OHIO STRAINS OF A VIRUS PATHOGENIC FOR INFANT MICE (COXSACKIE GROUP). SIMULTANEOUS OCCURRENCE WITH POLIOMYELITIS VIRUS IN PATIENTS WITH "SUMMER GRIPPE"*

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Several immunologically distinct viruses characterized by myositis with accompanying paralysis or death, which they cause in infant mice, have recently been found to be widespread throughout the country (1–3). Recoveries of these viruses have been made from feces or throat swabs of patients with illnesses resembling poliomyelitis (1, 2) and epidemic myalgia (9, 10, 3), as well as from sewage and flies (2, 3). Tentatively these agents have been designated the "Coxsackie group" and will be referred to as "C" virus.

As reported earlier (2), one of the sources from which the C virus was isolated included specimens of feces collected in Akron, Ohio, in 1947. A pool of fecal samples collected from 6 hospitalized patients with a diagnosis of non-paralytic poliomyelitis was found to contain both poliomyelitis virus and C virus. It was not possible to determine whether the individual patients who contributed to the pool were *infected* with one virus or the other, or both. The possibility exists that a single patient may excrete both viruses simultaneously.

During the same summer of 1947, when the above specimens were obtained, Sabin and Steigman (4) collected fecal samples and serial samples of serum from patients with a diagnosis of "summer grippe" as well as from patients with illnesses diagnosed as paralytic or non-paralytic poliomyelitis in this same epidemic area (Cincinnati and Akron). Actually it appeared that thousands of cases of similar mild illnesses were prevalent in Akron and Cincinnati (as well as in other parts of the country including Delaware and Connecticut) during the summer and early fall when these samples were collected. Sabin and Steigman have described their attempts to recover poliomyelitis virus from such patients in Ohio (4, 5). Fecal specimens collected within the 1st week of disease from 4 of their patients with "summer grippe" and 4 with "poliomyelitis" were kindly made available by Dr. Sabin for the present study.[1] In addition, frozen samples of acute and convalescent serum were also provided from these patients as well as from 10 other Ohio patients with mild aseptic meningitis

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[1] Clinical descriptions of patients with "summer grippe" as well as those with "poliomyelitis" from this area have been reported by Sabin and Steigman (4, 5).

diagnosed as non-paralytic poliomyelitis, especially selected by Dr. Sabin because no poliomyelitis virus was recovered from their alimentary tract (5).

This paper reports:—(*a*) the isolation of 3 strains of C virus from the acute phase stool specimens of patients with non-paralytic illness, (*b*) the development of complement-fixing and neutralizing antibodes to this virus in the serum of these 3 patients but not in the serum of the remaining 5 patients whose feces yielded negative tests for C virus, and (*c*) the demonstration of an increasing titer of antibodies in 4 of the 10 convalescent serum samples which were obtained from patients with aseptic meningitis unassociated with demonstrable poliomyelitis virus in the alimentary tract.

Materials and Methods

Isolation of Virus from Feces.—Frozen suspensions (approximately 10 percent) of feces from 8 patients listed in Table I were shipped to New Haven on dry ice. There they were thawed and samples of about 50 ml. were centrifuged in the cold at 18,000 R.P.M. for 20 minutes in the International PR-1 centrifuge. To 1 ml. of the supernatant fluid of each suspension was added 0.1 ml. of a solution containing 2,000 units of penicillin and 10 mg. of streptomycin. This mixture was inoculated into 8 to 9 infant mice. Swiss albino mice were injected intraperitoneally with 0.02 ml. within 48 hours of birth.

A portion (30 ml.) of the remaining supernatant fluid was spun in the ultracentrifuge at 33,000 R.P.M. for 60 minutes (average gravitational field ×60,000). Previous work had shown that C virus is thereby sedimented, so the supernatant fluid following the ultracentrifugation was discarded. The sediment was suspended in 3 ml. of water and centrifuged for a few minutes at 2,000 R.P.M. to remove aggregated material. Penicillin and streptomycin were added to the supernatant fluid in the same concentrations as indicated above, and the mixture promptly inoculated into newborn mice from 2 litters.

Neutralizing Antibody Tests.—On the basis of earlier investigations the following techniques were found to be satisfactory for the detection of neutralizing antibodies. Preliminary tests were conducted in which undiluted samples of serum were mixed with varying dilutions of virus (from 10 to 10,000 ID_{50}). In some instances, the sera were tested both before and after heating at 56° for 30 minutes and this treatment was without marked effect on the antibody levels. In all cases acute and convalescent sera from the same patient were tested simultaneously. Equal parts of serum and virus were mixed and the mixture held for 1 hour at room temperature. Seven to 10 newborn mice were then inoculated intraperitoneally with 0.02 ml. Mice were observed daily for 2 weeks for paralysis or death. Mice which died within 2 days of the inoculation and those mice which disappeared (eaten) were not included in the tabulations.

Complement-Fixing Antibody Tests.—Antigen was prepared from the bodies (exclusive of head and viscera) of suckling mice infected with the Ohio Red. strain. The eviscerated bodies were weighed and homogenized with 3 volumes of cold saline in a Waring blendor. The resulting suspension was centrifuged lightly to remove coarse particles, and the supernate was then centrifuged at 18,000 R.P.M. for 1 hour in a refrigerated centrifuge (International PR-1). This supernate was then spun in the cold in the Spinco Model E ultracentrifuge at 39,460 R.P.M. for 1 hour (130,000 × gravity at bottom of tubes). The sediment was resuspended in pH 7.3 veronal buffer[2] in an amount 1/20 of the volume of the original suspension, and centrifuged

[2] 100 ml. of this buffer contains 0.85 gm. NaCl, 57.5 mg. 5,5-diethyl barbituric acid, 37.5 mg. sodium-5,5-diethyl barbiturate, 16.8 mg. $MgCl_2 \cdot 6H_2O$, and 2.8 mg. $CaCl_2$. We have used this buffer in the complement fixation test as recommended by Mayer *et al.* (6).

(PR-1) in the cold at 18,000 R.P.M. for 30 minutes. The supernatant fluid was treated with protamine sulfate following the suggestion of Warren (7). 5 mg. of protamine were added per ml. of ultracentrifuged virus suspension. This was allowed to remain at 4°C. for 1 hour, and the precipitate was then removed by centrifugation at 18,000 R.P.M. for 30 minutes in the cold. The clear supernatant fluid constituted the antigen. In the presence of specific hyperimmune sera, this antigen still reacted in the complement fixation test, when diluted sixteen-fold and was therefore considered to contain 16 units. In the tests below it was used at a dilution of 1:2 (8 units).

Antigens prepared in this way retain their infectivity. As a result of the concentration they actually titer higher in mice than the original crude suspensions of infectious tissue. When

TABLE I

Virus Isolations from Patients with "Summer Grippe" and "Poliomyelitis," Cincinnati, Ohio, 1947

Clinical diagnosis	Name	Age	Pleocytosis*	Poliomyelitis virus (myelitis in monkeys)‡	C virus (myositis in mice)§	Incidence of infection in mice	
						18,000 R.P.M. Supernate	36,000 R.P.M. Sediment
Summer grippe	Moh.	8	0	+ (P)	−	0/7	0/6 0/9 1/7(0) 1/9(0)
	Mac.	5	40	+ (NP)	−	0/8	0/8 1/7(0)
	Hux.	3	3	+ (NP)	+	5/5(2)	5/5(2) 9/9(3)
	Lin.	12	150	+ (NP)	+	7/9(3)	7/7(4) 2/8(2)
Non-paralytic poliomyelitis	Vau.	8	734	+ (P)	−	0/7	0/8 0/6
	Wal.	3	27	+ (P)	−	0/9	0/7 0/9
	Red.	7	70	−	+	7/7(7)	3/3(2) 7/7(3)
Paralytic poliomyelitis	Ris.	1	107	+ (P)	−	0/8	0/7 0/8

* Pleocytosis. This column indicates the total white blood cells per cubic millimeter of cerebrospinal fluid.

‡ These tests were carried out and reported by Sabin and Steigman (4, 5).

+ (NP) = positive isolations of strain of low virulence, characterized by limited lesions in the CNS (non-paralytic poliomyelitis).

+ (P) = positive isolations of strain of typical virulence, characterized by extensive lesions in the CNS and clinical paralysis in the test monkey.

§ +5/5(2) 5/5(5) 9/9(3) = positive isolation of myositis virus in 3 different litters of mice. 5/5(2) = of 5 satisfactory animals in the test, 2 developed paralysis and 3 were found dead without signs of the disease having been observed.

tested with the purified antigen, homologous hyperimmune serum had a complement-fixing titer of 1:28.

The complement fixation tests were carried out on lucite plates according to the method of Fulton and Dumbell (8). Tests done in tubes in the conventional manner gave comparable results. In the first phase of the reaction the mixture (antigen, complement, and serum) was held overnight at 4°C. During the second phase, following the addition of the hemolytic system, the mixture was incubated for 30 minutes at 37°C. Titers presented in Table IV represent the serum dilution at which 1.5 units of complement were fixed. One unit of complement was taken to be that amount of complement at which 50 per cent hemolysis occurs in the presence of antigen alone.

Further details of antigen preparation and performance of the complement fixation test will be published in a later report.

Criteria for Isolation of a New Strain.—Each of the three strains recovered from the feces of patients fulfilled the following criteria: (*a*) production of paralysis of extremities in infant

mice after an incubation period of about 3 to 10 days, (*b*) fatal termination of the disease in mice usually within 1 to 2 days from onset, (*c*) reproduction of the disease in other infant mice on passage of bacteriologically sterile, infectious tissues (muscles, brains), and, (*d*) production of characteristic myositis in sick mice (degeneration of muscle fibers and cellular infiltrations). Supporting evidence that the virus was isolated from the feces of the patients was found in the rise in titer of specific antibodies, both complement-fixing and neutralizing, in the convalescent serum of each patient from whom these three strains of virus were isolated. Furthermore, one of the strains was passed through an EK Seitz, bacterial filter.

RESULTS

Of the 4 patients diagnosed as "summer grippe", 2 were found to be excreting C virus in their feces (Table I). One of these patients had a pleocytosis in the spinal fluid and the other did not. It is noteworthy that these 2 patients excreted simultaneously with C virus what appears to have been a strain of poliomyelitis virus of low virulence (4). The other 2 patients with "summer grippe" also excreted a poliomyelitis strain of low virulence, and their feces gave negative tests for C virus. It should be pointed out that these 4 patients were selected for study because Sabin and Steigman had previously obtained positive tests for poliomyelitis virus from their specimens (mixed throat swabs and stools).

C virus was isolated from the stools of one of 4 patients with a diagnosis of poliomyelitis. This patient (Red.) had a non-paralytic disease and poliomyelitis virus was not found in his feces. The 3 remaining patients in this group who yielded negative tests for C virus had, in Cincinnati (5), previously given positive tests for poliomyelitis virus (strains of high virulence).

The amount of C virus present in these human stools was obviously more than the minimum required to infect infant mice. The samples from each patient infected the majority of mice in each litter inoculated. This was true whether or not the virus was concentrated by ultracentrifugation. Of 60 mice inoculated with the virus-containing feces, 28 manifested signs of paralysis and 24 died without signs of paralysis but after a proper incubation period. In contrast, of 131 mice inoculated with stools giving negative tests, only 3 mice died (without signs) during the same period of observation. Titrations of positive stools in mice gave the following end points (ID_{50}): Hux, $10^{-2.6}$; Lindsay, $10^{-1.3}$; Redmond, $10^{-2.1}$.

Each strain was carried in passage and produced titers of 10^{-4} to 10^{-6} in infant mice. Like strains isolated from other parts of the country, each of these 3 strains failed to produce obvious disease in monkeys, 4 monkeys being used on each strain.[3] By cross-neutralization tests, it was shown that the 3 strains are related immunologically to each other but are different from the Connecticut, the North Carolina, and the Texas strains (2, 3). One Ohio strain (Red.)

[3] *Cynomolgus* monkeys, however, may be *infected*, as evidenced by fever, by virus carrier states in the throat and intestines, and by a sharp antibody response (3).

was arbitrarily selected for use in the neutralization tests with the patients' sera. This strain was found to be readily filterable through an EK Seitz, bacterial filter.

TABLE II

Neutralization Index of Acute and Convalescent Sera of 3 Patients Found to be Excreting C Virus, and 5 Similar Patients Whose Stools Gave Negative Tests for C Virus

Patient	C virus	Poliomyelitis virus	Day of disease serum collected	Concentration of infected brain Red. strain					Negative log of ID_{50}	Neutralization index
				10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}		
Hux.	+	+ (NP)	1	7/7 (1)	8/8 (7)	3/8 (2)			4.0	10
			28	0/8	3/7 (0)	0/7			<2.0	**>1000**
Red.	+	−	5	Inc.	0/8	0/8			<3.0	**>100**
			28	3/9 (0)	0/8	0/8			<2.0	**>1000**
Lin.	+	+ (NP)	5	0/9	1/8 (1)	0/9			<2.0	**>1000**
			28	Inc.	0/8	2/8 (0)			<3.0	**>100**
Moh.	−	+ (P)	1	6/6 (3)	7/7 (5)	6/8 (4)			>4.0	—
			28	8/8 (5)	8/8 (6)	6/7 (6)			>4.0	—
Mac.	−	+ (NP)	1	6/6 (3)	4/4 (0)	6/9 (2)	4/9 (2)		4.6	3
			28	5/5 (1)	8/9 (5)	5/7 (3)	4/9 (2)		4.6	3
Ris.	−	+	3	8/8 (6)	6/6 (3)	3/6 (3)			4.0	10
			28	7/7 (3)	5/6 (3)	5/8 (2)			4.0	10
Vau.	−	+	5	8/8 (4)	6/6 (2)	6/6 (0)			>4.0	—
			28	6/6 (5)	5/6 (2)	6/6 (4)			>4.0	—
Wal.	−	+	2		5/5 (3)	9/9 (5)	6/8 (2)		>4.0	—
			28		9/9 (8)	3/6 (3)	1/8 (1)		3.0	10
Normal monkey						7/7 (4)	4/8 (2)	0/7	5.0	

Neutralization index = difference in titer of virus between that in presence of control normal serum and test serum. Significant neutralization is indicated by figures in bold-faced type.

Inc. = incomplete test due to non-specific deaths.

Table II presents the results of the *neutralization tests* carried out on the sera of the patients whose stools were examined for C virus. It will be seen that the only significant neutralization indices were obtained from the 3 patients who had positive stools. In order to obtain a better evaluation of the level of antibodies—in particular to determine whether these patients actually had a rise

TABLE III

Titrations of Acute and Convalescent Sera against 50 to 100 ID_{50} of Red. Virus

Patient	Day of disease serum collected	Serum dilutions						Highest serum dilution found to neutralize virus completely
		1:10	1:50	1:100	1:250	1:1000	1:1250	
Hux.	1	4/6(3)		6/9(3)		9/9(6)		0
	28	**0/8**		**0/9**		**0/9**		1000
Red.	5	**0/7**		**1/8(0)**		4/10(4)		100
	28	**0/5**		**0/7**		**0/10**		1000
Lin.	5	**0/5**	**0/5**		4/8(2)			50
	28		**0/7**		**0/7**		4/8(2)	250
Bow.	2	6/7(6)						0
	28	**0/5**	**0/5**		5/6(1)			50

Groups of mice inoculated with neutralized virus are in bold-faced type.

TABLE IV

Antibodies to Red. Strain of C Virus in Acute and Convalescent Sera of Patients with "Summer Grippe" and "Poliomyelitis", Ohio, 1947

Clinical diagnosis	Name and city	Age	Pleocytosis	Poliomyelitis virus (Monkey)	C virus (Mice)	Day of disease serum collected		Neutralization index (Undiluted serum *vs.* varying dilutions of virus		Neutralizing titer (Constant amount of virus *vs.* varying dilutions of serum)		Complement fixation titer (8 units of antigen *vs.* varying dilutions of serum)	
						Acute	Convalescent	Acute	Convalescent	Acute	Convalescent	Acute	Convalescent
Summer grippe	Moh.-C	8	0	+ (P)	−	1	28	0	0			0	0
	Mac.-C	5	40	+ (NP)	−	1	28	3	3	0	0	0	0
	Hux.-C	3	3	+ (NP)	+	1	28	10	**>1000**	0	**1000**	0	**40**
	Lin.-C	12	150	+ (NP)	+	5	28	**>1000**	**>100**	**50**	**250**	0	**24**
Paralytic poliomyelitis	Ris.-C	1	107	+ (P)	−	3	28	10	10				0
Non-paralytic poliomyelitis	Vau.-C	8	734	+ (P)	−	5	28	0	0			0	0
	Wal.-A	3	27	+ (P)	−	2	28	0	10			0	0
	Red.-C	7	70	−	+	5	28	**>100**	**>1000**	**100**	**1000**	0	**96**
	Bow.-C	4	104	−		2	28	8	**>300**	0	**50**	0	**24**
	Bru.-C	15	40	−		1	28	**>300**	**>300**	**50**	**1250**	0	**64**
	Cah.-C	3	220	−		2	28	**>300**	**>300**	**50**	**250**		
	Gil.-C	11	60	−		2	28	0	0			0	0
	Hal.-C	12	105	−		3	27	**>300**	**>300**	**250**	**250**		
	Cur.-A	3	343	−		4	28	6	**>300**	0	**250**		
	Fru.-A	12	20	−		4	28	**>300**	**>300**	**50**	**50**	0	0
	Hud.-A	11	85	−		3	31	**>300**	**>300**	**250**	**250**		
	Let.-A	5	93	−		1	28	−5	−3			0	0
	Wor.-A	7	87	−		1	28	7	8			0	0

C = Cincinnati; A = Akron.

Significant figures for antibodies are shown in bold-faced type.

50 to 100 doses of Redmond strain were used in the serum titer tests.

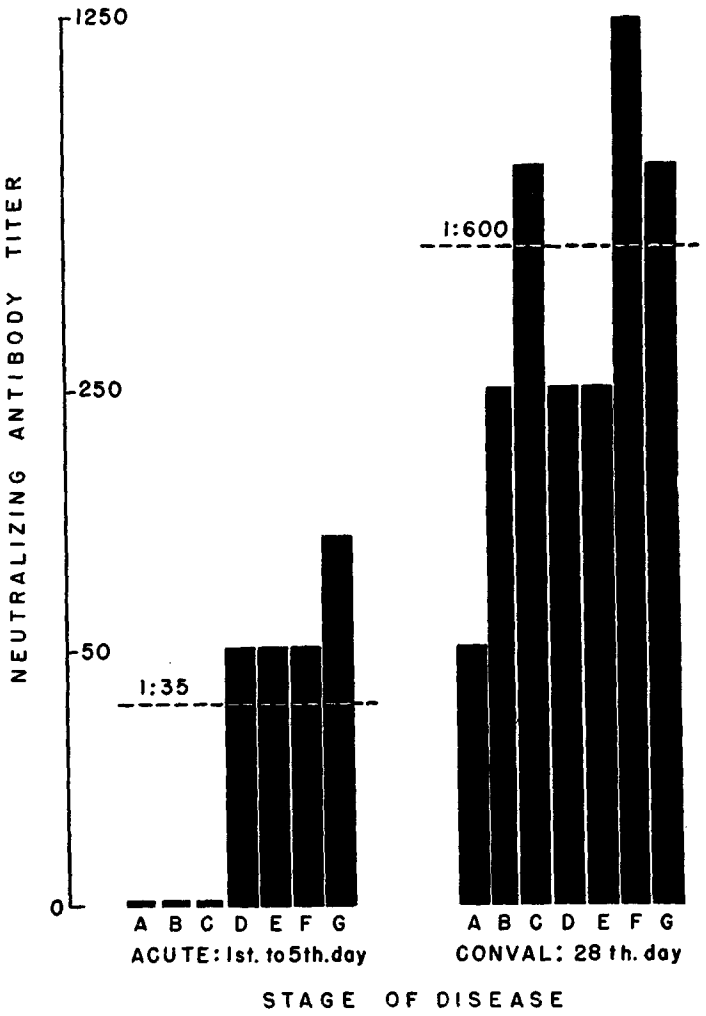

FIG. 1. Development of neutralizing antibodies to C virus (Red. Ohio strain) in 7 patients (A to G) with a diagnosis of "summer grippe" or "non-paralytic poliomyelitis." A constant amount of virus (50 to 100 ID_{50}) was mixed with varying dilutions of serum. The average dilution of serum capable of neutralizing this amount of virus was 1:35 in the acute stage and 1:600 in convalescence.

in antibodies following the illness during which C virus was recovered from their feces—titrations were carried out using various dilutions of serum against a constant amount of virus. The results of such tests with diluted sera are shown in Table III. It is clear that a rise in the antibody level occurred in all instances. It is obvious from these data and from those in Table IV, which

summarizes the neutralization tests on these sera and on those from 10 other non-paralytic patients in this area, that antibodies develop early in the disease, and often are present at the time of onset. This is shown in Fig. 1. It can also be seen that antibodies which may be found early in the disease increase in concentration during the next few weeks.

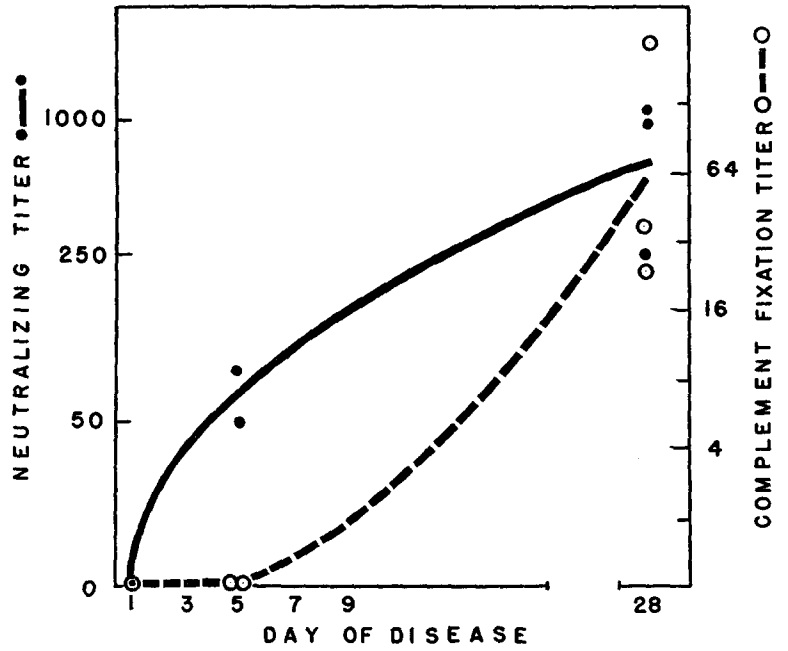

FIG. 2. Comparative development of neutralizing and complement-fixing antibodies to C virus. These data were obtained from 3 patients proven to be intestinal carriers of C virus during the acute stage of illness.

For the 3 patients from whom C virus was isolated, serum antibody titers in the acute phase were 0, 50, and 100. These titers increased in convalescence so that on the 28th day after onset they were 1000, 250, and 1000 respectively.

Of the 10 patients with a diagnosis of non-paralytic poliomyelitis whose stools gave negative tests for poliomyelitis (in Dr. Sabin's laboratory), 7 had significant amounts of neutralizing antibody in their sera. Of these, however, only 4 showed a rise in antibody titer during convalescence. The values in the acute phase for the latter patients were: 0, 0, 50, and 50, and 4 weeks later they had increased to 50, 250, 250, and 1250 respectively.

The *complement fixation tests* gave results which were on the whole in good agreement with the neutralization titers. Thus the 3 patients from whom C virus was isolated had serum titers of 0 in the acute phase, and these increased

to 24, 40, and 96, on the 28th day after onset. The data are summarized together with the neutralizing titers in Table IV. From the curves drawn in Fig. 2 based on the data collected in these 3 cases of proven infection with C virus, it seems that neutralizing antibodies may appear before complement-fixing antibodies. Thus antibodies were not detected in the one sample taken on the 1st day of disease, and only neutralizing antibodies (in low titer) were recognized, in the 2 samples collected on the 5th day. As already mentioned, both types of antibody were present in high titer on the 28th day of disease.

It is also noteworthy that 2 additional patients (Bow. and Bru.) developed complement-fixing antibodies and both of these had significant increases in neutralizing antibodies. Furthermore, 9 patients who failed to develop neutralizing antibodies also had no complement-fixing antibodies.

DISCUSSION

Evidence which has been accumulating in this as well as in other laboratories (1–3, 9) indicates that infection with C virus is not only present in many areas of this country, but that it may actually have accounted for an appreciable, though unknown, proportion of cases of mild illnesses (and perhaps severe ones as well) in 1947 and 1948. The evidence for this occurring in Ohio in 1947 is furnished by the data in this paper: (*a*) the isolation of 3 related strains from 8 patients examined,[4] and (*b*) the serological evidence of an increase in both neutralizing and complement-fixing antibodies in these 3 patients as well as in 4 of 10 others. These last 10 patients were selected because Sabin and Steigman were unable to recover poliomyelitis virus from their alimentary tract. But it should be emphasized that among 23 non-paralytic patients (including the above 10) in the same area, these workers (5) found poliomyelitis virus in 9.

Some important questions have been raised by this research: When 2 viruses are being simultaneously excreted in the intestinal tract, is the host infected by both or is one merely in passive transit? And if such excretion of 2 viruses represents a combined infection, which was inapparent and which gave rise to symptoms? Two patients (Hux. and Lin.) were found to be harboring both C virus and poliomyelitis virus in the intestines. The fact that they had a rise in antibody level to C virus would suggest that the current illness was due to infection with C virus. We do not know whether there occurred a simultaneous rise in poliomyelitis antibodies, for the latter were not determined. Even though the poliomyelitis strains found in these 2 patients were of the low virulent type, it would seem worth while to measure in such instances antibody levels against the 3 known types of poliomyelitis virus, particularly using a strain recovered in 1947 from Cincinnati. Experiments with chimpanzees indicate that intes-

[4] Of these 8 *selected* patients, Sabin and Steigman found 3 to be excreting a virulent strain of poliomyelitis virus and 4 others, a mild (non-paralyzing) strain of apparently the same virus.

tinal carrier states with C virus and poliomyelitis virus may exist simultaneously (3).

It should be noted that the 2 strains of poliomyelitis virus isolated in Cincinnati from Hux. and Lin. were of low virulence, so low in fact that they failed to produce paralysis in monkeys and could be identified only by the limited lesions produced in the CNS (4). In view of the fact that C virus has not been found to produce lesions in monkey CNS, we must for the present accept the findings of such lesions as due to poliomyelitis virus and not to C virus. Consideration should be given to a possible modification of the reaction of poliomyelitis virus in primates when it is inoculated together with C virus. Yet the following facts appear to militate against this possibility: (*a*) three different pools of stools from patients in areas as scattered as Ohio, North Carolina, and Texas have yielded both C virus and virulent, monkey-paralyzing poliomyelitis virus (2, 3), (*b*) simultaneous inoculation of C virus and Lansing strain of poliomyelitis virus into 4 week old mice did not affect the infective titer of the latter virus or the nature of the disease produced. However, temporal and quantitative relationships between the two viruses must be given further consideration, and further experiments on possible interference between strains of C virus and poliomyelitis virus should be performed.

SUMMARY

Further evidence for the widespread occurrence of Coxsackie or C virus is presented in this paper. This virus is characterized by paralysis and myositis produced in infant mice.

An epidemic of mild illnesses diagnosed as "non-paralytic poliomyelitis" and as "summer grippe" occurred during the summer of 1947 in Akron and Cincinnati, Ohio. From the pooled feces of such patients both poliomyelitis virus and C virus were obtained. From the samples of single patients, 3 immunologically related strains of C virus were isolated.

One patient from whom virus was isolated had an illness which was diagnosed as non-paralytic poliomyelitis (with pleocytosis). Although poliomyelitis virus could not be isolated from this patient, previous tests (5) from similar non-paralytic patients in the same area revealed that 9 of 23 were infected with poliomyelitis virus. C virus could not be recovered from the stools of 3 of these poliomyelitis virus-infected patients.

Four patients with an illness diagnosed as "summer grippe" were tested. Two harbored poliomyelitis virus of low virulence (4) as well as C virus, and two harbored poliomyelitis virus without any evidence of infection with C virus, either by virus isolation or by serological tests.

The patients from whom C virus was isolated developed complement-fixing antibodies 4 weeks after onset. Neutralizing antibodies appeared within the first 5 days of disease (before complement-fixing antibodies) and increased in

titer during the next 3 to 4 weeks. The patients from whom C virus could not be recovered failed to develop either neutralizing or complement-fixing antibodies. Other patients in the area were infected with C virus as indicated by their serological response to the agent.

Addendum.—Since this paper was submitted, we have tested a number of fecal samples collected from poliomyelitis patients in Pennsylvania during the 1949 epidemic. It is pertinent to the work reported here that both poliomyelitis virus (monkey paralytic strains) and C virus have been isolated from each of four patients. Three of the patients had a paralytic disease, and one a non-paralytic illness.

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