

Etiology of 1954-55 Poliomyelitis Epidemic in Puerto Rico

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DURING the latter part of 1954, paralytic poliomyelitis appeared in epidemic form on the island of Puerto Rico. This epidemic became one of the largest outbreaks of poliomyelitis in the recorded history of the island with some 500 cases reported from November 1954 through June 1955.

At the request of the Secretary of Health, Commonwealth of Puerto Rico, plans were made early in December 1954 for viral studies on a representative group of pediatric patients. The laboratory data obtained from this study during the height of the epidemic supplements a preliminary epidemiological report (1).

Methods

Cases of paralytic poliomyelitis admitted to the pediatric service of Bayamon District Hospital in Puerto Rico relatively soon after onset of their disease were chosen for study. The hospital staff selected 16 patients, ranging in age from 1½ months to 7 years, collected the appropriate specimens, and prepared case summaries. Materials for collection of specimens

were supplied by the Tropical Research Medical Laboratory, United States Army, San Juan, Puerto Rico. The responsibility for handling, storing, and shipping specimens fell to this same installation. Diagnostic laboratory studies for poliomyelitis were performed by the Department of Virus Diseases, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C.

Initial blood samples were drawn shortly after hospital admission, and subsequent specimens were taken on the 14th to 21st days of disease. Throat and rectal swabs were obtained during the first few days of hospitalization. In fatal cases, generous blocks of tissue were taken from several areas of the brain in addition to a portion of the cervical spinal cord.

Blood drawn in Keidal vacuum tubes was stored overnight at 4° C. Serum was then separated from the clot and maintained in the frozen state until used for serodiagnostic procedures. Throat and rectal swabs were individually placed in sterile screw-capped tubes containing 1 milliliter of veal infusion broth. These were promptly frozen and maintained at -70° C. (dry ice) until thawed for tissue culture inoculation. Central nervous system tissues were aseptically removed at time of autopsy, placed in tightly sealed (screw cap) wide-mouth bottles, and also stored at -70° C. Materials thus collected, and accompanying clinical and laboratory summaries, were periodically shipped in dry ice via air express to the

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Walter Reed Army Institute of Research for laboratory study.

Serums were tested for neutralizing antibodies against the three types of poliomyelitis virus by a modification of the tissue culture metabolic inhibition procedure described by Salk and associates (2). The test, as performed, contained serial twofold dilutions of the patient's serum, approximately 100 to 300 tissue culture ID₅₀ (3) of one of three prototype poliomyelitis viruses (Mahoney, type 1; Y-SK-7, type 2; and D-3-83, type 3), and a suspension of trypsin-dispersed monkey kidney epithelial cells (4).

For attempted isolation of virus, broth suspensions of throat and rectal swab specimens were first centrifuged at 2,000 r.p.m. for 30 minutes. The supernatant fluids were treated with sufficient penicillin and streptomycin to bring the final concentration to 1,000 units and 1,000 micrograms per milliliter, respectively. After incubation from 30 minutes to 1 hour at room temperature, individual suspensions were inoculated in 0.1 milliliter amounts into each of 2 or 3 tubes containing tissue cultures from monkey kidneys. Blocks of central nervous system (CNS) tissue from the various anatomical areas were pooled, made into a 20 percent suspension with chilled distilled water, and then processed and inoculated as above.

Inoculated tissue culture tubes were observed daily for microscopic evidence of cytopathogenic effect. If no degeneration was seen after 14 to 21 days, one blind passage of cells and fluid was made and these subcultures observed

for a similar period of time. When definite signs of specific cellular degeneration occurred, the tissue culture fluid was harvested and inoculated into a "spot neutralization typing test" as described by Melnick and others (5). In this procedure, samples of infected "unknown" fluid were mixed separately with poliomyelitis type-specific monkey hyperimmune serums. Following incubation, these mixtures were each inoculated into two tissue culture tubes and incubated at 37° C. for several days. Preliminary identification was accomplished if protection occurred in the tubes containing one monotypic antiserum but not in the tubes containing the other two antisera or in the control cultures. In each instance, the procedure promptly identified the cytopathogenic isolate as a strain of poliomyelitis virus, and it was unnecessary to perform quantitative neutralization tests.

Specimens from the 16 patients studied were obtained during December 1954 and January 1955. Nine of these patients died while the seven survivors exhibited definite paralytic manifestations. All 16 patients lived within a 10-mile radius of the hospital, but several cities were represented; 7 resided in Toa Baja, 5 in Catano, 3 in Bayamon, and 1 in San Juan.

Results

Virus isolation attempts using central nervous system tissue were successful in 6 of the 9 fatal cases, with recovery of type 1 poliomyelitis virus in each instance (table 1).

Throat and rectal swab materials were avail-

Table 1. Poliomyelitis virus isolations from 9 fatal cases

Patient No.	Patient's age	Results of virus isolation attempts on.		
		CNS	Throat swab	Rectal swab
1.....	1½ years.....	Type 1.....	Negative.....	Negative.
2.....	1½ months.....	Type 1.....	Negative.....	Negative.
3.....	1½ years.....	Type 1.....	Negative.....	Negative.
4.....	16 months.....	Type 1.....
5.....	2½ years.....	Type 1.....
6.....	1 year.....	Type 1.....
7.....	4½ years.....	(1)
8.....	10 months.....	Negative.....
9.....	3 years.....	Negative.....

¹ Results unsatisfactory because of bacterial contamination.

NOTE: Leaders (..) indicate swabs were not available.

Table 2. Poliomyelitis studies of 7 surviving (paralytic) cases

Patient No.	Patient's age	Results of virus isolation attempts		Neutralizing antibody titer against type: ¹		
		Throat swab	Rectal swab	1	2	3
10.....	5 years.....	Negative.....	Type 1.....	64	<4	<4
				128	<4	<4
11.....	16 months.....	Type 1.....	Negative.....	64	<4	<4
				128	<4	<4
12.....	1½ years.....	Negative.....	Negative.....	128	<4	<4
				512	<4	<4
13.....	7 years.....	Negative.....	Negative.....	16	1024	1024
				64	256	256
14.....	3½ years.....	Negative.....	Negative.....	<4	8	32
				32	<4	32
15 ²	8 months.....	Negative.....	Negative.....	256	<8	<8
16 ²	8 months.....	Negative.....	Negative.....	32	<4	<4

¹ Upper row represents tests with acute phase serum; lower row represents convalescent phase serum.

² Single serum samples only were available.

able from 3 of the patients who died and from all 7 surviving patients (tables 1 and 2). In only two instances were isolation attempts successful with these specimens; one strain was obtained from rectal swab material and another from a throat swab. Each of the two agents proved to be type 1 poliomyelitis virus (table 2). Although throat and rectal swabs failed to yield virus in the three fatalities, viral recovery was possible when CNS tissue specimens were used.

Paired serums were available for 5 of the 7 paralytic patients (table 2). Serologic tests revealed a significant rise in neutralizing antibody titer (fourfold or greater) in 3 of these instances (patients 12, 13, and 14). Although no significant increase in antibody was shown with the remaining two paired serums (patients 10 and 11), it should be noted that only type 1 antibody was present, and in each case a type 1 virus was recovered from either the rectal or throat swab. Convalescent serums only were obtained from the remaining two paralytic patients. Both were 8-month-old infants and their serums neutralized type 1 but not types 2 or 3 poliomyelitis virus.

Discussion

All 6 of the 9 fatal cases from whom virus was recovered from the CNS died by the third day of hospitalization, which was still within a few days following onset of illness. Two of

the three patients from whom isolation attempts were unsuccessful died relatively late in their illness (one on the 21st and the other on the 57th day of disease). The one remaining CNS specimen was considered unsatisfactory for study because of heavy bacterial contamination. In all nine of the fatal cases microscopic examination of brain and cord tissue resulted in a pathological diagnosis of poliomyelitis. (Gross and microscopic pathological examinations were done under the supervision of Dr. Gerardo B. Polanco, Bayamon District Hospital). It is of course not surprising that no virus could be recovered from the two patients dying late in the course of the disease since general experience has shown that virus can rarely be recovered from the CNS after the second week of disease (6).

It is of some interest that isolation attempts were successful in only two instances with throat and rectal swab specimens although presumably all such samples were taken from paralytic poliomyelitis patients. One must consider the possibility that the nasopharyngeal secretions contained little or no virus or perhaps neutralizing antibodies when these samples were obtained; yet, no such explanation suffices for the rectal swabs. More plausible explanations are, perhaps, that only small amounts of throat secretions or fecal materials were obtained thus causing virus dilution to be a critical factor (1) or viral inactivation

occurred in the tubes of broth under conditions of subsequent handling and shipment (2), or both. These observations appear in general agreement with those of Godenne and Riordan in a recent publication (7). They noted that throat and rectal swabs were not ideal specimens for routine poliomyelitis virus isolation attempts.

Demonstration of a significant antibody titer rise between paired serum specimens enabled a diagnosis in three surviving patients. The four remaining surviving patients were shown to possess neutralizing antibodies for type 1 virus alone. This assumes additional significance when one considers that each was convalescing from an attack of acute paralytic disease, and furthermore, in two such instances the children were less than 1 year of age.

Summary

Type 1 poliomyelitis virus was isolated from 6 of 9 patients who died and from 2 of 7 survivors studied in the 1945-55 outbreak of acute paralytic disease in Puerto Rico. A diagnostic increase in neutralizing antibody titer for type 1 virus was demonstrated in 3 of the 7 surviving

patients, while the remaining 4 possessed antibodies for this type virus alone.

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National Advisory Council on Health Research Facilities

Marion B. Folsom, Secretary of Health, Education, and Welfare, has appointed 12 members to the new National Advisory Council on Health Research Facilities. The council assists the Public Health Service in administering a program of Federal grants for construction of medical research facilities.

The advisory council, established in the new law, includes 8 leading medical, dental, and scientific authorities and 4 members to represent the public. The Surgeon General of the Public Health Service and an official of the National Science Foundation are *ex officio* members, with the former serving as chairman.

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