Summary. The results indicate that the culture of *L. arabinosus* used in these studies for microbiological assay was not easily affected by frequency of transfer of stock cultures or by incubation of assay tubes at fixed temperatures between 30°C and 37°C. In the case of the culture of *L. casei,* both the dilution of the inoculum and the age of the culture from which it was prepared affected assay titration values.

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Recovery of Virus from Throats of Poliomyelitis Patients.*

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In discussions of the epidemiology of poliomyelitis much importance has been placed on the presence or absence of virus in the nose and throat of patients and of associates in so far as such findings might support theories of transmission of the disease by means of droplets. A review of literature on the subject in 1938[1] indicated that virus was reported in the nasopharynx, tonsils or trachea of patients in 15% of 105 examinations made during the first 5 days of illness and in 7% of 182 made later. Conflicting evidence has been presented during the last decade. Sabin and Ward found virus in the pharyngeal wall of 4 of 7 patients dead within 6 days of onset,[2] and Kessel *et al.* reported positive results with tonsil-adenoid tissue in 3 of 6 autopsy specimens.[3] Howe and Bodian detected virus in pooled throat swabs from at least 2 of 28 healthy children and from 1 of 6 juvenile, familial associates of cases; the positive associate may have been a nonparalytic case since the child had fever and diarrhea at the time of collection of the specimen.[4] Oropharyngeal swabs obtained by the same authors, within 3 days after onset of illness from 10 of 23 patients yielded virus, but none of 13 swabs taken between the 4th and 9th days was positive.[5] In a similar study by Horstmann, Melnick and Wenner, only one of 19 swabs and one of 15 washings obtained during the first week of illness contained virus;[6] the specimens from 10 of the 19 patients in the study were obtained within 3 days after onset of symptoms. Kramer, Hoskwith and Grossman[7] tested 18 washings of the nasopharynx and recovered virus from 2 which had been collected 5 and 9 days after onset, respectively. Ward and Walters tested material from the nose and throat of 19 patients between the second and fourth days of illness. Virus was isolated from cloth masks over the nose and mouth of two patients, from a nasal swab of one of these, from pharyngeal swabs of the second and from 5 others.[8]

This report describes attempts to recover virus from pharyngeal washings of a group of poliomyelitis patients during the outbreak

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TABLE I.

Dallas Throat Washings.																	
Day after onset of symptoms specimen was collected	3	4	6	7	7	7	8	9	9	11	11	11	11	13	13	13	
Age of patient	6	4	11	3	11	14	4	3	13	3	4	11	15	5	13	14	
Virus present	—	—	—	—	—	—	—	—	—	—	+	+	—	—	—	—	
Detroit Throat Washings.																	
Day after onset	All 1 to 3																
Age	7	7*	12	13	15	15	15	16	18	3	4	5	6	7*	7	10	
Virus present	(+	—	—	—	+	—	—	+	—)	(+	+	+	—	—	—	+)	
	Washing									Swab							

* Washing and Swab combined.

in Dallas, Texas, 1943 and from swabs and washings from patients in Detroit, Michigan during 1944.

Materials and Methods. The washings from patients in Dallas were obtained by expressing 3 to 5 ml of saline into each nostril with a needleless syringe and collecting the fluid in a paper cup after it ran into the throat. The patient then gargled 20 to 30 ml of saline which also were collected in the cup. Washings in Detroit consisted of 20 ml of gargled, distilled water. Swabs were obtained by use of cotton on applicator sticks. These were rolled over the tonsillar areas and the posterior oropharynx then twirled in 1 to 2 ml of saline contained in a lusteroid vial and finally pressed against the side of the tube to express the saline. All specimens were stored under dry ice refrigeration until prepared for inoculation into rhesus monkeys.

Throat washings from Dallas were concentrated by centrifugation of one or two 7 ml samples per specimen in an air-driven centrifuge at 30,000 r.p.m. for one hour.† Supernatant fluid was removed, leaving 0.5-0.75 ml in the tube, and saved for intraperitoneal injection in a monkey that also received the sterile sediment intracerebrally. The sediment was resuspended in the fluid remaining by use of a glass rod; sediments from the companion tubes were combined when there were 2 samples. Bacteria were inactivated by addition of ether which was evaporated *in vacuo* after 1 to 7 days of contact in the refrigerator. In some specimens bacteria survived the treatment with ether and were inactivated by the addition of phenol to a final concentration of 0.5%. One to 1.5 ml of each specimen was injected intracerebrally into a rhesus monkey.

The throat swab fluids from Dallas and the throat washings from Detroit were treated with ether or ether and phenol and inoculated intracerebrally. The latter, however, were first concentrated to a volume of 1 to 2 ml by evaporation from a cellophane "Visking" casing placed before an electric fan.

Results. All monkeys were observed for a period of one month after injection. Animals that developed paralysis were considered positive for poliomyelitis if histological examination of the nervous system revealed characteristic pathology of the disease. The results of the tests are shown in Table I.

Virus was detected in 2 of 16 Dallas patients, one of the spinal and one of the bulbar type. Both specimens were collected on the eleventh day after onset of symptoms. Eleven other specimens obtained between the 3rd and 11th days were negative. Of the specimens collected in Detroit all within 3 days after onset, 3 of 9 throat washings contained virus as did 4 of 7 throat swabs. One of the 4 positive swabs was from a bulbar paralytic and one from a nonparalytic patient.

Discussion. The results obtained tend to support the impression that swabs are as good, if not a better method of recovering virus from the throat than throat washings even though, as in this study, the latter are concentrated before testing.

The evidence to date shows that poliomyelitis virus is present in the throats of patients and may be readily detected early in the illness. The present study duplicates the report of Howe, Bodian and Wenner[5-b] in demonstrating virus in 43% (7 of 16) of those

† The centrifugation was kindly made by Dr. R. W. G. Wyckoff of this laboratory.

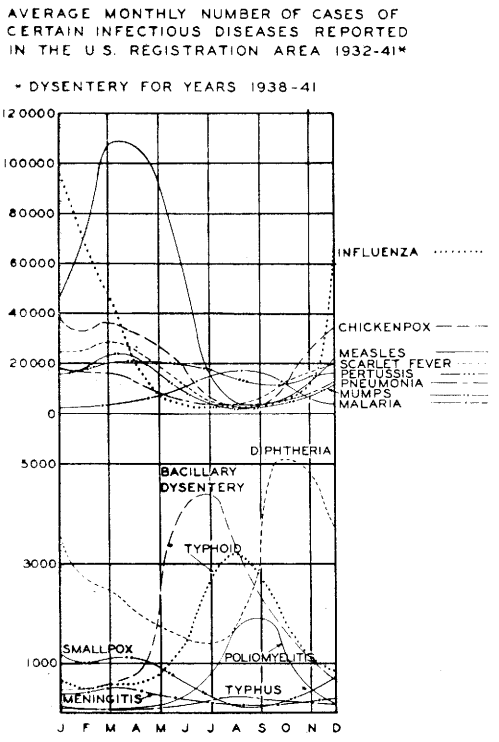

FIG. 1

patients tested within 3 days of onset. However, the fact that virus was detected in two throat washings 11 days after onset suggests that its persistence may be quite variable.

The presence of an agent in the throat does not necessarily mean that the nose and mouth are the chief portals of exit for the germ. For example bacilli are reported present in the mouths of approximately 50% of typhoid patients[9] yet the stools and urine are regarded as the chief routes of contamination from the patient. Likewise the more frequent recovery of poliomyelitis virus from the bowel than from the throat has in recent years tended to emphasize the former as the more important portal of exit.

Another argument favoring enteric over respiratory spread is the seasonal prevalence of the disease. As shown in the figure, poliomyelitis has a seasonal occurrence similar to typhoid, dysentery, typhus and malaria and unlike the disease spread by the respiratory route; diphtheria has a unique curve that perhaps may be explained as resulting in part from increased incidence associated with reopening of schools. The temptation exists to reason from analogy and to suggest that outbreaks of poliomyelitis like those of typhoid-dysentery are started by enteric carriers; the subsequent spread of infection may depend on either or both respiratory or enteric contamination of the environment. More data are needed particularly in regard to the relative importance of the case and of the asymptomatic carrier in spreading the illness and in regard to physiologic effects of nutrition and of environmental temperature on susceptibility to the disease. Meanwhile, until evidence to the contrary is obtained it would seem necessary to consider the throats of poliomyelitis patients as important potential sources of infection.

Summary. Virus was recovered from 4 of 7 throat swabs and from 3 of 9 throat washings of poliomyelitis patients collected during the first 3 days after onset of symptoms. In another series of 16 throat washings collected from 3 to 13 days after onset, virus was isolated from 2 patients 11 days after onset of disease.

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