AN EPIDEMIC OF PARALYTIC POLIOMYELITIS CHARACTERIZED BY DUAL INFECTIONS WITH POLIOMYELITIS AND COXSACKIE VIRUSES*

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Coxsackie viruses have been isolated from patients with a variety of illnesses, and at times have been particularly associated with poliomyelitis, aseptic meningitis, epidemic myalgia or pleurodynia (Bornholm disease), and herpangina (1–11, 30). In certain instances, patients with paralytic or non-paralytic poliomyelitis have been found to be harboring in their intestinal tract both poliomyelitis and Coxsackie viruses (12–15, 3).

This paper presents a study of the first epidemic of poliomyelitis which appeared in Easton, Pennsylvania, during the summer of 1949. It was characterized by the high ratio of paralytic to non-paralytic cases, by the high proportion of fatal cases, and by the isolation of *both* poliomyelitic and C viruses from more than half of the patients studied. Twenty-eight strains of C virus were isolated and were classified according to antigenic type.

The Epidemic

Easton has a population of about 50,000 and is part of a "metropolitan" area of about 100,000 population, including Phillipsburg, New Jersey. The Easton Hospital serves the entire metropolitan area.

The history of poliomyelitis in this area, recorded in Fig. 1, indicates that prior to 1949 the disease was known to the community but at a low endemic rate. During the first half of the summer of 1949, the disease was at its usual low level, with only a single case in June and another in July. Explosively the epidemic broke in the 2nd week of August (13 cases) and a high incidence was maintained for the subsequent 5 weeks (16, 21, 10, 15, and 18 cases, respectively), as shown in Fig. 2. Cases continued to appear regularly through October and November (2 to 3 per week), and sporadically into the winter months. All

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patients diagnosed as suffering from poliomyelitis were admitted to the Easton Hospital, where they were classified as paralytic or non-paralytic. In order to

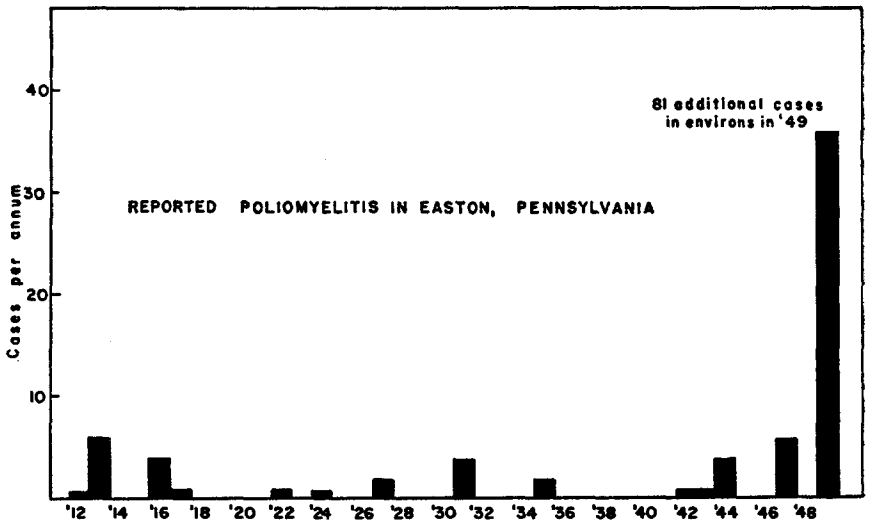

FIG. 1. History of poliomyelitis in Easton, Pennsylvania.

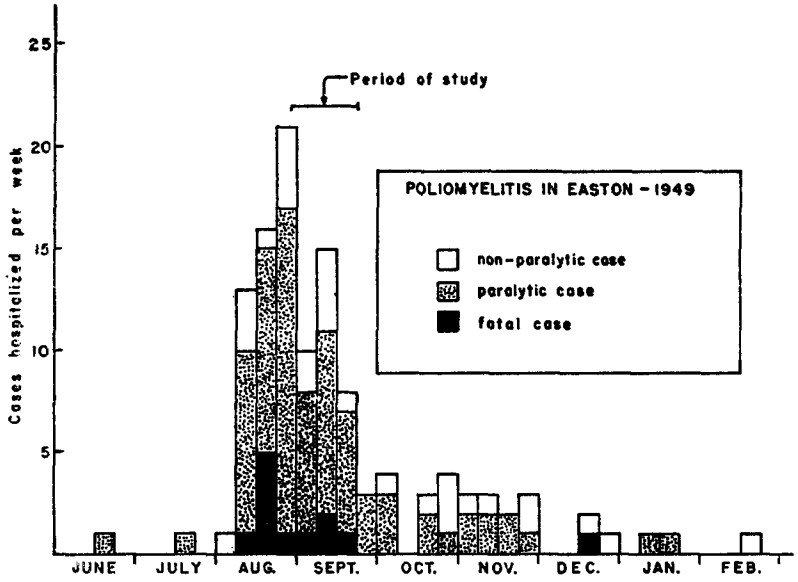

FIG. 2. Distribution of cases of poliomyelitis by week.

be included in the latter category, a patient must have had the symptoms of non-paralytic poliomyelitis plus a pleocytosis of the cerebrospinal fluid. As

shown in Fig. 2, all patients who entered the hospital in consecutive fashion between August 26 and September 23 were included for virological and serological study. In addition we included for study 4 patients who entered the hospital as suspected cases of poliomyelitis, but in whom the cerebrospinal fluid findings were normal.

Because of the explosive nature of the onset of the epidemic an analysis was made of the distribution of cases according to sex in the first and second halves

TABLE I

Distribution of Cases According to Sex in the First and Second Halves of the Epidemic

1st half				
Age	Male	Female	Total	Total cases
yrs.				*per cent*
0–4	10	8	18	31
5–9	5	8	13	22
10–14	8	6	14	24
15–20	4	3	7	12
21+	2	5	7	12
Total	29	30	59	
Per cent	49	51		

nd half				
Age	Male	Female	Total	Total cases
yrs.				*per cent*
0–4	11	5	16	27
5–9	8	4	12	20
10–14	5	4	9	15
15–20	5	4	9	15
21+	8	4	13	22
Total	37	21	59	
Per cent	63	37		

of the epidemic. The figures listed in Table I show that a slightly higher percentage of adults was involved in the latter part of the epidemic, and that the usual predominance of males over females found during poliomyelitis epidemics was absent during the first half of the epidemic.

Materials and Methods for Virological Studies

Stools and throat swabbings were collected daily during the first 3 days of hospitalization. The throat swabs were immersed in 0.5 ml. of water. These materials were frozen at −20°C. and kept at this temperature until tested. Serum was obtained on the 1st or 2nd day of hospitalization, and later samples collected 4 weeks, 12 weeks, and in some instances 18 months after onset. Serum was also kept frozen until used.

The methods used in the preparation and testing of these samples for poliomyeltis and C viruses, as well as the criteria for positive isolations, have been described in detail previously (12, 16, 17). For poliomyelitis virus, one monkey was usually employed and the animal was inoculated intracerebrally with a fecal extract which had been concentrated and partially purified by ultracentrifugation. For C virus, 2 litters of 8 newborn mice each were inoculated for each test.

Neutralization tests were carried out as described (18) using a fixed dose of virus (100 ID_{50}) against varying dilutions of serum.

The viruses were typed in part by cross-neutralization tests but chiefly by the plate complement fixation test as adapted to C viruses (19). Antigens were prepared from virus in the second or third generation in mice and run against 9 or 10 prototype sera.

Neutralizing antibody tests were performed to the Lansing strain in qualitative fashion only: 0.2 ml. of undiluted serum was added to 0.2 ml. of virus containing about 200 ID_{50} per 0.03 ml. Following incubation for 1 hour at room temperature, 0.03 ml. was inoculated intracerebrally into each of 8 young adult mice. Antibody was regarded as present if 5 or more mice survived for 21 days after the inoculation.

RESULTS

The details on the virus isolations from individual patients are shown in Table II, with an analysis of the data in Table III.

Poliomyelitis virus was found in the stools of 27 hospitalized patients in a series of 36 who were examined for virus. It was found in 90 per cent (18 of 20) of the samples obtained from children under 8 and in 56 per cent (9 of 16) of those 8 years and over. The throat swabbings of 4 children, 3 of whom gave positive stool tests, were tested for poliomyelitis virus with negative results. These results are similar to earlier ones from this laboratory in experiments in which virus was sought in the fecal and throat specimens (20, 21).

C virus was found in 64 per cent (27 of 42) of the fecal specimens collected in the 1st week of the disease. None of 38 throat swabbings and none of the 30 serum samples collected at this time yielded virus. During the 4th week of disease, fecal samples were collected again, and 22 samples from patients yielding positive tests in their 1st week samples were tested. Of these 22, only 3 now contained virus in detectable amounts. During the acute illness, C virus was found to be present with the same frequency in those under 8 as in those 8 years and over.

In Table IV, the results are shown of the tests on 4 patients who were seen at the hospital and then sent home because of the negative findings in the cerebrospinal fluid. C virus was isolated from only one of these patients, an individual with myalgia. In this instance it was isolated from both the stool and the throat swab. Attempts to detect poliomyelitis virus in these specimens were unsuccessful. Of the 28 patients from whom C virus was isolated, it is noteworthy that virus was found in the throat only in this single instance.

Thirty-six patients were studied for both viruses. With 75 per cent of the patients excreting poliomyelitis virus and 72 per cent C virus, it is obvious that a number must have been excreting both agents simultaneously. The extent to

TABLE II

*Isolation of Poliomyelitis and Coxsackie Viruses from Hospitalized Patients**

Patient					Poliomyelitis virus‡		Coxsackie virus§							
					Acute stage		Acute stage (1st wk.)						Late stage (4th wk.)	
No.	Name	Age	Pleocytosis	Type of disease	Stool	Throat	Stool		Throat		Serum		Stool	
		yrs.	*cells per c.mm.*											
1	J. A.	16	128	P	−, −		10/15(9)	+	0/8	−	Inc.			
2	B. A.	7	372	P	+	−	12/12(9)	+	1/14	−	0/6	−	0/8	−
4	J. B.	5	394	P	−		0/8	−	0/14	−	0/10	−		
5	A. M. C.	5	161	NP	+		3/23(0)	−	0/16	−	0/8	−		
6	G. C.	4	12	P	+		13/13(10)	+	2/8(0)	−	0/8		0/16	−
7	M. A. C.	28	2	P	−, −, −	−	8/8(8)	+	0/15	−			0/7	−
8	M. D.	1	75	NP	+		0/14	−	1/15(0)	−	1/8(0)	−		
10	S. DiG.	9/12	146	P	−, −, −		17/17(13)	+	0/16	−	0/14	−	0/13	−
11	D. D.	3	52	P	+		7/12(6)	+	0/18	−	0/6	−	0/6	−
12	C. A. F.	5		P	+		15/15(13)	+	1/15(0)	−			5/16(3)	+
13	C. F.	13	132	NP	−, −		12/17(10)	+	0/15	−	0/14	−	0/16	−
14	J. M. F.	4	88	P	+		9/9(9)	+	5/17(0)	−	1/14(0)	−	3/14(0)	−
15	J. F.	6	32	P	+		7/7(1)	+	0/16	−	0/13	−	4/7(3)	+
16	D. G.	11	146	NP	−		11/11(5)	+	0/16	−	0/13	−	1/8(0)	−
17	A. G.	20	62	P	+	−	18/18(11)	+	0/16	−				
18	J. H.	37	209	NP	+	−	4/4(4)	+			1/11(0)	−		
19	K. J.	12	119	P	+		5/7(5)	+					1/8(0)	−
20	C. K.	3	30	P	+		12/13(11)	+	0/8	−	1/9(0)	−	8/8(4)	+
21	R. L.	11	294	P	+		16/16(13)	+	0/8	−	1/7(0)	−	0/16	−
22	M. L.	8	80	NP	−		1/8(0)	−	0/16	−	0/8	−		
23	L. M.	6	340	P	+		11/11(9)	+	0/8	−			0/16	−
24	N. M.	18	211	P	+		16/16(15)	+	0/9	−			3/12(0)	−
25	J. M.	9/12	13	P	+		0/16	−	0/11	−	0/8	−		
26	E. O.	16	79	P	−		2/22(1)	+	0/8	−	0/6	−		
28	B. R.	4	243	NP	+		11/13(8)	+	3/8(0)	−	0/14	−	2/13(0)	−
29	L. R.	28	610	P			0/15	−	0/7	−	0/4	−		
30	K. R.	6	110	P	+		1/40(0)	−	0/8	−	0/8	−		
31	C. R.	11		P	+		1/23(0)	−	1/8(0)	−	0/8	−		
32	F. S.	4	25	P	+		18/18(16)	+	0/7	−	0/10	−	0/8	−
33	D. S.	7	175	P	+		14/14(9)	+	0/7	−	4/18(0)	−	0/12	−
34	J. S.	10	357	P	+		7/7(7)	+	0/8	−	0/14	−	1/9(0)	−
35	C. S.	10/12	38	P	+		0/15	−	0/8	−				
36	M. S.	12	158	P			0/8	−	0/8	−	0/8	−		
37	J. A. S.	1	91	P	+		0/15	−	0/7	−	0/8	−		
39	S. S.	11	173	NP	+		13/13(12)	+	1/6(0)	−	2/8(0)	−	0/16	−
40	E. S.	2	70	P			3/11(0)	−	0/7	−	0/9	−		
41	C. T.	13	62	P	−, −		15/15(11)	+	0/6	−	Inc.			
42	M. A. T.	14	395	P			0/8	−	0/6	−	Inc.			
43	F. T.	16	180	NP			0/16	−	0/6	−	0/10	−		
44	J. V.	4	95	P	+		15/15(12)	+	0/8	−	0/14	−	2/11(0)	−
45	B. V.	13	139	P	+		14/14(11)	+			Inc.		0/14	−
47	W. W.	28	241	P			3/17(1)	+			0/9	−		

P, paralytic poliomyelitis; NP, non-paralytic poliomyelitis.

* These patients were admitted to the Easton Hospital in the period Aug. 26 to Sept. 23, 1949.

‡ One negative sign indicates a negative test in one monkey; a positive sign, the recovery of poliomyelitis virus in the test monkey.

§ 10/15(9). Of 15 satisfactory newborn mice in the test, 10 developed paralysis and/or died within 2 weeks of inoculation. Of these 10 mice, 9 were found with paralysis. The interpretation of this test is positive for C virus isolation. The interpretation of all C virus tests is to the right of the column listing the fate of the test mice.

TABLE III

Isolation of Poliomyelitis and Coxsackie Viruses from Hospitalized Patients Tested for Both Viruses

No. of cases	Total	Total +P	Per cent	Total +C	Per cent	+P +C	Per cent	+P −C	Per cent	−P +C	Per cent	−P −C	Per cent
Paralytic	28	22	79	21	75	17	61	5	18	4	14	2	7
Non-paralytic	8	5	63	5	63	3	38	2	25	2	25	1	13
Total	36	27	75	26	72	20	56	7	19	6	16	3	8
Under 8 yrs.													
Paralytic	17	15	88	12	71	11	65	4	23	1	6	1	6
Non-paralytic	3	3	100	1	33	1	33	2	67	0	0	0	0
Total	20	18	90	13	65	12	60	6	30	1	5	1	5
8 yrs. or over													
Paralytic	11	7	64	9	82	6	55	1	9	3	27	1	9
Non-paralytic	5	2	40	4	80	2	40	0	0	2	40	1	20
Total	16	9	56	13	81	8	50	1	6	5	31	2	13

P, poliomyelitis virus; C, Coxsackie virus; +, presence of virus; −, absence of virus.

TABLE IV

Isolation of Poliomyelitis and Coxsackie Viruses from 4 Non-Hospitalized Patients

Patient				Poliomyelitis virus*		Coxsackie virus					
				Acute stage		Acute stage (1st wk.)					
						Stool		Throat		Serum	
No.	Age	Cerebrospinal fluid	Symptoms	Stool	Throat						
	yrs.	*cells per c.mm.*									
3	24		Stiff neck, headache			0/8	−	0/7	−	0/8	−
9	10	3	Pain in right arm and right leg, spasms in back, right shoulder, and right leg	−, −	−	14/14(12)	+	16/16(8)	+	1/9(0)	−
27	7	4	Headache, vomiting, difficulty in walking	−		0/8	−	0/4	−	0/8	−
38	27		Nausea, vomiting, fever			2/9(0)	−	Inc.		Inc.	

* See footnote in Table II.

which this was true is shown in Fig. 3 and is given in more detail in Table III, in which the results are presented on 28 paralyzed and 8 non-paralyzed patients. Both viruses were sought in the samples collected from each of these 36 patients, and both agents were found in 20 (56 per cent). Only poliomyelitis virus was found in 7 (19 per cent), only C virus in 6 (16 per cent), and neither virus in 3 (8 per cent). It is worth pointing out that in 4 paralytic patients excreting C virus, 2 to 3 monkeys were used for each of the poliomyelitis virus tests, with negative results.

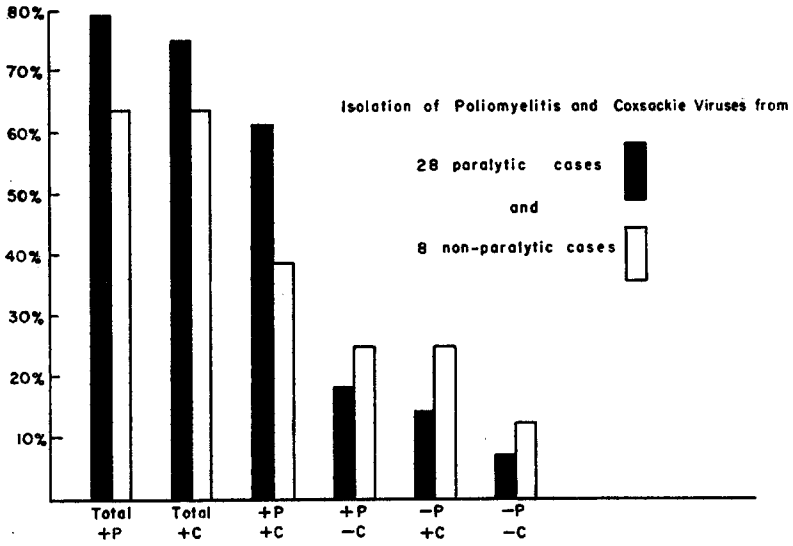

FIG. 3. Poliomyelitis and Coxsackie virus isolations from paralytic and non-paralytic poliomyelitis patients. +P, poliomyelitis virus isolated; +C, C virus isolated; −P, poliomyelitis virus not isolated; −C, C virus not isolated.

As noted above poliomyelitis virus was recovered less readily in patients 8 years and over than in the younger children, but this was not found for C virus. Mixed infections of poliomyelitis and C viruses were found to about the same degree in both age groups, 12 of 20 (60 per cent) in the younger, and 8 of 16 (50 per cent) in the older group. Poliomyelitis virus alone was detected more often in the younger group, 6 of 20 (30 per cent) as against 1 of 16 (6 per cent) for the older group. The reverse was true for the finding of C virus alone, 1 of 20 (5 per cent) for the younger, as against 5 of 16 (31 per cent) for the older group. In view of the fact that we had only 8 non-paralytic patients in this series, it is not possible to evaluate any differences in rates between the paralytic and non-paralytic groups. However, it is noteworthy that mixed virus infections were found in both groups: 17 of 28 (61 per cent) for those with paralysis and 3 of 8 (38 per cent) for those without paralysis.

TABLE V

Persistence of Excretion of C Virus in Stools

No. of patients in group	No. of patients yielding positive tests for poliomyelitis virus in 1st wk. specimens	Isolation of C virus			
		1st wk.		4th wk.	
		+	−	+	−
14	14	14	0	2	12
8	0	8	0	1	7
Totals..... 22		22	0	3	19

TABLE VI

Antibody Responses in Patients from Whom Both Poliomyelitis Virus and C Virus Were Isolated

Age	Disease	Cerebrospinal fluid	Easton-2		Easton-14		Homologous C		Lansing poliomyelitis			Homologous poliomyelitis	
			Acute	Convalescent	Acute	Convalescent	Acute	Convalescent	A	C_1	C_2	Acute	Convalescent
yrs.		*cells per c.mm.*											
3	P	52	0	50	0	0	0	50	−	−		0	30
3	P	30	0	1250	0	0			−	−	−		
4	P	12	0	250	0	0	0	250	−		−		
4	P	88	0	0	1250	1250	1250	1250	−	−	−		
4	NP	243	50	50	0	0	25	250	−	−	−		
4	P	25	0	250									
4	P	95	50	250	250	250			−	−	−		
5	P		0	50	250	250	0	50	−	−	−	10	270
6	P	32	>50	>250	0	0	50	1250	−	−			
6	P	340	0	50					+	+	+		
7	P	175	0	50					±	−	−		
7	P	372	100	500	250	250	100	500	−	−	−		
10	P	357	0	250					+	+	+		
11	P	294	0	0	0	0	0	0	−	−	−		
11	NP	173	0	50					+	+	+		
12	P	119	0	100					−	−	−		
13	P	139	0	250					−	−	−		
18	P	211	0	250					±	+	+		
20	P	62	10	50					+	+	+		
37	NP	209	10	250	0	0							

A, acute, C_1, early convalescent serum, 4th week, C_2, late convalescent serum, 3rd to 4th month.

Attention has been directed to the finding of C virus more often in the acute phase of illness than later. The data in Table V show that the simultaneous excretion of poliomyelitis virus did not appear to influence the duration of the C virus carrier state. Of 14 patients excreting both agents in the 1st week of

disease, 2 (14 per cent) were still excreting C virus 4 weeks after onset. Of 8 patients who in the acute phase yielded negative tests for poliomyelitis virus and positive tests for C virus, one (13 per cent) was still positive in the 4th week.

Neutralizing Antibodies.—Antibodies to the Easton-2 strain (isolated from patient 2 listed in Table II) were determined for all patients from whom acute

TABLE VII

Antibody Responses in Patients from Whom C Virus but Not Poliomyelitis Virus Was Isolated

Age, yrs.	Disease	Cerebrospinal fluid	Easton-2		Easton-14		Homologous		Lansing poliomyelitis		
			Acute	Convalescent	Acute	Convalescent	Acute	Convalescent	A	C_1	C_2
9/12	P	146	0	0	0	0	50	500	−	−	
11	NP	146	10	10	1250	1250	10	250	+	+	+
13	NP	132	10	10	1250	1250	50	50	+	+	+
13	P	62	50	250					−	−	+
16	P	128	0	10	0	0	0	50	−	−	−
28	P	2	0	50	250	250	0	50	+	+	+

TABLE VIII

Antibody Responses in Patients from Whom Poliomyelitis Virus but Not C Virus Was Isolated

Age, yrs.	Disease	Cerebrospinal fluid	Easton-2		Easton-14		Lansing poliomyelitis		
			Acute	Convalescent	Acute	Convalescent	A	C_1	C_2
9/12	P	13	0	0			−	−	−
10/12	P	38	250	250			−	−	
5	NP	161	0	250	1250	1250	−	−	−
6	P	110	0	10			−	−	−
11	P		0	250			−	−	−

and convalescent sera had been collected. In addition a number of determinations were carried out for antibodies to an antigenically different strain, Easton-14 (isolated from patient No. 14). A selected group of 13 patients was studied for the development of antibodies to their homologous strain; *i.e.*, to the very strain isolated from the patient. The antibody level was determined by titrating the serum in the presence of a fixed amount of virus (100 ID_{50}) (18).

Tables VI to X show the results of these tests in relation to the finding of C virus and/or poliomyelitis virus in the specimens from the patient. In addi-

tion, we have listed the results of the Lansing poliomyelitis neutralizing antibody tests on these patients. For 2 patients on whom we carried out homologous strain poliomyelitis antibody tests, these results are also included. As summarized in Table XI, Easton-2 type infection appeared to be commonplace in these patients, with 19 of 26 patients from whom C virus was isolated showing an antibody rise during their disease. Of the 7 patients who failed to show an antibody rise to Easton-2, 4 showed specific rises to the strain isolated from their

TABLE IX

Antibody Responses in Patients from Whom neither Poliomyelitis Virus nor C Virus Was Isolated

Age, yrs.	Disease	Cerebrospinal fluid	Easton-2		Easton-14		Lansing poliomyelitis		
			Acute	Convalescent	Acute	Convalescent	A	C_1	C_2
5	P	394	50	50	50	50	−	−	−
7	NP	4	0	0			−	−	−
8	NP	80	250	250			±	+	+
16	P	79	0	0			+	+	+

TABLE X

Antibody Responses in Patients from Whom C Virus Was Not Isolated and for Whom Poliomyelitis Virus Tests Were Not Done

Age, yrs.	Disease	Cerebrospinal fluid	Easton-2		Easton-14		Lansing poliomyelitis		
			Acute	Convalescent	Acute	Convalescent	A	C_1	C_2
2	P	70	0	0			−	−	±
12	P	158	0	0			−	−	−
14	P	395	0	0			±	+	+
16	NP	180	0	0			+	+	+
24	NP		10	10	10	10	+	+	+
27	NP		50	50			+	±	+

own fecal specimens. In only 3 patients from whom virus was isolated were we unable to demonstrate an antibody rise, even to the patient's own strain. On the other hand, of 15 patients in whom C virus was sought but not isolated, only 3 showed rises to the Easton-2 strain. 16 of the patients from whom C virus was isolated were also studied for development of Easton-14 antibodies and not one showed such a rise, not even the patient who was the source of the Easton-14 strain.

Lansing Poliomyelitis Neutralization Tests.—Lansing antibodies did not increase in these patients except for one child, who also showed an increase in C

virus antibodies. However, Lansing antibodies in this paralytic patient were not found until the 3rd month of convalescence.

The details of the Lansing tests are shown in Table XII. The results for the acute, 1 month convalescent, and 3rd to 4th month convalescent sera are listed for each patient in chronological order together with the viruses which were found in the stools. The distribution of the Lansing tests into negative, positive, and questionable results is shown in Table XIII, which shows that only 5 tests gave a questionable result according to the criterion used here (4 or 8 mice developing poliomyelitis). Even though the numbers are small, the results on 40

TABLE XI

Demonstrable Increases in Antibodies

Viruses isolated	Coxsackie antibodies			Poliomyelitis antibodies	
	Easton-2	Easton-14	Homologous C	Homologous poliomyelitis	Lansing poliomyelitis
+P +C	16/20	0/11	6/8	2/2	1/18
−P +C	3/6	0/5	4/5		0/6
+P −C	3/5	0/1			0/5
−P −C	0/4	0/1			0/4
ndP −C	0/6	0/1			0/6
	Antibody rise to E-2 or homologous C virus				
+P +C	18/20 } 23/26				
−P +C	5/6 }				
+P −C	3/5 }				
−P −C	0/4 } 3/15				
ndP −C	0/6 }				

+P, poliomyelitis virus isolated, +C, C virus isolated, −P, polio virus not recovered, −C, C virus not recovered, nd P, no test made for poliomyelitis virus.

16/20 means that of 20 patients tested, the sera of 16 showed increases in antibodies.

sera (see Table XIV) show a definite trend, which was reproducible on sera collected from the same individuals 1 month and again 3 to 4 months later. Only 2 of 20 patients under 10 years of age had Lansing antibodies. In the 10 to 14 age group, 5 of 12 were positive, and this increased to 7 of 8 in the group above 15. These data indicate that the inhabitants of Easton have had previous exposure to Lansing type virus, although spread of infection seems to be more limited than that found in certain other parts of this country, and especially so when compared with results obtained on sera collected from certain Southern communities.

From the neutralization tests with Lansing virus, it is apparent that this agent, or one of like antigenic constitution, was not the cause of poliomyelitis

TABLE XII

Lansing Neutralizing Antibodies in Patients Excreting Poliomyelitis Virus and/or Coxsackie Virus

Age	Patient No.	Poliomyelitis virus excreted	C virus excreted	Lansing neutralizing antibodies*		
				Acute	Convalescent (1 mo.)	Convalescent (3 to 4 mos.)
yrs.						
9/12	10	−	+	−	−	
9/12	25	+	−	−	−	−
10/12	35	+	−	−	−	
2	40		−	−	−	±
3	11	+	+	−‡	−‡	
3	20	+	+	−	−	−
4	6	+	+	−		−
4	14	+	+	−	−	−
4	28	+	+	−	−	−
4	44	+	+	−	−	−
5	4	−	−	−	−	−
5	5	+	−	−	−	−
5	12	+	+	−‡	−‡	−
6	15	+	+	−	−	−
6	23	−	+	+	+	+
6	30	+	−	−	−	−
7	2	+	+	−	−	−
7	27	−	−	−	−	−
7	33	+	+	±	−	−
8	22	−	−	±	+	+
10	9	−	+	−	−	
10	34	+	+	+	+	+
11	16	−	+	+	+	+
11	21	+	+	−	−	−
11	31	+	−	−	−	−
11	39	+	+	+	+	+
12	19	+	+	−	−	−
12	36		−	−	−	−
13	13	−	+	+	+	+
13	41	−	+	−	−	+
13	45	+	+	−	−	−
14	42		−	±	+	+
16	1	−	+	−	−	−
16	26	−	−	+	+	+
16	43		−	+	+	+
18	24	+	+	±	+	+
20	17	+	+	+	+	+
24	3		−	+	+	+
27	38		+	+	±	+
28	7	−	+	+	+	+

* + indicates that of 8 mice inoculated, 5 to 8 survived; − indicates 0 to 3 survived; ± indicates 4 survived.

‡ For tests with patient's strain of poliomyelitis virus, see Table XVII.

in this epidemic. Our findings are unlike those reported by Steigman and Sabin (22). These workers found an increase in Lansing antibody in 6 of 24 patients,

TABLE XIII

Criteria of Lansing Neutralization Tests and Pattern of Results

Group	No. of sera in each group	Result	Per cent
8/8	42	−	61
7/8	17		
6/8	11		
5/8	2		
4/8	5	±	4
3/8	7	+	35
2/8	14		
1/8	10		
0/8	11		
Total	119		

The numerator indicates the number of dead or paralyzed mice; the denominator indicates the number of mice inoculated.

TABLE XIV

Lansing Neutralizing Antibodies by Age in Easton Poliomyelitis Patients

Age group, *yrs.*	0–4	5–9	10–14	15–20	21+
Acute serum:					
No. patients	10	10	12	5	3
No. positive	0	2	5	4	3
Positive, *per cent*	0	20	42	80	100
Convalescent serum: (1 mo.)					
No. patients	9	10	12	5	3
No. positive	0	2	5	4	3
Positive, *per cent*	0	20	42	80	100
Convalescent serum: (3 to 4 mos.)					
No. patients	7	10	11	5	3
No. positive	0	2	5	4	3
Positive, *per cent*	0	20	45	80	100

particularly if sera obtained 3 months after convalescence were compared with acute specimens. In the present study only one of the Easton patients developed such an increase even when sera obtained as late as 4 months after onset were

tested. Our results are in accord with those reported from other laboratories (23, 24).

When the age distribution pattern of 2 C viruses is tabulated together with the Lansing results, as in Table XV, it is seen that infection with Easton-2 and

TABLE XV

Development of Neutralizing Antibodies to Lansing Poliomyelitis Virus and to Easton-2 and Easton-14 Coxsackie Viruses in Different Age Groups

Patients in epidemic		Lansing neutralizing antibodies				Easton-2 neutralizing antibodies				Easton-14 neutralizing antibodies			
		Acute sera		Convalescent sera		Acute sera		Convalescent sera		Acute sera		Convalescent sera	
Age, *yrs.*	Total	No. tested	Per cent +	No. tested	Per cent +	No. tested	Per cent +	No. tested	Per cent +	No. tested	Per cent +	No. tested	Per cent +
0–4	34	10	0	9	0	10	30	10	70	7	29	7	29
5–9	25	10	20	10	20	9	33	9	67	5	80	5	80
10–14	23	12	42	12	42	9	44	9	89	3	67	3	67
15–20	16	5	80	5	80	5	20	5	60	1	0	1	0
21+	19	3	100	3	100	4	75	4	100	3	67	3	67

TABLE XVI

Family Infection with Poliomyelitis and Coxsackie Viruses

	Age, *yrs.*	Cerebrospinal fluid	Disease	Poliomyelitis virus	C virus
G. C.	4	12	P	+	+
A. M. C.	5	161	NP	+	−

Neutralizing antibody response

Virus used in neutralization test	G. C.		A. M. C.	
	1st wk.	4th wk.	1st wk.	4th wk.
Easton-2	0	250	0	250
Easton-14	0	0	1250	1250
Easton-6 (G. C.)	0	250	0	250
Lansing poliomyelitis	0	0	0	0

Easton-14 C viruses took place earlier, and that of these three antibodies only those to Easton-2 increased significantly during the epidemic.

Family Infection with Both Viruses.—Two patients were admitted to the hospital from the same family, with G. C., aged 4, diagnosed as paralytic, and A. M. C., aged 5, as non-paralytic poliomyelitis (as shown in Table XVI). Poliomyelitis virus was isolated from both, and C virus only from the paralytic

child. The neutralizing antibody study, however, showed that C virus infection with the E-2 type occurred in both children at the time of their illness, for both had a serum titer of zero in the acute phase serum and a titer of 250 in the serum collected 4 weeks later. These levels were also found with the Easton-6 strain (which had been isolated from G. C.) as the virus in the neutralization test. and subsequent work showed that the E-2 and Easton-6 strains are antigenically related. However, when the unrelated Easton-14 strain was used, G. C.'s acute and convalescent sera were negative, while A. M. C.'s two sera contained the same high level of 1250, indicating previous infection with an E-14 type.

Dual Antibody Studies in Patients Simultaneously Excreting C and Poliomyelitis Virus.—Two patients (Nos. 11 and 12) with paralytic poliomyelitis were studied in detail using as virus in the neutralization test the patient's own stool. Each serum-virus mixture was inoculated into 3 monkeys to test for poliomyelitis antibodies and into 2 litters of newborn mice to test for C virus antibodies. In addition, for the C virus antibody tests, the mouse passage virus was used. Sera were used in serial threefold dilutions as shown in Table XVII, which also gives the results of these tests.

Similar findings were obtained on both patients. Antibodies were negligible or present in low titer on the 3rd or 4th day of disease and these had increased significantly for both poliomyelitis and C viruses by the 32nd day. The result with the mouse passage C virus was similar to that found with the C virus in the patient's stool.

Typing of C Viruses Isolated during the Epidemic.—From the frequent antibody rise to Easton-2 strain (Table XI), we suspected that this strain would be the one most commonly obtained from the patients during this outbreak. Also in view of the fact that some patients failed to produce antibodies to Easton-2 and Easton-14 types but did to their homologous strain, we had reason to believe that other types were also present in Easton in 1949. A systematic investigation was made by the complement fixation test (19) of 28 strains isolated from as many patients. The details of the typing carried out on these and a number of other strains isolated from various parts of this country and abroad will be described elsewhere. For the present purposes, it is sufficient to point out that the plate complement fixation test was used with antigens being made from each of the 28 strains. Antigen was prepared by inoculation of newborn mice (less than 24 hours old) with 10^{-1} suspensions of infected murine torsos, and harvesting the recipient mice 48 hours later regardless of whether they had developed disease. These antigens were run against the following prototype sera: Texas-1, Conn.-5, Ohio-1, Easton-2 (Albany type 1), Albany type 2,[1] Albany type 3, Nancy, Easton-10, and Easton-14. Certain strains, including

[1] We wish to acknowledge Dr. Gilbert Dalldorf's kindness in sending us the three strains which he isolated in Albany, and which he designated as types 1, 2, and 3.

all those used as prototypes, were also run against serum to Alaska-5 type. The list includes three types which were encountered in Easton, one of which, Easton-2, was found to be related to Albany type 1 and two of which, Easton-10 and Easton-14, were found to be hitherto unknown types. This was established by the fact that Easton-10 and Easton-14 antigens failed to react with the other prototype sera and conversely that Easton-10 and Easton-14 sera

TABLE XVII

Simultaneous Development of Antibodies to Poliomyelitis Virus and C Virus

Patient No.	Day of disease serum collected	ID_{50} in 10 per cent suspension of patient's stool	Final serum dilutions				Highest serum dilution neutralizing virus completely	Dilution of serum protecting 50 per cent of animals	Neutralization of 100 ID_{50} doses of passaged C virus	
									Highest serum dilution neutralizing virus completely	Dilution of serum protecting 50 per cent of animals
			1:10	1:30	1:90	1:270				
		1	C virus: in mice							
	3		0/8	4/15(3)			10	α50	0	0
11	32		0/15	0/8	0/14	3/12(3)	90	α300	50	150
		10	Poliomyelitis virus: in monkeys							
	3		0/3	2/3			10	20		
	32		0/3	0/3	0/3	0/3	270	>270		
		100	C virus: in mice							
	4		3/12(2)	7/14(7)			0	30	0	0
12	32		0/6	0/6	0/14	4/7(3)	90	230	50	110
		10	Poliomyelitis virus: in monkeys							
	4		1/3	3/3			0	15		
	32		0/3	0/3	1/3	1/3	30	270		

Denominator indicates the number of animals used per serum dilution. The numerator indicates the number of animals with disease. The number in parentheses indicates the number of mice with observable paralysis. All monkeys with disease showed some degree of paralysis (mild to severe) and all sick animals had typical poliomyelitic lesions in the spinal cord. No CNS lesions were found in monkeys which failed to develop paralysis.

reacted specifically with Easton-10 and Easton-14 antigens respectively, and not with the other prototype antigens.

It was found of the 28 strains tested, that 24 were related to Easton-2, 2 to Easton-14, 1 to Easton-10, and 1 to Nancy. Of the 24 patients who were found infected with Easton-2 type virus, 22 were studied to determine whether or not they showed a rise in neutralizing antibodies to the Easton-2 strain. It is noteworthy that 18 of the 22 developed Easton-2 antibodies at the time when

an Easton-2 type of virus was being excreted. Thirteen of these patients were also studied for Easton-14 neutralizing antibodies and none showed a rise. Conversely, 4 patients were found infected with one of the following types: Nancy,

TABLE XVIII

Classification by Complement Fixation Reaction of 28 Strains Isolated during Easton 1949 Epidemic, and Correlation with Rise in Neutralizing Antibodies

Type of strain by complement fixation	Patient No.	Age, *yrs.*	Type of disease	Rise in neutralizing antibodies		
				Easton-2	Easton-14	Homologous
Easton-2 (Albany-1)	11	3	P*	+	0	+
	20	3	P	+	0	nd
	6	4	P	+	0	+
	32	4	P	+	nd	nd
	44	4	P	+	0	nd
	28	4	NP	0	0	+
	12	5	P	+	0	+
	15	6	P	+	0	+
	23	6	P	+	nd	nd
	2	7	P	+	0	+
	33	7	P	+	nd	nd
	34	10	P	+	nd	nd
	21	11	P	0	0	0
	39	11	NP	+	nd	nd
	19	12	P	+	nd	nd
	13	13	NP	0	0	0
	45	13	P	+	nd	nd
	41	13	P	nd	nd	nd
	1	16	P	+	0	+
	26	16	P	0	nd	nd
	24	18	P	+	nd	nd
	7	28	P	+	0	+
	47	28	P	nd	nd	nd
	18	37	NP	+	0	nd
Easton-14	14	4	P	0	0	0
	9	10	Myalgia	nd	nd	nd
Easton-10	10	9/12	P	0	0	+
Nancy	16	11	NP	0	0	+

* P, paralytic; NP, non-paralytic poliomyelitis; nd, not done.

Easton-10, or Easton-14. Three of these patients were studied for Easton-2 and Easton-14 antibodies and none responded with antibodies to these types, while 2 of 3 tested showed a rise in neutralizing antibodies to their homologous type.

In Table XVIII we have grouped the Easton patients according to the type of strain isolated, development of neutralizing antibodies, and clinical illness.

RECAPITULATION

1. Easton, Pennsylvania, and its environs suffered their first known epidemic of poliomyelitis in 1949, an outbreak characterized by a high proportion of paralytic to non-paralytic cases and by the isolation of both poliomyelitis and Coxsackie, or C, viruses from more than half the patients studied. All patients who entered the hospital during the height of the epidemic were included for virological and serological study.

2. Poliomyelitis virus was found in the stools of 27 patients of a series of 36 who were examined (75 per cent). Throat swabbings of 4 children, 3 of whom gave positive stool tests, were tested for poliomyelitis virus with negative results.

3. C virus was found in 27 of 42 (64 per cent) fecal specimens collected during the 1st week of disease. None of 38 throat swabbings and none of 30 serum samples collected at this time yielded virus.

4. During the 4th week of disease, fecal samples were again collected. Of 22 samples from patients yielding positive tests in their early sample, only 3 specimens were positive.

5. Of 4 patients with a minor disease (not poliomyelitis according to our criteria), one with a diagnosis of myalgia yielded C virus in specimens collected from the throat as well as from the intestines. Attempts to detect poliomyelitis virus in these specimens were unsuccessful.

6. Both poliomyelitis and C virus were sought in the fecal samples collected from each of 36 patients (28 paralytic and 8 non-paralytic). Both agents were found in 20 (56 per cent), poliomyelitis virus alone in 7 (19 per cent), C virus alone in 6 (16 per cent), and neither virus in 3 (8 per cent). Dual virus infections were found in both paralytic and non-paralytic groups: 17 of 28 (61 per cent) for the former and 3 of 8 (38 per cent) for the latter.

7. Twenty-eight strains isolated from as many patients were classified according to antigenic type. Twenty-four were found to belong to one antigenic type, Easton-2 (related to the Albany type 1 virus); 1 to the Nancy type virus; and 3 to two hitherto unknown types (2 to Easton-14 and one to Easton-10). None of the strains reacted with the following prototypes: Conn.-5, Ohio-1, Albany type 2, Albany type 3, Alaska-5, and Texas-1.

8. Of 22 patients infected with the Easton-2 type, 18 developed Easton-2 neutralizing antibodies. Thirteen of these patients were also studied for Easton-14 antibodies and none showed a rise. Of 3 patients from whom a C virus other than Easton-2 was isolated and who did not respond with Easton-2 antibodies, 2 developed antibodies to the strain isolated from their fecal specimens.

9. Of 26 patients from whom C virus was isolated and antibody determina-

tions made, 23 showed a rise either to the Easton-2 or the homologous type antibody. On the other hand, of 15 patients from whom C virus was not isolated, only 3 showed rises to the Easton-2 strain.

10. With the exception of one patient, the presence of Lansing antibodies did not increase; these tests were carried out on acute, 1 month convalescent, and 3rd to 4th month convalescent sera.

11. Apparently Lansing type virus had been present in Easton prior to this epidemic. While only 2 of 20 patients under 10 had Lansing antibodies, these increased to 5 of 12 in the 10 to 14 age group and to 7 of 8 in the group above 15. Infection with Easton-2 and Easton-14 viruses seems to take place at an earlier age than was the case for Lansing virus.

12. A family infection occurred in which 2 members were infected with both poliomyelitis and C viruses.

13. Two patients with paralytic poliomyelitis were studied for the development of both poliomyelitis and C virus antibodies. Using the neutralization test, a simultaneous rise in antibodies against both agents was observed.

DISCUSSION

The simultaneous presence of poliomyelitis virus and C virus in patients has been observed by Rhodes (13) and Dalldorf (3) as well as in our own laboratory (5, 12, 14, 15). In addition we have also found that both viruses may be found in the same specimen of sewage or flies (5, 25). The present study is unique in that over half of the poliomyelitis patients examined, particularly those with the paralytic form of the disease, were found to be excreting both viruses. The rise in antibodies during convalescence showed that most of the patients excreting C virus had been infected with this virus at the time of their acute onset. These patients undoubtedly also had an antibody rise to the poliomyelitis type with which they were infected. However, because at the present time monkeys are required for these tests, we carried them out in only 2 patients: both showed a rise in poliomyelitis antibodies which paralleled the rise in C virus antibodies. Thus patients who excrete both viruses during acute paralytic disease may develop antibodies against both agents.

In the main, these patients were infected with one type of C virus, Easton-2, which is related to Albany type 1 of Dalldorf. If infection with this antigenic type in any way influenced the development of paralysis in these patients, it is pertinent to compare this C virus type with another which was isolated during an epidemic of mild poliomyelitis. In the latter epidemic which occurred in Ohio in 1947, both poliomyelitis virus and C virus were also isolated from the same patient. The epidemic was characterized by high incidence of minor illnesses ("summer grippe"). From 3 patients in this epidemic, the Ohio-1 type of C virus was isolated and all 3 developed Ohio-1 antibodies (12). Two of these patients had been shown previously to have been excreting poliomyelitis

virus in the identical fecal specimen (26). Other patients with non-paralytic illnesses in Ohio were found to be excreting poliomyelitis virus and the Conn.-5 type of C virus. In Connecticut during the summer of 1948, patients with aseptic meningitis (from whom we were unable to obtain poliomyelitis virus) were also found infected with the Conn.-5 type (6). The Easton-2 type of virus (the one isolated most often from paralytic patients during the Easton epidemic) is not only antigenically different from the Ohio-1 and Conn.-5 viruses, but has different biological properties. In the infant mouse Easton-2 virus produces lesions restricted to the muscle (so-called group A of Dalldorf) while Ohio-1 and Conn.-5 viruses produce lesions in other tissues as well, particularly in the fat pads, brain, and pancreas (group B). A histopathological description of these types can be found in another publication (27). The question may be raised as to whether in a patient infected with poliomyelitis virus, simultaneous infection with an Easton-2 type virus tips the balance in favor of paralysis, while infection with an Ohio-1 type virus (or Conn.-5 type) limits the spread of poliomyelitis virus within the body. Although our attempts (5) to demonstrate either enhancement of or interference with poliomyelitis infection in mice or primates have not yielded positive results, Dalldorf (28) has reported some success with certain C viruses interfering with poliomyelitis virus.

The epidemic in Easton was characterized by an abrupt onset (Fig. 2). During the first half of the epidemic the usual preponderance of males over females was not present, although the ratio of male to female patients in the second half of the epidemic was 1.7 to 1 (Table I). These observations suggest that something unusual occurred in Easton when the epidemic flared up, and the possibility must be considered that this was an outbreak of C virus infection in the community. It is noteworthy that in 1949 poliomyelitis had a higher prevalence than usual in New York City (29), but the outbreak was of a much milder nature than that which occurred in Easton. In another study from this laboratory (15), 20 representative paralytic and non-paralytic patients, suspected of having poliomyelitis during the New York City 1949 outbreak, were examined for C virus. Virus, also belonging to the Easton-2 type, was recovered from the stools of 2 of these patients, both paralytic cases. Both patients were found to be excreting poliomyelitis virus together with C virus. Thus in the same year in different epidemics, Easton-2 type C virus and poliomyelitis virus were found associated in the same patients with paralytic poliomyelitis. However, in Easton where the incidence of paralytic poliomyelitis was high, the recoveries of Easton-2 virus were also high. In New York City with a lower incidence of paralytic poliomyelitis, Easton-2 virus, although present, was found less frequently.

SUMMARY

The first known epidemic of poliomyelitis in Easton, Pennsylvania, occurred in 1949, and was unusual in the high proportion of paralytic to non-paralytic

cases. Both poliomyelitis and Cosxackie, or C, viruses were isolated from more than half the patients studied during the acute stage of the disease. One month later C virus was only occasionally recovered. Classification of the 28 strains of C virus which were isolated revealed that 24 belonged to one antigenic type, Easton-2 (related to Albany type 1 virus).

Patients from whom C virus was isolated showed a rise during convalescence to the Easton-2 or homologous type antibody. Two patients with paralytic poliomyelitis were studied for the quantitative development of antibodies to the poliomyelitis virus and to the C virus found in their stools. Using the neutralization test in monkeys and in newborn mice, respectively, a simultaneous rise in antibodies to both agents was observed.

The situation at present can be summarized as follows:—Poliomyelitis virus or C virus may produce infection in man, with a specific antibody response. Both agents may be carried, particularly in the intestines, without causing any serious illness and healthy carrier states have been observed for each. Both viruses can be found in nature in flies and in sewage. However there has been no evidence to suggest that these two viruses bear a relationship to each other, even when isolated from the same patient. Thus, when both viruses are found in a patient with paralysis, it is not yet possible to say with any degree of accuracy to what extent each is responsible in the over-all pattern of the disease. How frequently dual infections of this nature may occur remains for future investigations to determine. Certainly all cases of poliomyelitis are not complicated by a superimposed infection with a C virus. However, this will have to be one more item to consider in *epidemic* poliomyelitis.

BIBLIOGRAPHY

1. Dalldorf, G., Sickles, G. M., Plager, H., and Gifford, R., *J. Exp. Med.*, 1949, **89**, 567.
2. Melnick, J. L., Shaw, E. W., and Curnen, E. C., *Proc. Soc. Exp. Biol. and Med.*, 1949, **71**, 344.
3. Dalldorf, G., *Bull. New York Acad. Med.*, 1950, **26**, 329.
4. Curnen, E. C., *Bull. New York Acad. Med.*, 1950, **26**, 335.
5. Melnick, J. L., *Bull. New York Acad. Med.*, 1950, **26**, 342.
6. Curnen, E. C., Shaw, E. W., and Melnick, J. L., *J. Am. Med. Assn.*, 1949, **141**, 894.
7. Howitt, B. F., *Fed. Proc.*, 1950, **9**, 574.
8. Kilbourne, E. D., *Fed. Proc.*, 1950, **9**, 581.
9. Shaw, E. W., Melnick, J. L., and Curnen, E. C. *Ann. Int. Med.*, 1950, **33**, 32.
10. Huebner, R. J., Cole, R. M., Beeman, E. A., Bell, J. A., and Peers, J. H., *J. Am. Med. Assn.*, 1951, **145**, 628.
11. Weller, T. H., Enders, J. F., Buckingham, M., and Finn, J. J., Jr., *J. Immunol.*, 1950, **65**, 337.
12. Melnick, J. L., Ledinko, N., Kaplan, A. S., and Kraft, L. M., *J. Exp. Med.*, 1950, **91**, 185.

13. Rhodes, A. J., Clark, E. M., Knowles, D. S., Shimada, F. S., Ritchie, R. C., Donohue, W. L., Armstrong, M. P., Wilson, F. H., McLean, W. J., and Silverthorne, N., *Canad. J. Pub. Health*, 1950, **41,** 183.
14. Melnick, J. L., and Kaplan, A. S., *Proc. Soc. Exp. Biol. and Med.*, 1950, **74,** 812.
15. Curnen, E. C., and Melnick, J. L., *Pediatrics*, 1951, **8,** 237.
16. Melnick, J. L., *Proc. 4th Internat. Cong. Trop. Med. and Malaria*, 1948, 401.
17. Paul, J. R., Diagnostic Procedures for Virus and Rickettsial Diseases, New York, American Public Health Association, 1948, 165.
18. Melnick, J. L., and Ledinko, N., *J. Exp. Med.*, 1950, **92,** 463.
19. Kraft, L. M., and Melnick, J. L., *J. Exp. Med.*, 1950, **92,** 483.
20. Horstmann, D. M., Ward, R., and Melnick, J. L., *J. Clin. Inv.*, 1946, **25,** 275.
21. Horstmann, D. M., Melnick, J. L., and Wenner, H. A., *J. Clin. Inv.*, 1946, **25,** 270.
22. Steigman, A. J., and Sabin, A. B., *J. Exp. Med.*, 1949, **90,** 349.
23. Turner, T. B., and Young, L. E., *Am. J. Hyg.*, 1943, **37,** 67.
24. Brown, G. C., and Francis, T., Jr., *J. Immunol.*, 1947, **57,** 1.
25. Unpublished data.
26. Sabin, A. B., and Steigman, A. J., *Am. J. Hyg.*, 1949, **49,** 176.
27. Godman, G. C., Bunting, H., and Melnick, J. L., *Am. J. Path.*, in press.
28. Dalldorf, G., *J. Exp. Med.*, 1951, **94,** 65.
29. Greenberg, M., Siegel, M., and Magee, M. C., *New York State J. Med.*, 1950, **50,** 1119.
30. A more complete bibliography may be found in Melnick, J. L., *Ann. Rev. Microbiol.*, 1951, **5,** in press.