis was recovered from human and animal sources, exclusive of the central nervous system, has yielded a surprisingly large number of so called takes. We have attempted to tabulate the efforts of the various previous investigators to recover the virus from nasal secretions and from intestinal contents of man and animals in Tables I to IV. The total number of such attempts is probably larger than indicated on these tables, because occasionally only positive findings have been reported and reference made in the text of the paper to other similar attempts, whose outcome was negative.

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TABLE I

*Isolation of Poliomyelitis Virus from Nasopharynx of Human Beings**

Author	Stage of disease	At-tempts	Takes	Pas-sage
(1) Landsteiner, Levaditi and Pastia, 1911	Post mortem	1	1	—
(2) Rosenau, Sheppard and Amoss, 1911	Acute	8	0	—
	Convalescent	10	0	—
(3) Kling, Pettersson and Wernstedt, 1912	Acute	12	7	—
	Contact	12	3	—
	Convalescent	31	17	—
	Post mortem	11	7	—
(4) Thomsen, 1912	Post mortem	2	2	1
(20) Kling and Levaditi, 1913	Acute	12	0	—
	Abortive	9	0	—
(5) Kling and Pettersson, 1914	Contact	4†	1	1
(6) Flexner, Clark and Fraser, 1913	Contact	2‡	1	1
(7) Lucas and Osgood, 1913	Convalescent	4‡	2	2
(8) Dubois, Neal and Zingher, 1914	Convalescent	1	1	1
(21) Sawyer, 1915	Convalescent	4	0	—
(9) Taylor and Amoss, 1917	Convalescent	3	2	1
	Abortive			—

* Explanation of terms and symbols used in Tables I to IV inclusive:

Takes = successful infection of animals with human material.

Passage = successful infection of a second animal with cord suspension from the primary take.

0 = an attempted passage with a negative result.

— = no mention of attempt or no attempt was made to pass the infection to another animal.

The numbers indicating the number of attempts are the closest approximations obtainable from the original papers. Frequently reference is made to other attempts without stating the exact number, and numbers in such instances are derived from protocols and the text.

† Pooled.

‡ From the same patient.

TABLE I—*Concluded*

Author	Stage of disease	At-tempts	Takes	Pas-sage
(10) Flexner and Amoss, 1919	Contact	27	0	—
	Acute			
	Convalescent	14	5	—
	Post mortem			
(11) Kling, 1928	Acute	84	3	—
	Post mortem			
(12) Levaditi, Schmultz and Willemin, 1931	Acute	—	1	—
(13) Levaditi and Willemin, 1931	Acute	12	3	0
(14) Frontali, 1932	Acute	8	1	—
(15) Paul and Trask, 1932	Abortive	12	2	2
(16) Kramer, 1935	Healthy carrier	156	1	1
(17) Paul, Trask and Webster, 1935	Acute abortive	27	1	1
	Contacts	14	0	0
(18) Kramer, Sobel, Grossman and Hoskwith, 1936	Convalescent	20	2	2
(19) Stillman and Brodie, 1937	Acute	15	1	1
(22) Harmon, 1937	Acute	20	0	0

Furthermore, although it is quite probable that the number of takes reported by some of these authors, particularly during the last decade, represent true poliomyelitis, this cannot be said to apply to the findings of a number of the earlier reports. When these reports are analyzed according to the criteria which we now believe essential for positive identification of the virus, (*a*) the production of the typical disease with paralysis in the experimental animal, (*b*) typical histopathologic changes in the cord, (*c*) successful passage of the disease to a second monkey, which at autopsy will present the characteristic pathologic changes in its cord,—the number of positive results rapidly dwindles.

In Table I are summarized 535 individual attempts to recover the

TABLE II
*Isolation of Poliomyelitis Virus from the Intestinal Tract of Human Beings**

Author	Stage of disease	At-tempts	Takes	Pas-sage
(3) Kling, Pettersson and Wernstedt, 1912	Acute	10	9	—
	Convalescent	30	18	—
	Post mortem	10	9	—
(20) Kling and Levaditi, 1913	Acute	5	1	—
	Abortive	1	0	—
(21) Sawyer, 1915	Convalescent	4	1	1
(8) Dubois, Neal and Zingher, 1914	Acute	1	0	0
(11) Kling, 1928	Post mortem	54	5	—
	Abortive			
	Carriers			
	Acutely ill			
(13) Levaditi and Willemin, 1931	Acute	4	0	0
(22) Harmon, 1937	Acute	20	5	—
(23) Trask, Vignec and Paul, 1938	Acute	5	3	3
	Convalescent			
(from one child)				

* See explanatory note under Table I.

TABLE III
*Isolation of Poliomyelitis Virus from Nasopharynx of Monkeys**

Author	Stage of disease	At-tempts	Takes	Pas-sage
(24) Landsteiner and Levaditi, 1909	Acute	1	1	—
(25) Leiner and von Weisner, 1910	Acute	1	1	—
(26) Flexner and Lewis, 1910	Convalescent	1	1	—
(27) Landsteiner, Danulesco and Levaditi, 1911	Acute	2	2	—
(30) Osgood and Lucas, 1911	Convalescent	2	2	2
(28) Flexner and Clark, 1911	Convalescent	1	1	—
(4) Thomsen, 1912	Acute	2	1	1
(29) Levaditi and Danulesco, 1912	Acute	13	2	—

* See explanatory note under Table I.

virus from the nasopharynx in the human disease; 64 positive takes are reported, but only 14 passages to second animals are mentioned.

In Table II are reviewed 144 individual attempts to recover the virus from patients' feces or from the intestinal contents at post mortem. In only 4 of the 51 takes reported, were the strains passed on to second animals; three of these being done on the stools of the same patient (Trask, Vignec and Paul, 23).

TABLE IV
*Isolation of Poliomyelitis Virus from the Intestinal Tract of Monkeys**

Author	Stage of disease	At-tempts	Takes	Pas-sage
(31) Flexner, Clark and Dochez, 1912	2 hrs. after feeding (50 cc. virus)	2	2	—
(32) Clark, Schindler and Roberts, 1930	48 hrs. after feeding concentrated 10 cc.	3†	1	1
	Acutely ill	24	0	—
(33) Kling, 1931	24 and 48 hrs. after feeding	3	1	—
(34) Levaditi, Kling and Lepine, 1931	24 and 48 hrs. after feeding	2	2	—
(35) Clark, Roberts and Preston, 1932	After injecting virus into small intestines	2	2	—
	After feeding 60 cc. for 3 days	8	5	—
	Acutely ill	3	0	—

* See explanatory note under Table I.

† Pooled.

In Table III are presented the records of 23 attempts to detect the virus from the upper respiratory tract of monkeys. 11 positive takes were cited. In only 3 instances, however, were the strains passed to a second monkey, and 2 of these were recovered from the washings of a single animal.

In Table IV are tabulated 47 attempts to detect the virus in the intestinal tracts of monkeys. In 20 instances the feces were obtained following feeding experiments in which varying amounts of virus

were administered to normal monkeys by mouth or by stomach tube; and in the remaining 27 experiments, the feces and intestinal contents were obtained from acutely ill animals. All 13 positive takes were in animals which had been fed large doses of virus. It will be noted, however, that in no instance was the virus recovered later than 48 hours following the feeding, and that only one passage to a second animal is recorded. We furthermore found no record of recovery of the virus from feces or intestinal contents obtained during the acute stage of the experimental disease.

From these tables, it can be seen that the virus of poliomyelitis has been recovered from the upper respiratory tract of both man and the monkey appreciably oftener than from the gastro-intestinal tract, except when the animals were fed large quantities of virus. It would seem probable, in recognition of the normal physiologic passage of oral and nasal secretions into the gastro-intestinal tract by reflex swallowing and the relative bacterial content of the materials from these two sources, that the small number of successful recoveries of the virus from the latter was perhaps due to the greater difficulties encountered in removing bacterial contaminants from the feces. We believe that the positive data included in these tables are significant and that further studies along these lines are indicated.

The occurrence of poliomyelitis in Brooklyn in the summers of 1935 and 1937 afforded an opportunity to carry on such investigations. In our first communication (18), based on the 1935 cases (included in Table I), we reported the detection of the virus of poliomyelitis in the nasal washings of 2 of a total of 20 children studied during convalescence. In the present paper, which comprises the results of the 1937 occurrence, we have included examination of the stools, as well as the oral and nasal secretions of patients in varying stages of the disease; and we have also studied the nasal washings and intestinal contents from 7 monkeys sacrificed at the height of the disease.

EXPERIMENTAL

Except for the modifications necessary for the handling of the fecal material and intestinal contents, the procedures employed were essentially those outlined in our previous communication (18).

Collection of Human Nasal Washings.—With the patient lying prone with his head turned to one side, a soft rubber catheter was inserted 1 to 2 inches into one nostril compressed against the catheter to prevent leakage. 50 to 100 cc. of sterile distilled water were gently introduced by means of a large syringe. The return fluid from the opposite nostril and the mouth was caught in a sterile pus basin, and then transferred to a sterile bottle.

Collection of Human Fecal Material.—Fecal material was obtained on the same day as the nasal washings. If the feces were from a spontaneous bowel movement, 100 cc. of sterile distilled water were added; if an enema were given, 100 cc. of sterile distilled water were employed. The feces were then transferred to a 250 cc. sterile centrifuge bottle and shaken vigorously in an eccentric shaker for 1 hour. The well macerated material was then centrifuged at 1250 R.P.M. for 1 hour and the supernatant fluid, usually quite opalescent, was drawn off into another sterile centrifuge bottle.

Collection of Nasal Washings and Tissue from Monkeys.—The animals employed in these experiments were infected either by the intranasal route with the Armstrong strain of virus, or by the intracerebral route with our stock laboratory VM strain.

Nasal washings were obtained at the height of the disease. The animal was quickly sacrificed with ether anesthesia; its face was washed with 5 per cent phenol followed by ether; and with the head held downward over a pus basin, the same procedure was used as was employed for obtaining nasal washings from patients. About 40 cc. of sterile distilled water were used. The margin of the nostrils was then cut, and by means of a fine curette the nasal cavity and posterior pharynx were carefully curetted. The resulting tissue, consisting of the mucous membrane, adenoid tissue, cartilage and some bone fragments, was thoroughly ground with sterile sand in a mortar and added to the nasal washing. This mixture of washing and ground tissue was shaken for 30 minutes in an eccentric mechanical shaker and centrifuged at 1250 R.P.M. for a half hour. The supernatant was poured off into a sterile Erlenmeyer flask.

Collection of Fecal Material from Monkeys.—Immediately after the nasal washing and curetting was completed, the abdominal cavity of the animal was opened and segments of the intestine were tied off. One ligature was applied at the pyloric end of the stomach, two ligatures at the junction of the ileum and cecum, and a single ligature at the anal end of the rectum. The intestines were then dissected away from the omentum, removed from the abdominal cavity and placed on sterile towels or in sterile Petri dishes. The single length of gut was then divided between the second pair of ligatures, resulting in two segments of gut; the first comprising the pylorus, the jejunum and ileum, and the second including the colon, the sigmoid and the rectum. The ligatures were then removed and the contents of each section of gut squeezed into separate containers. 50 cc. of sterile distilled water were then introduced into each segment and, by means of gentle massage, forced through the gut and added to the corresponding intestinal

contents or fecal material. Each section of gut was then slit longitudinally, exposing the mucous membranes, and these were gently curetted. The scrapings were added to the corresponding container. The combined material from each section of gut was placed in 250 cc. centrifuge bottles and shaken vigorously in an eccentric shaker for 1 hour and centrifuged for 30 minutes at 1250 R.P.M. The supernatant liquid was poured off into sterile centrifuge bottles.

Sterilization of Washings and Fecal Material.—The method described by us in a previous communication (18) and recently successfully employed by Trask *et al.* (23) was used in removing bacterial contaminants from human and monkey material. From 10 to 20 per cent of anesthetic ether was added to the flask containing nasal washings, and approximately 40 per cent ether was added to the supernatant material obtained from the intestinal tract. The flasks were tightly

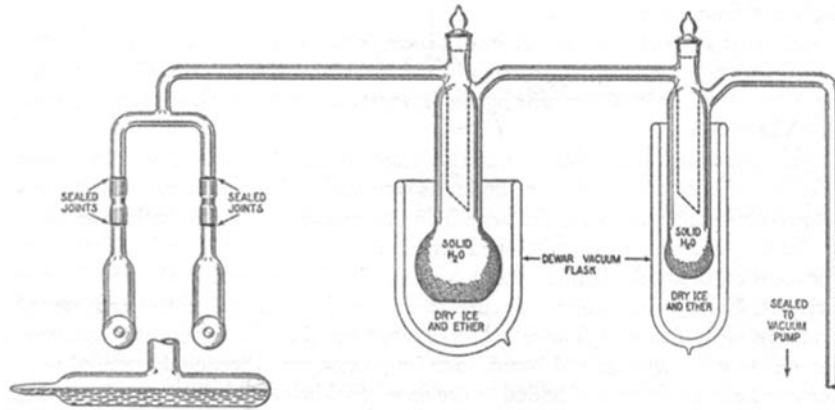


FIG. 1

stoppered and shaken mechanically for from 30 minutes to an hour and left overnight in the ice chest.

Concentration of Materials.—The following morning the materials were re-centrifuged and the aqueous portions were transferred by sterile technique to a specially constructed sterile container and attached to the vacuum system (see Fig. 1). The material in each container was frozen solid by immersion in CO_2 -acetone mixtures, and the vacuum was started. In 6 to 8 hours the material was concentrated to from 2 to 5 cc., depending on the original volume (50 to 100 cc.).

Although we had previously established (18) that the virus would survive the manipulation involved in our procedure, we again included 3 specimen runs at the beginning of our concentrations. Each of these specimens consisted of 75 cc. of sterile distilled water to which had been added 0.5 cc. of a 5 per cent suspension of our stock VM virus. 10 per cent of anesthetic ether was then added, the mixture well shaken and left overnight in the refrigerator. The following morning

the specimens were placed in the special containers, attached to the desiccating outfit, then frozen and desiccated. All 3 monkeys (Nos. 3-38, 3-39 and 3-46, Table V), inoculated with concentrations from these specimens, succumbed to the experimental disease in from 6 to 10 days.

Inoculation of the Concentrates.—The concentrates were removed from the special containers by cutting the constricted end of the tube and withdrawing the material through this opening with a sterile pipette. 1 to 2 cc. of sterile distilled water were usually added to the emptied container and the walls of the tube were gently rubbed with either a pipette or a glass rod to release any adherent material, which was then added to the concentrate. The fecal concentrates had to be recentrifuged to throw down the heavy particles which appeared during the concentration, and the supernatant liquid was used for inoculation. From 2 to 4 cc. of each concentrate were inoculated intracerebrally into a fresh monkey, and the remainder of the material was inoculated intraperitoneally into the same animal.

RESULTS

Nasal washings and stool or intestinal contents were obtained from 20 patients in varying stages of convalescence, and from 7 monkeys during the height of the disease. 5 strains of virus were recovered, 4 strains from 3 patients and 1 strain from a monkey. The pertinent data are tabulated in Tables V and VI.

The first strain was isolated from the feces of a 14 year old child (S.W.), 7 days after the onset of the illness. A test animal (3-48) inoculated on Aug. 28, was paralyzed on Sept. 17. Histologically, the cord showed typical poliomyelitis. A passage from the cord of this animal intracerebrally into monkey 3-77 on Sept. 17 resulted in paralysis and prostration Sept. 23. Histologically the cord showed typical poliomyelitis.

The second human strain was isolated from the nasal washings of a 6½ year old child (D.E.), 5 days after the onset of illness. The test animal (3-50) was inoculated on Aug. 31, and developed weakness of both lower extremities and the left arm on Sept. 7. It was found dead on the morning of Sept. 8. A cord suspension from this animal inoculated on the same day into monkey 3-68, resulted in paralysis on Sept. 21. Both animals presented typical histologic findings in their cords.

The third and fourth human strains were recovered from a 2½ year old boy (J.C.), 9 days after the onset of the illness. One strain was obtained from the nasopharynx and the other from the feces. The 2 test animals (3-55 and 3-56, Table V) inoculated on Sept. 2 with the concentrates from these sources showed temperature and paralysis on the 11th. Histologic examination of sections from the cords of both these animals showed typical poliomyelitis. Intracerebral inoculations of emulsions of the cords on Sept. 11 into 2 fresh test animals (3-67

TABLE V
*Outcome of Inoculations of Concentrates from Human Sources
 (Nasal Washings and Feces)*

Case	Patient	Age	Date of onset	Date of washings	Time from onset to washings	Date of inoculation	Source of concentrates	Cultures*	Amount of inoculum	Monkey No.	Outcome			
											Fever	Symptoms	Post mortem and histological findings	Passage
1	S. W.	14 yrs.	8/21 1937	8/27 1937	7 days	8/28 1937	N†	16 colonies	4 cc.	3-47	None	None		
							F†	Sterile	4	3-48	Weakness of all extremities. Sacrificed	Typical poliomyelitis	M. 3-77, 9/17 inoculated. 9/23 prostrated. Hist. typical poliomyelitis	
2	A. D.	17 yrs.	8/20	8/30	10	8/31	N F	Not done "	4 3	3-49 3-52	None "	None "		
3	D. C.	7 mos.	8/19	8/27	8	8/31	N F	Sterile 34 colonies	4 4	3-51 3-54	" "	Lethargic refused to climb. 9/7 died	Brain abscess. Organisms recovered	
4	D. E.	6 yrs.	8/24	8/28	5	8/31	N	Sterile	3	3-50	9/5, 105° 9/7, 101°	Generalized weakness. 9/8 found dead	Typical poliomyelitis	M. 3-68, 9/8 inoculated. 9/21 prostrated. Hist. typical poliomyelitis
							F	50 colonies	4	3-53	None	Animal in stupor, crouched in cage. 9/1 found dead	No brain abscess	

5	J. C.	2½	8/25	9/2	9	9/3	N	Not done	4	3-55	9/10, 106° 9/11, 106°	9/11 upper limbs involved. Sacrificed	Typical poliomyelitis	M. 3-69, 9/11 inoculated. 9/21 reinoculated. 10/6 reinoculated. 10/11 prostrated. Hist. typical poliomyelitis.
							F	"	4	3-56	9/10, 106°	9/11 right upper weak. Sacrificed	"	M. 3-67, 9/11 inoculated. 9/22 prostrated. Hist. typical poliomyelitis
6	M. O.	9	8/25	9/2	8	9/3	N	Sterile 100 colonies	4	3-60 3-62	None "	None Animal drowsy for 2 days. Recovered		
7	M. F.	6	8/28	9/2	10	9/3	N	19 colonies	4	3-59	"	9/17 generalized weakness. Recovered		
							F	8 colonies	4	3-57	"	None		
8	E. H.	4	8/22	9/2	11	9/3	N	Not done	4	3-58	"	"		
							F	"	4	3-61	"	9/4 weakness of upper limbs. 9/7 found dead	No brain abscess	
9	C. J.	17	8/30	9/7	9	9/8	N	Not done	4	3-63	None	None	Suggestive poliomyelitis	M. 3-89, 9/23 inoculated. 10/6 reinoculated. 10/20 reinoculated. No take
							F	"	4	3-64	9/21, 106.8° 9/22, 106.4°	Upper left limb paralyzed. Sacrificed		
10	R. L.	8	9/4	9/12	8	9/13	N	"	4	3-66	9/17, 100.4° 9/18, 104.5° 9/25, 106°	None 9/26 generalized weakness. Recovered		
							F	"	4	3-65	"	"		

* 0.5 cc. to 1 cc. of concentrates used for cultures. The numerals indicate colonies on pour plate.

† N, nasopharynx. F, fecal material.

TABLE V—*Concluded*

Case	Patient	Age yrs.	Date of onset 1937	Date of washings 1937	Time from onset to washings days	Date of inoculation 1937	Source of concentration	Cultures*	Amount of inoculum cc.	Monkey No.	Outcome			
											Fever	Symptoms	Post mortem and histological findings	Passage
11	S. S.	7	9/11	9/14	4	9/15	N	Sterile	4	3-71	None	9/23 left upper and lower paralyzed	Typical cord lesions	M. 3-86, 9/23 inoculated. 10/16 reinoculated. 10/20 reinoculated. No take
							F	100 colonies	3	3-72	"	9/16 animal lethargic. Died	No brain abscess	
12	M. F.	3	9/2	9/14	12	9/16	N	Sterile	4	3-73	"	9/22 animal in stupor. 9/23 died	Brain abscess. Organisms recovered. Lesions not characteristic	
							F	4 colonies	4	3-74	"			
13	S. D.	2	9/9	9/15	6	9/17	N	Sterile	4	3-76	"	None	No gross brain abscess	
							F	47 colonies	4	3-75	"	9/18 drowsy. Refused to climb. All limbs weak. 9/22 died		
14	J. C.	8	9/11	9/16	6	9/18	N	Sterile	4	3-79	"	None	Tuberculosis	
							F	"	4	3-78	"	Generalized weakness. 9/22 died	No brain abscess. Acute suppurative meningitis	
15	R. D.	3	9/14	9/19	6	9/22	N	"	4	3-81	10/16, 104.4° 10/18, 99.8° 10/19, 101.6°	9/23 lethargic. Died		
							F	60 colonies	3	3-80	None			

16	G. A.	10	9/16	9/21	6	9/23	N F	Sterile "	4 3	3-82 3-83	None 9/26, 100°	None 9/23 crouched in cage. Drowsy. Generalized weakness. 9/27 died	No brain abscess
17	M. H.	21	9/14	9/22	9	9/24	N F	" "	4 3	3-84 3-85	None 9/25, 100°	None 9/25 generalized weakness. Lethargic. Died	Acute suppurative meningitis
18	M. P.	3	9/14	9/23	10	9/25	N F	" "	4 2	3-87 3-88	9/29, 105° 9/30, 101° 9/30, 101° 10/1, 105.6°	None Tremor for 4 days after inoculation. Disinclination to climb. Recovered	
19	E. B.	7	9/21	9/24	4	9/25	N F	" "	4 2	3-90 3-91	9/28, 105° 10/1, 101° None	None "	
20	J. C. (repeat)	2½	8/25	10/3	41	10/4	N F	" "	4 3	3-95 3-96	" "	" "	

Controls

Monkey No.	Date of inoculation	Type and amount of inoculum	Outcome
3-38	1937 8/21	0.5 cc. of a 5 per cent cord suspension diluted with 75 cc. of sterile distilled water, frozen and concentrated <i>in vacuo</i> to about 4 cc.	Poliomyelitis 8/31/37
3-39	8/26		Poliomyelitis 9/2/37
3-46	8/26		Poliomyelitis 9/1/37

TABLE VI
*Outcome of Inoculations of Concentrates from Animal Sources
 (Nasopharynx and Intestines)*

Acutely ill monkey	Date of washings	Date of inoculation	Source and amount of inoculum	Cultures*	Monkey No.	Outcome			
						Fever	Symptoms	Histological findings	Passage
2-75	1937 9/27	1937 9/29	Nasal washing 2 cc.	Sterile	3-92	None	None		
			U. intest. seg. † 2 cc.	"	3-93	10/16, 105.8° 10/18, 101.6°	"		
			L. intest. seg. ‡	"	3-94	10/19, 106.4°	Animal excited. Recovered		
8-6	10/9	10/11	Nasal washing 4 cc.	6 col.	4-03	None	None		
			U. intest. seg. 3 cc.	23 "	4-01	"	Generalized weakness. 10/17 died	Tuberculosis	
			L. intest. seg. 3 cc.	17 "	4-02	10/19, 104.8°	Limbs weak. 10/19 died	Questionable polio	—
2-7	10/10	10/12	Nasal washing 3 cc.	Sterile	4-05	10/25, 104.8° 10/26, 101.8°	Generalized weakness. No paral. Recovered		
			U. intest. seg. 3 cc.	2 col.	3-70	None	None		
			L. intest. seg. 3 cc.	25 "	4-04	"	"		
3-52	10/13	10/15	Nasal washing 4 cc.	Sterile	4-07	11/5, 105.8°	Lower limbs weak. Recovered		
			U. intest. seg. 3 cc.	"	4-06	10/18, 106.4° 10/19, 106.8°	Continued high temp. 11/21 prostrated	Suggestive polio	M. 4-09, 11/21 inoculated. 12/19 paralyzed. Hist. typical polio
			L. intest. seg. 3 cc.	8 col.	4-08	11/3, 106° 11/5, 106°	Generalized weakness. Recovered		
3-99	11/21	11/23	Nasal washing 3 cc.	Sterile	4-10	12/29, 104.2°	12/10 lethargic, refused to climb. 12/29 limbs weak. 12/30 died	Questionable polio	—

* 0.5 cc. to 1 cc. of concentrates for cultures. The numerals indicate colonies on pour plate.

† Upper intestinal segment.

‡ Lower intestinal segment.

TABLE VI—*Concluded*

Acutely ill monkey	Date of washings	Date of inoculation	Source and amount of inoculum	Cultures*	Monkey No.	Outcome			
						Fever	Symptoms	Histological findings	Passage
3-99 (con- tin- ued)	1937	1937	U. intest. seg. 3 cc.	"	4-11	None	None		
			L. intest. seg. 3 cc.	"	4-12	12/2, 105°	Generalized weakness. Recovered		
4-00	11/25	11/27	Nasal washing 2 cc.	Sterile	4-13	None	None		
			U. intest. seg. 3 cc.	49 col.	4-14	12/1, 105°	12/12 general- ized weakness. 12/14 died	Question- able polio	—
			L. intest. seg. 3 cc.	Sterile	4-15	None	None		
3-94	11/25	11/28	Nasal washing 2 cc.	"	4-18	"	"		
			U. intest. seg. 3 cc.	"	4-16	"	12/12 drowsy. Died	No gross ab- scess. Question- able polio	—
			L. intest. seg. 3 cc.	"	4-17	"	None		

and 3-69), resulted in the typical disease in both animals. Monkey 3-67 inoculated with a suspension of the cord from the primary take of fecal origin succumbed in 11 days. Monkey 3-69 inoculated with a suspension of cord from the primary take of the nasal washings succumbed after the third reinoculation of heavy cord suspensions (10, 20 and 20 per cent respectively), the second and third inoculations being given at 2 week intervals, and the animal succumbing 5 days after the third inoculation.

As is indicated in Table V, a number of additional monkeys inoculated with concentrates from human sources presented suggestive elevations in temperature or showed some weakness. One such animal (3-71), sacrificed when it developed paralysis, showed what appeared to be a typical histologic picture of poliomyelitis, yet we were unsuccessful in transmitting the disease to a second monkey, even after repeated reinoculation with heavy suspension of cord. A second monkey (3-64) showed elevation in temperature and some weakness

in the right leg and presented suggestive, though not typical histologic findings of poliomyelitis. We were unable to obtain a successful passage into a second monkey.

The fifth and only strain of virus recovered from the experimental animal came from the concentrate of the material from the upper intestinal segment of monkey 3-52, Table VI, sacrificed at the height of the disease. Monkey 4-06, Table VI, was inoculated with the concentrate from monkey 3-52 on Oct. 15. 3 days later, the animal showed temperature elevation and was prostrate on the 21st. The histologic findings were compatible but not typical of poliomyelitis. 2 cc. of a 20 per cent suspension of cord were inoculated into monkey 4-09 and resulted in the experimental disease after a prolonged incubation period. Histologic examination showed typical poliomyelitic changes in its cord.

The remaining 2 concentrates from monkey 3-52 produced symptoms suggestive of the experimental disease in the respective test animals. Both monkey 4-07, which was inoculated with the concentrate of the nasal washing, and monkey 4-08, which received concentrate from the lower intestinal segment, showed elevation in temperature and some suggestive weakness, but no extensive involvement of any of the extremities. Both these animals recovered without residual paralysis.

Bacteriology of Concentrates.—30 of the 40 concentrates from human material, and all of the 21 concentrates from the animal material, were cultured on pour plates, broth in Dunham tubes, and blood plates. 0.5 to 1 cc. of the sediment from each concentrate was used for culture. Since our primary interest was to determine whether or not the concentrates were sterile, no special effort was made to identify the organisms beyond morphology and reaction to Gram stain. The organisms were usually Gram-negative rods, presumably colon bacilli. In one instance a Gram-positive coccus was found, and in two instances yeasts were contaminants.

It will be noted in Table V that of the 15 cultures from the concentrates of the human nasal material, 2 were slightly contaminated, while the remaining 13 were sterile. Of the 15 cultures from the fecal concentrates, 8 were found to contain bacteria.

Of the 21 cultures obtained from animal sources, 7 were slightly contaminated; of these one was a nasal concentrate, the remaining 6 from intestinal contents.

Fifteen animals died from causes other than poliomyelitis; 2 died from tuberculosis, 2 from brain abscesses from which positive cultures

of organisms were recovered. 7 animals, all presenting a similar clinical picture, died from 1 to 5 days following inoculation. These animals apparently never recovered from the inoculation: they appeared lethargic, crouched in their cages with drooped heads, responded poorly to stimuli, and finally died. It is furthermore interesting to note that these animals had all been inoculated with concentrates from intestinal contents. It was our impression that the fecal concentrates contained some toxic factor, as has been suggested by Toomey (36) and others.

It can furthermore be seen that 5 animals which had been inoculated with contaminated concentrates survived. Indeed, one animal (3-62) survived an intracerebral inoculation of a heavily contaminated fecal concentrate, which showed more than 100 colonies on the pour plate.

SUMMARY AND COMMENT

Five strains of virus were recovered from nasal washings and feces. Four strains were of human origin, the fifth strain came from a monkey sacrificed at the height of the disease. Of the four human strains the first was isolated from the feces of a 14 year old child 7 days after the onset of illness. The second strain was from the nasal washings of a 6½ year old child, 5 days after the onset of illness. The third and fourth strains were recovered from the same patient, a 2½ year old child, 9 days after the onset of illness. One of these strains was obtained from nasopharyngeal washings and the other from the feces. The single monkey strain was isolated from the upper intestinal segment and appears to be the only instance of its isolation from this source in the literature.

We believe that the detection of the virus in the nasal washings of two additional patients during convalescence lends further support to the belief that the virus of poliomyelitis is spread by human contact.

Furthermore, the recovery of the virus from the gastro-intestinal tract with as great or greater frequency as from the upper respiratory tract, need not, it appears to us, alter our concept of the mode of entrance of the virus into the body, namely, by way of the upper respiratory tract. If the presence of the virus is conceded, then a

consideration of the physiologic passage of nasal and oral secretions into the gastro-intestinal tract by reflex swallowing would serve to explain adequately the presence of the virus in those organs. It might even be further predicated that since the gastro-intestinal tract functions as a temporary reservoir for secretions from the upper respiratory tract, the gut should, after a time, contain the virus in higher concentration than any single sample of secretion obtained from the upper respiratory tract by nasal washing. It appears to us that failures to detect the virus in the gastro-intestinal tract are perhaps more indicative of inadequate procedures for its detection than of its absence.

The recovery of the virus from the feces 7 and 9 days after the onset of illness takes on added significance. It indicates first, that the virus withstands the gastric acidity which under normal physiological conditions tends to keep gastric contents relatively free of bacteria. It further suggests that improper disposal of feces from patients with poliomyelitis may have serious public health consequences, particularly in smaller communities where inadequate sewage disposal may result in contamination of surrounding beaches or even local water systems.

BIBLIOGRAPHY

1. Landsteiner, K., Levaditi, C., and Pastia, C., *Semaine méd.*, Paris, 1911, **31**, 296.
2. Rosenau, M. J., Sheppard, P. A., and Amoss, H. L., *Boston Med. and Surg. J.*, 1911, **164**, 743.
3. Kling, C., Pettersson, A., and Wernstedt, W., *Communications Inst. méd. État Stockholm*, 1912, **3**, 5.
4. Thomsen, O., *Berl. klin. Woch.*, 1912, **49**, 63.
5. Kling, C., and Pettersson, A., *Deutsch. med. Woch.*, 1914, **40**, 320.
6. Flexner, S., Clark, P. F., and Fraser, F. R., *J. Am. Med. Assn.*, 1913, **60**, 201.
7. Lucas, W. P., and Osgood, R. B., *J. Am. Med. Assn.*, 1913, **60**, 1611.
8. Dubois, P. L., Neal, J. B., and Zingher, A., *J. Am. Med. Assn.*, 1914, **62**, 19.
9. Taylor, E., and Amoss, H. L., *J. Exp. Med.*, 1917, **26**, 745.
10. Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1919, **29**, 379.
11. Kling, C., *Office Int. hyg. Pub.*, 1928, **20**, 1790.
12. Levaditi, C., Schmultz, L., and Willemin, L., *J. Am. Med. Assn.*, 1931, **96**, 126.
13. Levaditi, C., and Willemin, L., *Ann. Inst. Pasteur*, 1931, **46**, 233.
14. Frontali, G., *Atti. Cong. nag. microbiol.*, 1932, **4**, 319.

15. Paul, J. R., and Trask, J. D., *J. Exp. Med.*, 1932, **56**, 319.
16. Kramer, S. D., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 1165.
17. Paul, J. R., Trask, J. D., and Webster, L. T., *J. Exp. Med.*, 1935, **62**, 245.
18. Kramer, S. D., Sobel, A. E., Grossman, L., and Hoskwith, B., *J. Exp. Med.*, 1936, **64**, 173.
19. Stillman, M., and Brodie, M., *Proc. Soc. Exp. Biol. and Med.*, 1937, **37**, 382.
20. Kling, C., and Levaditi, C., *Ann. Inst. Pasteur*, 1913, **27**, 718.
21. Sawyer, W. A., *Am. J. Trop. Dis. and Prev. Med.*, 1915, **3**, 164.
22. Harmon, P. H., *J. Am. Med. Assn.*, 1937, **109**, 406, 1061.
23. Trask, J. D., Vignec, A. J., and Paul, J. R., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 147.
24. Landsteiner, K., and Levaditi, C., *Compt. rend. Soc. biol.*, 1909, **67**, 787.
25. Leiner, C., and von Wiesner, R., *Wien. klin. Woch.*, 1910, **23**, 817.
26. Flexner, S., and Lewis, P. A., *J. Exp. Med.*, 1910, **12**, 227.
27. Landsteiner, K., Danulesco, L., and Levaditi, C., *Compt. rend. Soc. biol.*, 1911, **71**, 558.
28. Flexner, S., and Clark, P. F., *J. Am. Med. Assn.*, 1911, **56**, 585.
29. Levaditi, C., and Danulesco, L., *Compt. rend. Soc. biol.*, 1912, **72**, 651.
30. Osgood, R. B., and Lucas, W. P., *J. Am. Med. Assn.*, 1911, **56**, 495.
31. Flexner, S., Clark, P. F., and Dochez, A. R., *J. Am. Med. Assn.*, 1912, **59**, 273.
32. Clark, P. F., Schindler, J., and Roberts, D. J., *J. Bact.*, 1930, **20**, 213.
33. Kling, C., *Bull. Off. internat. Hyg. pub.*, 1931, **23**, 1228.
34. Levaditi, C., Kling, C., and Lepine, P., *Bull. Acad. méd.*, Paris, 1931, series 3, **105**, 190.
35. Clark, P. F., Roberts, D. J., and Preston, W. S., *J. Prevent. Med.*, 1932, **6**, 47.
36. Toomey, J. A., *J. Prevent. Med.*, 1932, **6**, 379.