THE SURVIVAL OF POLIOMYELITIS AND COXSACKIE VIRUSES FOLLOWING THEIR INGESTION BY FLIES*

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Within the past decade, it has become established that during typical summer epidemics of poliomyelitis the causative virus can be isolated readily from several different species of flies (1–4). This has been true of urban as well as rural epidemics. However, it is not possible to assess as yet the full significance of fly contamination as far as the spread of human disease is concerned, a major question being whether flies act as true hosts of the virus. Earlier work in this laboratory on the ingestion of virus by the blowfly, *Phormia regina*, has shown that human poliomyelitis virus, as naturally present in the stools of patients, may be excreted for 2 to 3 weeks after its feeding (5). As the early experiments were qualitative in design, no measure was made of the exact amounts of virus taken up by the flies or of the amounts excreted shortly after the feeding and in the 2nd and 3rd week thereafter.

The present experiments were designed to be quantitative in nature and are concerned with the survival of virus in the fly and its possible multiplication in this insect. In view of the recent discoveries of the simultaneous infection of patients with poliomyelitis virus and Coxsackie, or C, viruses (6) and of the simultaneous occurrence of these agents in flies trapped during poliomyelitis epidemics (4, 7), experiments were also carried out in which C virus alone or in association with poliomyelitis virus was fed to flies.

Materials and Methods

The manner of raising and handling flies in the laboratory is fully described in the accompanying paper (8). Also included there are details of the procedure for performing quantitative experiments such as are discussed in the present paper.

The poliomyelitis and Coxsackie viruses were obtained from patients during epidemics of poliomyelitis: North Carolina, 1944; New York City, 1944; Philippine Islands, 1945; Delaware, 1948; North Carolina, 1948; and Texas, 1948. Unless indicated otherwise, they were used as concentrates prepared by ultracentrifugation from stools of paralytic patients (9). All such specimens were kept frozen, usually at −20°C., until used. In addition, some ex-

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periments were carried out with: (*a*) the Lansing and Y-SK mouse-passaged strains of poliomyelitis virus, having titers of $10^{-3.5}$ in mice; (*b*) the TO type of Theiler's spontaneous encephalomyelitis virus of mice as obtained from the intestinal contents of naturally infected stock mice having a titer of $10^{-1.5}$; and (*c*) the Texas-1 mouse-passaged strain of Coxsackie virus which originally had been isolated from flies and which had a titer of $10^{-7.5}$ in mice.

For the demonstration of poliomyelitis virus in flies fed human stools, *rhesus* and *cynomolgus* monkeys were used as test animals, usually with 2 monkeys for each dilution. For the Lansing, Y-SK, and TO viruses, 8 young adult mice were used per sample. For C viruses, 16 baby mice within 48 hours of birth (and usually within 24 hours) were inoculated. Criteria for positive tests were similar to those used previously. All suspensions of flies and of their excreta were centrifuged at 18,000 R.P.M. for 20 minutes. Before inoculation 1,000 units of penicillin and 5 mg. of streptomycin contained in 0.05 ml. were added to each milliliter of supernatant fluid.

General Procedure of Experiments

The detailed procedures for feeding flies known amounts of virus suspension and for collecting their excreta quantitatively are given in the accompanying paper (8).

Flies in groups of 20 to 200 (with 60 being the usual number employed in an experiment) were fed singly with each fly limited to about 0.01 ml. of the virus. The number of flies used in each experiment and the total milliliters taken up by the group are listed in the tables giving the results of the experiments. The flies were observed and fed daily with a solution of half-molar or molar sucrose. Flies which died were frozen and other flies were taken for harvest as indicated. These were suspended in known volumes of M/100 Na_2HPO_4 and titrated to determine the ID_{50} of virus present. Each day the excreta were also collected in known volumes of the diluent and titrated as indicated in the tables. Qualitative tests for virus were first run and aliquots of the suspension kept frozen. If these were positive, then the suspension was titrated.

An aliquot of the virus suspension used for fly feeding in each experiment was placed in a sterile tube and attached to the rack on which the flies were kept. Samples were taken from this control tube periodically during the experiment to determine the natural persistence of the virus used at temperatures at which the flies were held.

RESULTS

Experiments with Poliomyelitis Virus from Human Feces

Effect of Dose of Virus Ingested by the Fly.—The results of Table I show that in order to detect virus in flies or their excreta beyond the 2nd day, the original virus-containing stool preparation had to have a titer of at least 1:100 per ml. when inoculated into monkeys by the intracerebral route. When a virus with a titer of 1:100 to 1:1000 was used in 14 experiments, virus was subsequently found in the flies between the 5th and 17th day in 8; and in their excreta between the 4th and 10th day in 9 of the experiments. In the control test tubes held at the same temperature as the flies, virus did not persist significantly longer than in the flies or their excreta and sometimes even less. This is the more striking when we consider that the volume of original virus suspen-

TABLE I

Persistence of Poliomyelitis Virus from Human Feces within Flies and in Their Excreta

Experiment No.	Species of fly	Temperature*	No.	Amount fed	Virus fed to flies*‡		Last day virus found in		
					Strain	Titer	Flies	Excreta	Control§
		°C.		ml.					
2	*Phormia*	9	100	0.57	NYC '44	1:100	**17**	**4**	**35**
25	*regina*	9→25	60	0.72	"	"	**10**	**10**	<13
46A		10→25	20	0.3	"	"	**14**	**20**	nd
26		25	60	0.65	"	"	**6**	**10**	nd
46B		27	200	2.0	"	"	**8**	**3**	nd
45		25	100	0.8	W-S '48	1:1000	<5	**7**	**4**
36		25	100	0.95	Del. '48	"	<8	<4	<4
29		9→25	30	0.3	Ph. Is. '45	1:10	<2	<2	nd
28		25	30	0.3	"	"	<4	<4	nd
41A		25	60	0.49	Texas '48	1:1000	<4	**8**	**8**
41B		25	60	0.42	"	1:100	<4	**2**	**8**
41C		25	60	0.30	"	1:10	<4	**2**	**8**
41D		25	60	0.42	"	1:1	<4	<2	<2
34	*Phaenicia*	25	130	1.2	N. C. '44	1:100	<5	**7**	<20
30	*sericata*	25	20	0.2	Ph. Is. '45	1:10	<7	<7	nd
42A		25	60	0.56	Texaxs '48	1:1000	**7**	**7**	**7**
42B		25	60	0.50	"	1:100	**7**	**7**	**7**
42C		25	60	0.44	"	1:10	<4	<2	**7**
42D		25	60	0.42	"	1:1	<4	<2	<2
40A	*Musca*	25	60	0.24	Texas '48	1:1000	**5**	<2	<2
40B	*domestica*	25	60	0.25	"	1:100	<2	**2**	**2**
40C		25	60	0.26	"	1:10	<2	<2	**2**

* 9 → 25° indicates flies were kept at a night temperature of 9° and day temperature of 25°.

‡ All strains were in form of virus concentrated by ultracentrifugation from stools obtained from acutely ill poliomyelitis patients. Titer refers to dilution capable of producing poliomyelitis when serial tenfold dilutions were tested in monkeys, usually 2 to a dilution. Monkeys were injected intracerebrally with 1.0 ml.

§ Numbers in bold-faced type (last 3 columns) indicate virus recovery was positive at that time. Other figures in these columns indicate that virus was not found on day indicated, and that tests of earlier samples were not done. Thus <5 indicates that flies tested on the 5th day did not contain a detectable amount of virus.

A control tube containing the virus suspension fed, was kept at same temperature as the infected flies. Samples were removed at intervals and frozen with the harvests of flies and their excreta.

nd, not done.

sion used in the control tests was usually somewhat larger than that used for feeding the flies.

When a virus with the low titer of 1:10 was used in 6 experiments, virus was never recovered from the flies and it was found only once in the excreta of these flies, on the 2nd day.

In 2 experiments with virus of such titer that it could be detected only in the dilution fed, virus was not found in the flies or their excreta.

Strain of Virus.—Of six strains used, none seemed to have special properties of survival or early death in the flies.

Species of Fly.—3 species of flies were used, *Phormia regina*, *Phaenicia sericata*, and *Musca domestica*. Virus was found on the 7th day and beyond with the blowflies (*Phormia* and *Phaenicia*), but not beyond the 5th day with the houseflies, in the few experiments with the latter species. As seen from Table I, blowflies consume larger amounts of food than houseflies.

TABLE II

Experiments with Viruses to Which Mice Are Susceptible

Species of fly	No. of experiments			
	Total	Lansing poliomyelitis	Y-SK poliomyelitis	TO encephalomyelitis
Phaenicia sericata	7	3	1	3
Phormia regina	4	2	1	1
Sarcophaga bullata	2			2

Effect of Temperature.—18 of the experiments were carried out at room temperature averaging 25°. In 3 of the 4 experiments with flies at lower temperature, either 9° constantly or in 12 hour cycles of 9 and 25°, virus was present for longer periods of time (up to 20 days) than when held constantly at room temperature (up to 10 days). As expected the control virus at 9° remained viable longer (35 days) than at 25° (4 to 8 days).

Experiments with Lansing and Y-SK Murine-Adapted Strains of Poliomyelitis Virus, and with the TO Type of Theiler's Mouse Encephalomyelitis Virus

The poliomyelitis strains were employed as 10 per cent suspensions of spinal cord obtained from infected mice, and the TO encephalomyelitis virus as an ultracentrifuged preparation of intestinal contents of mice obtained from a spontaneously infected stock. The procedure of concentration was similar to that used for the human stools (9).

Thirteen experiments were carried out on 3 species of flies listed in Table II. Inasmuch as the results are similar with the different viruses and the different fly species, these are summarized in Table III. Virus was recovered only within 5 days of its ingestion. When virus was recovered, it was present in larger amounts in the 1st or 2nd day than later as evidenced by the greater percentage of test mice developing disease in the tests carried out with the early samples.

TABLE III

Survival Time of Murine Infectious Viruses Fed to Flies

Day	Flies		Excreta	
	No. of experiments	No. positive	No. of experiments	No. positive
1	8	4	9	5
2	4	1	5	3
3	6	2	7	2
4	5	2	2	0
5	3	1	6	1
6–7	5	0	6	0
8–9	5	0	5	0
10–28	12	0	10	0

TABLE IV

*Persistence of Coxsackie Virus within Flies and in Their Excreta**

Experiment No.	Species of fly	Temperature	No.	Amount fed	Strain	Titer	Last day virus found in		
							Flies	Excreta	Control
		°C.		ml.					
44	*Phormia*	25	90	0.66	Texas-1	$10^{-7.5}$	>12	>12	>12
45	*regina*	25	100	0.8	W-S '48	1:100	7	7	9
41A		25	60	0.49	Texas '48	"	<4	8	2
41B		25	60	0.42	"	1:10	<4	2	2
41C		25	60	0.30	"	1:1	<4	<2	<2
41D		25	60	0.42	"	<1	<4	<2	<2
42A	*Phaenicia*	25	60	0.56	Texas '48	1:100	14	2	2
42B	*sericata*	25	60	0.50	"	1:10	<4	2	2
42C		25	60	0.44	"	1:1	<4	<2	<2
42D		25	60	0.42	"	<1	<4	<2	<2
40A	*Musca*	25	60	0.24	Texas '48	1:100	2	2	5
40B	*domestica*	25	60	0.25	"	1:10	<2	<2	<2
40C		25	60	0.26	"	<1	<2	<2	<2

* Explanation as for Table I, except that titer now refers to dilution capable of producing myositis in newborn mice, usually 16 being used for each serial tenfold dilution. Mice were injected subcutaneously with 0.02 ml.

Experiments with Coxsackie Viruses

These experiments were carried out at 25°, for the most part with infectious human stools obtained from patients in 1948 in Winston-Salem, North Carolina, and in the Rio Grande Valley, Texas. These patients were diagnosed as having poliomyelitis, and both poliomyelitis virus and Coxsackie virus were recovered from their stools. In addition, one experiment was carried out with the Texas-1 strain of C virus after several passages in mice. The results are listed in Table IV.

With a high titer ($10^{-7.5}$) virus, the agent was detected in both flies and their excreta through the length of the experiment, 12 days. The control virus held in a test tube at the same temperature as the flies was also active after this period. Indeed, this virus has been found to persist at room temperature for periods beyond this time. When the titer of C virus was comparable to that used in the poliomyelitis experiments, namely, 1:100 or less, then the results were also comparable,—with the virus being recovered for about a week when the titer of virus fed was 1:100, for about 2 days when the titer was 1:10, and not at all when the titer was only 1:1 or less.

In 2 experiments in which C virus could be detected in the temperature control tube for only 2 days, virus was found in 1 experiment with *Phormia regina* in excreta collected on the 8th day and in the other with *Phaenicia sericata* in flies harvested on the 14th day. The failure of virus to last as long in *Musca domestica* as in the blowflies is probably due to the smaller amount of virus taken up by the housefly.

Excretion of Carmine

In the course of this work several experiments were carried out on the feeding and excretion of the dye carmine. A saturated solution of carmine in molar sucrose was fed to groups of *Phormia regina*, following which the flies were fed daily on molar sucrose alone as in the virus experiments. Daily observations were made either of the excreta spots or of suspensions of the excreta in water. Carmine excretion began in some cases within an hour after its ingestion, although some flies did not excrete for 24 hours after feeding. At any rate, most of the carmine was excreted within 2 to 3 days, and thereafter smaller amounts appeared. Although it was unusual to find carmine excreted after the 5th day, an occasional fly would excrete the dye, in trace amounts, as long as 8 to 13 days after its feeding.

Virus Balance Studies

It was the design of these experiments to determine the number of infectious units of virus which were fed and which could be recovered from flies. In this manner it was hoped to determine whether virus merely persisted in the fly unchanged, or whether actual multiplication of virus occurred in this insect.

The data collected for one such experiment are presented in detail in Table V. Soon after emerging, 60 female *Phormia regina* were mounted on paraffin blocks. They were fed a total of 0.72 ml. of NYC '44 strain of poliomyelitis virus having a titer of 1:100 per ml. of inoculum. These flies were held at 9 and 25° in approximately 12 hour cycles. The recovery of virus was calculated as follows:

$$\text{Recovery} = \text{volume of suspension} \times \text{its titer per milliliter} \times \frac{\text{Total No. of flies fed}}{\text{No. of flies harvested}} \times \frac{1}{\text{Volume of virus fed}} \times \frac{1}{\text{Titer of virus fed}}$$

It will be seen that the calculated virus recovered from flies harvested 1 to 6 days after feeding was 28 times that fed, and for the excreta in this period, 11 times. During the period 7 to 13 days after feeding, for the flies the recovery was 3 times that fed, and for the excreta, 0.4 times that fed. A total of 42 times the amount of virus fed was calculated to have been recovered from the flies

TABLE V

Poliomyelitis Virus Balance Study in Phormia regina

60 flies fed 0.72 ml. of NYC '44 poliomyelitis virus.

Material tested	Day of harvest	No.	Suspension	Dilution	Results virus tests		Titer per ml.	Recovery
					Cynomolgus	*Rhesus*		
			ml.					
Flies	1–6	34	11.3	1:1	+	++	100	$11.3 \times 100 \times \frac{60}{34} \times \frac{1}{0.72} \times \frac{1}{100} = 28x$
				1:10	+−	+−		
				1:100	−−	++		
				1:1000	−−	−−		
Flies	7–13	26	8.7	1:1	++	+−	10	$8.7 \times 10 \times \frac{60}{26} \times \frac{1}{0.72} \times \frac{1}{100} = 3x$
				1:10	+			
				1:100	−−	−−		
Excreta	1–6	327	8.2	1:1	+	++	100	$8.2 \times 100 \times \frac{60}{60} \times \frac{1}{0.72} \times \frac{1}{100} = 11x$
				1:10	++	++		
				1:100	++	+−		
				1:1000	−−	−−		
Excreta	7–13	42	1.2	1:1	+−		10	$1.2 \times 10 \times \frac{60}{26} \times \frac{1}{0.72} \times \frac{1}{100} = 0.4x$
				1:10	+−	−−		
				1:100	−−			
Control	0		1	1:1			100	
				1:10	+	+		
				1:100	+−	+−		
				1:1000	−−	−−		
"	13		3	1:1	−	−−	<1	

$42x$

and their excreta. This is in contrast to the fact that the virus kept at the same temperature as the flies in the experiment decreased in virulence to less than 1 per cent of its original titer.

12 balance studies for poliomyelitis virus were carried out at 25° with the following results (see Table VI): When 3 to 800 infectious doses or "units" of virus were fed in 10 experiments, virus was recovered in the flies and their excreta in 8 of them. In 2 experiments in which only 0.4 unit of virus was fed,

no virus was found. The calculated amount recovered was 10 to 130 per cent of that fed. These experiments were carried out with *Phormia regina, Phaenicia sericata,* and *Musca domestica,* and the results were similar for each species. In the *one* experiment carried out with *Phormia regina* at 9–25° cycles, 42 times the titratable amount of virus fed was recovered.

13 balance studies for C virus were carried out at 25°. As shown in Table VII, it was necessary to feed at least 200 units of virus before virus could be recovered. 7 experiments were performed with 200 to 300,000,000 units of virus

TABLE VI

Poliomyelitis Virus Balance Studies on Flies

Species of fly	Virus	Temperature	Units* virus fed	Virus recovery‡		
				Flies	Excreta	Total
		°C.				
Phormia regina	NYC '44	9°→25	72.0	31.0	11.4	42.4
	Texas '48	25	500.0	0	0.7	0.7
	"	"	40.0	0	0.1	0.1
	"	"	3.0	0	1.0	1.0
	"	"	0.4	0	0	0
	W-S '48	"	800.0	0	0.4	0.4
Phaenicia sericata	Texas '48	"	560.0	0.01	0.6	0.6
	"	"	50.0	0.08	0.6	0.7
	"	"	4.0	0	0	0
	"	"	0.4	0	0	0
Musca domestica	Texas '48	"	240.0	1.3	0	1.3
	"	"	25.0	0	0.1	0.1
	"	"	3.0	0	0	0

* 1 unit is 1 infective dose.

‡ 0.7 indicates that 70 per cent of the virus fed was recovered as measured by titration.

being fed, and the amount of virus recovered for the flies and their excreta was the same for all, 2 to 10 per cent. In 6 experiments in which 2 to 120 units were fed, virus could not be recovered.

Dual Infections of Flies with Poliomyelitis and Coxsackie Viruses

In view of the fact that certain of the preparations of human stools used in these experiments contained both poliomyelitis virus and C virus, an opportunity was afforded for comparing the fate of these viruses when simultaneously ingested by flies.

Table VIII lists the pertinent data, showing the number of infectious units of poliomyelitis virus and of C virus fed to the 3 species of flies together with

TABLE VII

Coxsackie Virus Balance Studies

Species of fly	Virus	Units fed	Virus recovery		
			Flies	Excreta	Total
Phormia regina	Texas '48	2,500	0	0.02	0.02
	"	200		0.1	0.1
	"	15	0	0	0
	"	2	0	0	0
	W-S '48	200,000	0.02	0.02	0.04
	Texas—1	300,000,000	0.003	0.015	0.02
Phaenicia sericata	Texas '48	2,500	0.04	0.02	0.06
	"	250	0	0.06	0.06
	"	20	0	0	0
	"	2	0	0	0
Musca domestica	Texas '48	1,200	0.03	0.03	0.06
	"	120	0	0	0
	"	12	0	0	0

TABLE VIII

Dual Infection of Flies at 25° with Both Poliomyelitis and Coxsackie Viruses

Species of fly	No. of flies	Strain	Virus units fed		Virus recovery		Duration of virus persistence*	
			Polio-myelitis	C virus	Polio-myelitis	C virus	Polio-myelitis	C virus
					per cent	*per cent*	*days*	*days*
Phormia regina	60	Texas '48	500.0	2,500.0	70	2	8	8
	60	"	40.0	200.0	10	10	2	2
	60	"	3.0	15.0	100	0	2	0
	60	"	0.4	2.0	0	0	0	0
Phormia regina	100	W-S '48	800.0	200,000.0	40	4	7	7
Phaenicia sericata	60	Texas '48	560.0	2,500.0	60	6	7	14
	60	"	50.0	250.0	70	6	7	2
	60	"	4.0	20.0	0	0	0	0
	60	"	0.4	2.0	0	0	0	0
Musca domestica	60	Texas '48	240.0	1,200.0	130	6	5	2
	60	"	25.0	120.0	10	0	2	0
	60	"	3.0	12.0	0	0	0	0

* Longest persistence of virus, whether in flies or their excreta, is recorded for each experiment.

the percentage recovery and persistence of each virus in the fly. A higher percentage of the poliomyelitis virus ingested was recovered than was the case for C virus, although the viruses each persisted for about the same duration. As mentioned earlier there was a correlation between the amount of virus fed and its persistence.

Persistence of Poliomyelitis Virus and C Virus in Dried Fly Excreta, and Dried Human Feces

These experiments were devised to determine how long after virus is excreted by flies it may remain infectious. For this, a sample of human stool (W-S 1948) was purified and concentrated in the ultracentrifuge to yield a preparation with a poliomyelitis virus titer of 1:1000 in monkeys (inoculum of 1 ml.) and a C virus titer of 1:30,000 in infant mice (inoculum of 0.02 ml.). The units of virus were, therefore, 1000 units of poliomyelitis virus and 1,500,000 units of C virus per ml.

The stability of these viruses to drying was first studied as follows:

Several hundred drops, each containing 0.01 ml., were distributed on wax paper and allowed to dry for about 1 hour at room temperature. Similar drops were made of the virus suspensions which had been diluted 20 fold with water. The dried spots were divided into two groups, one of which was stored at 25° and one at 4°. At the end of varying periods of time (2 hours to 3 weeks) 40 spots were moistened and the material resuspended in 3 ml. of buffer. This was frozen until tested at a later date. As each dried droplet of the undiluted specimen contained 10 units of poliomyelitis virus, and 15,000 units of C virus, 40 dried droplets when resuspended in 3 ml. should have contained 130 units of poliomyelitis virus and 200,000 units of C virus per ml., if no virus had been destroyed or lost by the procedure. By the same reasoning, the droplets made with the 20 times diluted suspension should have yielded a suspension of 7 units of poliomyelitis virus and 10,000 units of C virus per ml. The results obtained are shown in Table IX.

At 25°, poliomyelitis virus was present in the dried droplets through the 3rd day, and C virus through the 15th day. With the diluted suspensions, poliomyelitis virus was recovered only in the sample collected 2 hours after drying, while C virus was found through the 3rd day. The amounts of virus recovered were small for C virus, being less than 1 per cent. Titrations of poliomyelitis were not carried out.

At 4° poliomyelitis virus and C virus both persisted in the dried droplets of the undiluted samples through the 21st day. Based on titration figures, the recovery of C virus, 25 to 125 per cent, was close to theoretical when one considers the biological variables inherent in titrations of infectious material. With the diluted samples, poliomyelitis virus was recovered through the 6th day, and C virus through the 15th day again, with the recoveries of the latter being 15 to 50 per cent in this period.

To determine the influence of actual passage through the fly intestinal tract

on the stability of poliomyelitis virus and of C virus, the following experiment was performed.

200 *Phormia regina* females were fed 2.0 ml. of W-S 1948, virus specimen used in the above experiment; this contained 1000 units of poliomyelitis virus and 1,500,000 units of C

TABLE IX

*Persistence in Dried Droplets of Poliomyelitis Virus and Coxsackie Virus from Human Stools**

Preparation	Temperature	Time	Poliomyelitis virus activity		C virus	
			Cynomolgus	*Rhesus*	Units per ml.‡	Recovery§
	°C.					*per cent*
Undiluted	25	2 hrs.	+	+	nd	
	"	3 days	−	+	500	0.25
	"	6 "	−	−	100	0.05
	"	9 "			25	0.01
	"	15 "			10	0.01
20 × diluted	25	2 hrs.	+	−	nd	
		3 days	−	−	50	0.5
		6 "			0	0
Undiluted	4	3 days	+	+	50,000	25.0
		6 "	+	nd	250,000	125.0
		9 "	+	+	nd	
		15 "	+	+	150,000	75.0
		21 "	+	+	50,000	25.0
20 × diluted	4	3 days	+	+	5,000	50.0
		6 "	−	+	5,000	50.0
		9 "	−	−	2,500	25.0
		15 "			1,500	15.0
		21 "			0	0

* 40 dried droplets were suspended in 3 ml. of buffer for each test.

‡ Units/50 = titer of virus using an inoculum of 0.02 ml. per mouse.

§ Units per ml. found/original activity of 200,000 units per ml. for undiluted and 10,000 units per ml. for 20 times diluted virus.

virus per ml. The flies were kept at 25° in groups of 20, each group having received about 0.2 ml. of virus containing 200 units of poliomyelitis virus and 300,000 units of C virus. The excreta were collected from groups of 20 flies for virus tests.

From one such group of 20 flies 1 day after ingestion of virus, thirty-three fecal spots were obtained and allowed to dry at room temperature. They were held at 25° for another day, after which the dry excreta were resuspended in 3 ml. of buffer and tested for both viruses. *Both tests were positive.* Poliomyelitis virus was not titrated. However, a total of 4500 units of C virus were recovered, the percentage recovery being 4500/300,000 × 100 or

about 1 per cent of that fed. This is in keeping with the results obtained in previous experiments in Table IX.

Two similar groups of excreta containing 34 and 24 dried spots were obtained from 2 other groups 1 day after virus feeding. These were held at room temperature for an additional 3 and 6 days, respectively, before they were resuspended in buffer for testing. Neither poliomyelitis virus nor C virus was recovered.

Other groups of excreta obtained 1 day after feeding virus and allowed to dry, were held at 4°. In these instances poliomyelitis virus and C virus were present in *dried* excreta which were resuspended *3 days after being placed in the ice box.*

Similar experiments were carried out on excreta deposited between the 24th and 48th hour after feeding on virus. When such dried excreta were allowed to stand 1 day at room temperature or 3 days at 4°, poliomyelitis virus was no longer detectable. However, C virus was found to survive in small amounts (about 0.01 per cent of that ingested) under these conditions, and even in a dried sample held for 5 days at 4°.

When the results with poliomyelitis virus and with C virus present in dried human stools are compared with those obtained when these viruses were present in dried fly excreta, it can be seen that the fly does not seem to confer any stabilizing influence on the virus. However, it is worthy of note that poliomyelitis virus, as present in human stools, may survive drying and storage at room temperature for at least 3 days and at 4° with little loss in titer. When the same stool sample was fed to flies and the excreta examined after drying, then poliomyelitis virus persisted for 2 days at room temperature and for 3 additional days when held at 4°, and C virus persisted for 2 days at room temperature and for 5 days at 4°.

Serial Passage of Poliomyelitis Virus and Coxsackie Virus through Flies

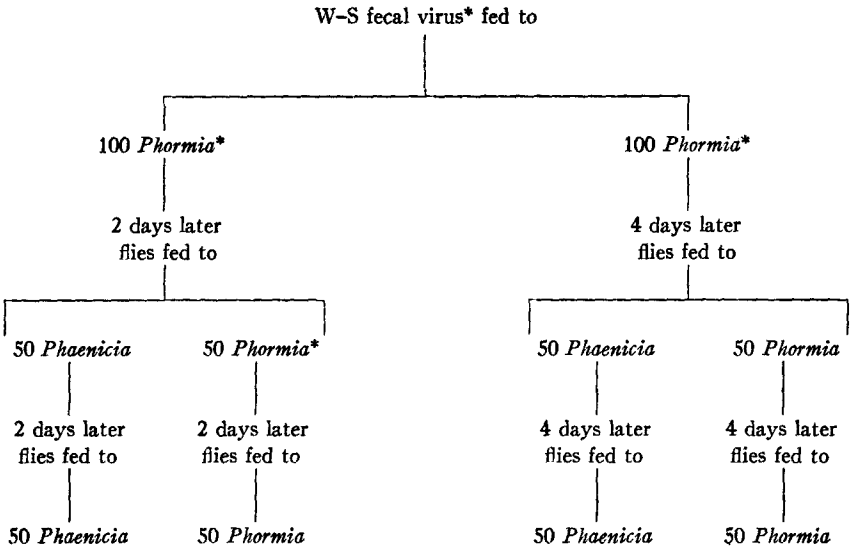
A further attempt to determine whether flies are true hosts of these viruses or merely serve as mechanical carriers was carried out by following the fate of these agents in fly to fly transfers, as outlined in Fig. 1.

100 *Phormia* females were fed 0.9 ml. of W-S fecal virus containing both poliomyelitis virus and C virus. They were then held at 25° for 2 days following which they were ground with 10 ml. of buffer and spun lightly for a few minutes to remove debris. 0.22 ml. of the fly suspension was fed to 50 *Phaenicia* females and 0.45 ml. to 50 *Phormia* females to serve as a second passage. The excreta of the latter *Phormia* collected 24 hours later contained both poliomyelitis virus and C virus. After another 24 hour period virus could no longer be detected in either the excreta or the flies that had been harvested at the end of the 2nd day. None of the *Phaenicia* excreta, nor the flies themselves, yielded either virus under similar circumstances.

The second passage *Phormia*, whose 1st day excreta yielded virus, were

ground in 5 ml. of buffer and fed to 50 new *Phormia* females (3rd passage). No virus was recovered in the excreta of the first 2 days, nor in the flies which were harvested on the 2nd day.

In a subsequent experiment 100 *Phormia* were fed 0.9 ml. of the W-S virus specimen. They were held for 4 days following which they were ground and passed to new *Phormia* and *Phaenicia*. No virus was recovered from the latter flies or their excreta.



* Indicates samples which were found to contain both poliomyelitis virus and Coxsackie virus. Other samples were negative.

FIG. 1

Poliomyelitis Virus Fed to Maggots

Bang and Glaser (10) attempted without success to recover the GDVII strain of Theiler's mouse encephalomyelitis virus and the Lansing strain of poliomyelitis virus from adult flies (*Musca domestica, Lucilia lepida,* and *Muscina stabulans*) which had developed from larvae fed these viruses with their food. Noguchi and Kudo (11) had earlier made a few attempts with unsuccessful outcome to recover poliomyelitis virus from houseflies which had developed from maggots fed on infectious monkey CNS. These findings were similar to those of Graham-Smith (12) who observed that it was difficult for many bacteria to survive the changes accompanying the metamorphosis of the fly. Our attempts in this direction with poliomyelitis virus are summarized below.

One group of 100 early third stage larvae of *Phaenicia sericata* was fed 5 gm. of virus-containing, solid human spinal cord obtained from a fulminating fatal case of poliomyelitis and a second group was fed on 5 gm. of mouse CNS obtained from Lansing-infected animals. The CNS tissues were consumed readily and no trace was evident in 24 hours. Larvae were harvested and frozen as indicated in Table X. Before testing they were washed with several changes of distilled water, then ground in 5 ml. of buffer, centrifuged at 18,000 R.P.M. for 20 minutes, and penicillin-streptomycin solution added.

In confirmation of earlier workers, we were unable to recover virus from the newly emerged flies. In fact the only recoveries of virus made were from the larvae 1 and 3 days after the feeding of Lansing virus, and in both instances only minimal amounts of virus were recovered.

TABLE X

Fate of Poliomyelitis Virus in Larvae

Day	Human cord from case of fatal poliomyelitis		Mouse cord (Lansing)	
	No. of insects	Results in monkeys	No. of insects	Results in mice
1	10 maggots		10 maggots	1/7*
3	10 prepupae	0/2‡	5 prepupae	1/10
6	10 puparia	0/2	10 puparia	0/9
7	10 "	0/2	5 "	0/10
9	10 "	nd	10 "	0/10
11	8 "	nd	10 "	0/8
11	31 flies	0/2	25 flies	0/8

* 1/7; of mice inoculated, 1 developed paralytic poliomyelitis.

‡ 0/2; of 2 monkeys inoculated neither developed poliomyelitis.

Can Flies "Reactivate" Virus Which Has Been "Inactivated" by Sludge?

Although poliomyelitis virus may be detected with some regularity from sewage during epidemic times, our attempts to isolate virus from sludge beds have consistently met with failure. The present experiments were set up to determine if the sojourn of "sludge-inactivated virus" through the fly would unmask such virus.

During the 1949 epidemic of poliomyelitis in Manchester, Connecticut, liquid, semidry, and dry sludge were collected from sludge beds at the sewage treatment plant. C virus was isolated from the liquid, semidry, and dry sludge. On the basis of past experience, it could be assumed that poliomyelitis virus was probably entering the treatment plant. Yet, in conformity with previous work, attempts to isolate poliomyelitis virus from the sludge collected during this epidemic met with failure. An attempt was made to determine whether flies may "reactivate" virus which was not detectable in sludge by conventional tests.

Semidry sludge was collected on August 8, 1949, from the sewage plant of Manchester, Connecticut. About 100 gm. were exposed to 200 newly emerged *Phormia regina* for a period of 2 days, following which the sludge was removed. Flies in groups of 20 were collected at the end of the 3rd, 5th, 7th, 9th, 10th, 12th, 14th, 16th, and 19th days. These samples were tested in monkeys for poliomyelitis virus and in mice for C virus; all were found negative.

Similar experiments with liquid and dry sludge were also negative.

Similar experiments with *Musca domestica* which were fed liquid and semidry sludge were also negative.

DISCUSSION

These experiments were designed to study the fate of poliomyelitis virus and of Coxsackie virus in laboratory-bred flies, chiefly in the blowflies, *Phormia regina* and *Phaenicia sericata*. Obviously, certain limitations exist in these attempts at a quantitative approach. When these experiments were carried out, titrations of poliomyelitis virus as it occurs in human stools required the use of monkeys limiting the number of animals in each titration series, and consequently the accuracy of the end-point determination. Other factors such as the sojourn of virus in the fly, the collection of the often times dried excreta, and the manipulations of the material in preparing the excreta in the fly bodies for inoculation, all tend to decrease the amounts of virus recovered. Thus, calculated recoveries of virus of 40 per cent and over in several experiments suggest that poliomyelitis virus passed through the flies unchanged with no apparent destruction. On the other hand, C virus usually underwent a significant loss in titer in these experiments, with only 2 to 10 per cent of the ingested virus being recovered. This is also evident in the experiments in which less than 200 "units" (infectious doses) of C virus were fed. In such cases, the virus could not be recovered from the flies or their excreta. With poliomyelitis virus, however, virus could be recovered when only 25 "units" (infectious doses) were fed and in one instance when only 3 units were used. The fact that virus could not be detected in flies fed subinfective doses offers further evidence for the passive transfer of virus through the fly with no multiplication when flies were kept at room temperature.

In reviewing these data, one experiment (see Table VI) stands out in pointing towards an apparent increase in virus in the fly. It is worth noting that this is the only balance experiment carried out in which flies were placed in the cold room each night. Whether this cycle was a determining factor remains to be determined. In view of the fact that this was the only experiment in this direction, it cannot be offered as crucial evidence. In view of the failure to demonstrate multiplication in the other experiments, one must also consider the possibility here of a virus inhibitor in stools having been destroyed in the alimentary tract of the fly.

Even though the fly has not proved to be a true host of either poliomyelitis virus or C virus, the present data emphasize anew the persistence of excretion of these agents for many days after the exposure of the fly. During such a period flies may travel distances up to several miles.

The persistence of human poliomyelitis virus and of C virus from stools in *dried* droplets is noteworthy. Poliomyelitis virus persisted in the dried form for 3 days at room temperature and for 21 days at ice box temperature. C virus fell off in titer but persisted for 21 days at room temperatures, and actually retained its titer when held at ice box temperature for this period. No attempt was made to determine the residual moisture content of the dried droplets (which before evaporation had a volume of only 0.01 ml. each). Certainly they looked dry. Dried excreta from flies fed the two viruses also retained infectivity when the dried specimens were held at room temperature for 1 to 2 days after excretion, and for a few days longer at ice box temperature. This observation means that consideration must be given to the possibility of food being contaminated by fly excreta in one location and then moved during a period of 1 or 2 days to another, where it may become a source of contamination.

SUMMARY

Poliomyelitis virus and Coxsackie (or C) virus were quantitatively fed to blowflies, *Phormia regina* and *Phaenicia sericata*, and to houseflies, *Musca domestica*. Naturally infectious human stools were the source of virus.

Poliomyelitis virus can be almost quantitatively recovered from flies and from their excreta collected over a period of several days following the feeding. C virus can also be recovered but in lesser yields. No conclusive evidence for virus multiplication in these laboratory-bred insects was obtained.

Poliomyelitis virus from human sources could be detected in flies between the 5th and 17th day and in the excreta between the 4th and 10th day. Murine-adapted strains of poliomyelitis virus and murine encephalomyelitis virus could not be detected beyond the 5th day, even though comparable amounts of virus were fed. The persistence of C virus excretion (2 to 12 days) varied directly with the amount of virus fed.

Poliomyelitis virus, as present in human stools, survived drying and storage at room temperature for at least 3 days and at 4° for 3 weeks. C virus from human stools under the same circumstances was detected for 15 days at room temperature (with marked drop in titer after the 3rd day) and for 21 days at 4° with little loss in titer. When stool samples were fed to flies and the dried excreta of the insects examined, it was found that (*a*) poliomyelitis virus persisted for at least 1 to 2 days at room temperature and for 3 to 4 days at 4°, and (*b*) C virus persisted for 1 day at room temperature and for 5 days at 4°.

Poliomyelitis virus could be carried through only two serial passages in adult flies. Flies emerging from maggots fed virus were free from the agent.

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