STUDIES OF THE FATE OF TYPE 1 POLIOVIRUSES IN FLIES*

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It has long been suspected that common species of non-biting flies might play a role in the natural history of poliomyelitis.

As early as 1911 Flexner and Clark determined that poliovirus fed to houseflies (*Musca domestica*) could be recovered from their carcasses 48 hours later (1). In 1941 three groups of workers, Paul *et al.*, Sabin and Ward, and Toomey, Takacs, and Tischer, reported that flies caught in nature during epidemics of poliomyelitis harbored polioviruses (2–4), and these findings have been confirmed on several occasions in recent years (5–9). Experiments by Bang and Glaser in 1943 and by Penner and Melnick in 1947 and 1952 showed that flies could remain infective over a period of 2 to 3 weeks after ingestion of the virus. However, no conclusive evidence of viral multiplication was obtained in investigations using intracerebral inoculation of mice and monkeys as a test for virus (10–12). The results obtained in the above studies supported the idea that flies act as mechanical carriers of polioviruses after contamination from infected human fecal material. The frequency of poliovirus isolations from wild flies and the fact that flies may carry polioviruses for 2 to 3 weeks after a single contact with infected material makes one wonder whether these insects might act as a natural reservoir for the viruses, and whether flies might not play a part in determining the seasonal incidence of the disease. These questions have never been answered although studies have been made of the correlation between the occurrence of poliomyelitis epidemics and the prevalence of certain flies (13–14). When live attenuated poliomyelitis vaccine is considered the question of an extrahuman host, capable of supporting multiplication of polioviruses becomes extremely important. The experiments described here were designed to see whether the more sensitive tissue culture methods now available would give any further information on the behavior of polioviruses in common flies and more particularly on any poliovirus multiplication in flies.

Material and Methods

Virus Strains.—The LSc attenuated type 1 poliovirus was chosen for these studies with the thought that any information obtained on the fate of the LSc strain in nature would be

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of significance since it is used as an oral vaccine. The LSc virus used was prepared by a single passage of oral vaccine in *rhesus* monkey kidney tissue culture.[1] For comparison, experiments were carried out with a virulent type 1 Mahoney strain also grown in *rhesus* monkey kidney culture.

Fly Species.—Flies of the species *Phormia regina*[2] were selected for most of these studies; houseflies, *Musca domestica* were used in one experiment. These flies have frequently been found to harbor polioviruses when caught in nature during epidemics and are fairly easy to

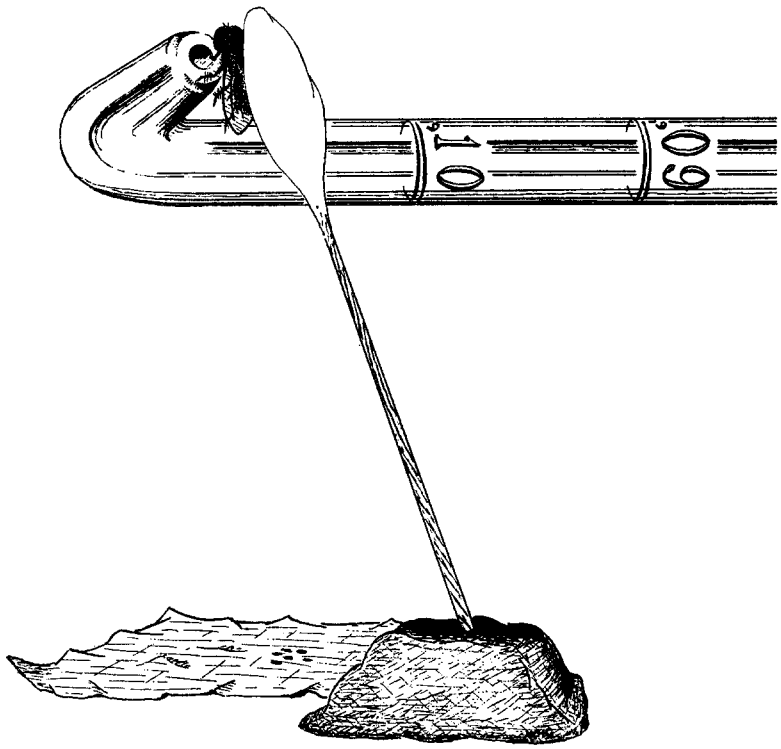

FIG. 1. Method of holding flies while quantitatively feeding virus suspension and collecting excreta on foil.

handle in the laboratory. Flies were raised on equal amounts of ground meat and rabbit pellets soaked in water. The fly colony was kept in an egg incubator at 27° C. with 70 per cent humidity. Samples of the uninfected flies were taken prior to each experiment and tested for the presence of poliovirus. They were found negative on all occasions.

Preparation of Flies Prior to Infection.—Immobilization of the flies was necessary in order to feed each fly the same amount of virus suspension and to collect the fecal material excreted by each fly in a given period of time. All flies used were therefore fixed by their wings and

[1] The author is indebted to Dr. A. B. Sabin for the LSc strain.

[2] The strain of *Phormia regina* was generously supplied by Dr. Charles C. Hassett of The Medical Laboratory, United States Army Chemical Center, Maryland.

thoraces on swab sticks, with hot candle wax. The other end of the stick was then stuck in a strip of modeling clay attached to the edge of a small tray which served as a moveable holder for the infected flies. A small piece of a household aluminum foil was placed on the tray below each fly so that fecal material could be collected at any desired time after infection (Fig. 1).

Virus Administration.—Tissue culture fluid containing a known amount of poliovirus was centrifuged at 3000 R.P.M. for 1 hour, then diluted in 10 per cent sucrose solution, and fed to the flies individually with a 0.2 ml. pipette. The tip of the pipette had been bent at a 90° angle. It proved impossible to make the flies feed on tissue culture fluid without sugar. The pipette was held in a horizontal position so that the fly sucked the virus suspension from the opening of the pipette, while the outside of the pipette remained dry. By this method it was possible to avoid external contamination of the flies, to measure accurately the amount of virus suspension fed to each fly, and to give several flies the same amount of virus for comparative studies. The pipettes used were graded to measure 0.01 ml. volumes which is the largest amount a *P. regina* fly can take regularly in one feeding (11). This volume was easily administered to well developed flies which had been fasted for 12 to 24 hours previously. If the flies were small, two of them were given 0.01 ml. in even portions and the two flies were handled as one unit from then on. A single virus administration was used in all the experiments. As a control the virus suspension fed was titrated in each experiment under the same conditions as the infected fly material. Some flies were also killed at the time of feeding and the initial virus intake was determined by titration of their carcasses. The difference in the estimated amount of virus fed as measured by these two methods was less than twofold.

Maintenance of Infected Active Flies.—For maintenance the infected flies were fed individually 2 to 3 times a day on a 5 per cent sucrose solution to which meat extract or powdered milk had been added. More frequent feedings were necessary if flies were to be kept at temperatures higher than 27° C. for any length of time. Since a high humidity is essential for the maintenance of flies, the trays with the infected flies were kept in tightly covered metal boxes, which excluded light, and in which humidity could be kept at 70 to 80 per cent during the course of an experiment. The high humidity also delayed the drying of the fecal material, but it was noted that the stool spots dried out quickly when the trays with the flies were taken out of the boxes. These artificial conditions are by no means at optimum for the activity of these insects.

Maintenance of Infected Hibernating Flies.—Hibernating flies were taken off the sticks and kept in glass jars at 5° — 10° C. An inch thick layer of plaster of Paris in the bottom of each container was moistened and kept the humidity high. The glass jars were covered with a piece of gauze, a ball of wet cotton, and aluminum foil. 15 to 20 flies were kept in each jar.

Preparation of Carcasses for Quantitative Studies.—Whole fly carcasses including heads, wings, and legs, were ground in a small mortar with a known amount of Hanks' balanced salt solution (BSS) to which 1000 units penicillin, 1000 μg. streptomycin, and 1000 units mycostatin per ml. had been added. One ml. BSS was used as a diluent for one or two flies. If a larger number of flies were ground together, 0.5 ml. diluent was added per fly. The flies were ground immediately after they were removed from the trays and the ground material frozen at −20° C. for storage until all samples to be compared had been harvested.

Collection of Fecal Material.—Fecal material was washed off the aluminum foil sheets by a capillary pipette with a known volume of BSS to which antibiotics had been added in the concentrations previously mentioned. If the stools from many flies were pooled, 0.1 ml. diluent was used per fly. Since the virus intake per fly was 0.01 ml., this stool suspension was considered 10^{-1} dilution of the excreted virus. It was considered more accurate to add diluent in proportion to the volume of virus fed than to go by the weight of the excreta which was found to vary considerably. This method gave a rough idea of the amount of virus excreted per fly. However, all detailed studies on fecal material were done on excreta from individual

flies, the findings being correlated with the amount of virus in carcasses of these same flies. If the fecal material was collected from flies individually, 1 ml. of Hanks solution was used per fly. The virus per milliliter therefore represented the virus excreted by one fly during the time periods observed.

Tissue Culture Methods.—Ground carcasses and stool suspensions to be tested in tissue cultures were thawed and centrifuged for 1 hour at 2500 R.P.M. The supernatant fluid was inoculated into *rhesus* monkey kidney tissue culture. When fly material was screened for the presence of virus, 1 to 1.5 ml. of supernatant fluid was inoculated into a 3 ounce tissue culture bottle, which thereafter was incubated at 37° C. for 2 hours, then washed twice in BSS to avoid toxic effects from this large inoculum; following this, 8 ml. of a maintenance media consisting of Earles' buffered salt solution and 2 per cent calf serum was added. All quantitative studies were done by the plaque assay method as described by Hsiung and Melnick (15). Fly materials to be compared were titrated on the same day in the same lot of tissue culture using the same overlay medium and temperature of incubation. Two bottles with a standard inoculum of 0.1 ml. per bottle were used for each dilution tested, starting with undiluted supernatant fluid from fly material in most of the experiments. Final plaque counts were made 4 days after inoculation, and the number of plaques was calculated for the whole volume of fly suspension or stool suspension tested. The number of plaque-forming units per fly was determined by dividing the number of plaque-forming units in the whole volume by the number of flies which had been ground in the sample.

$$\frac{\text{Plaque count per milliliter} \times \text{Volume in milliliters}}{\text{Number of flies}} = \text{Plaque-forming units (PFU) per fly}$$

The number of plaque-forming units per fly was used to compare the amount of virus from one time to another. Plaque counts of less than 10 plaques per bottle in the lowest dilution were not used for comparative studies if no samples were higher in the experiment.

RESULTS

Duration of Infectivity.—Fig. 2 shows the results of 11 experiments in which carcasses and fecal material from infected *Phormia* flies were tested for the presence of poliovirus on different days after administration of the attenuated type 1, LSc.

Four of the 11 experiments were carried out with low concentrations of virus, 50 PFU or less per fly, and seven experiments with a high concentration of virus, 10^5 PFU per fly. Eight of the 11 experiments were held at room temperature constantly but 3 were incubated at 36° C. for 2 hours a day. Of these 3, one experiment was carried out with only 10 PFU in the initial feeding. The flies in this experiment were found to harbor and excrete virus for a week, although the later titers were too low to be accurately quantitated. The number of squares in Fig. 2 represents the number of experiments.

After ingestion of 10 to 50 PFU per fly, all carcasses tested were positive for 4 days and 4 of 7 tests on the 5th to 7th day. After ingestion of 10^5 PFU per fly all fly carcasses that were tested within the first 11 days were positive. No virus was found in 5 attempts at isolation on the 12th to the 17th day. The flies excreted virus as long as their carcasses contained virus. The mortality rate among the infected flies in these experiments was high. There were surviving flies in the 2nd week in only 2 of the 11 experiments and in only one

in the 3rd week. Eight flies in this one experiment remained alive on the 16th and four on the 17th day. The diminishing number of surviving flies may have influenced the rate of virus isolations late in the study.

Hibernating flies, kept at 5°C., remained infected for 3 months with very little change in viral content during this time. Flies which had hibernated for

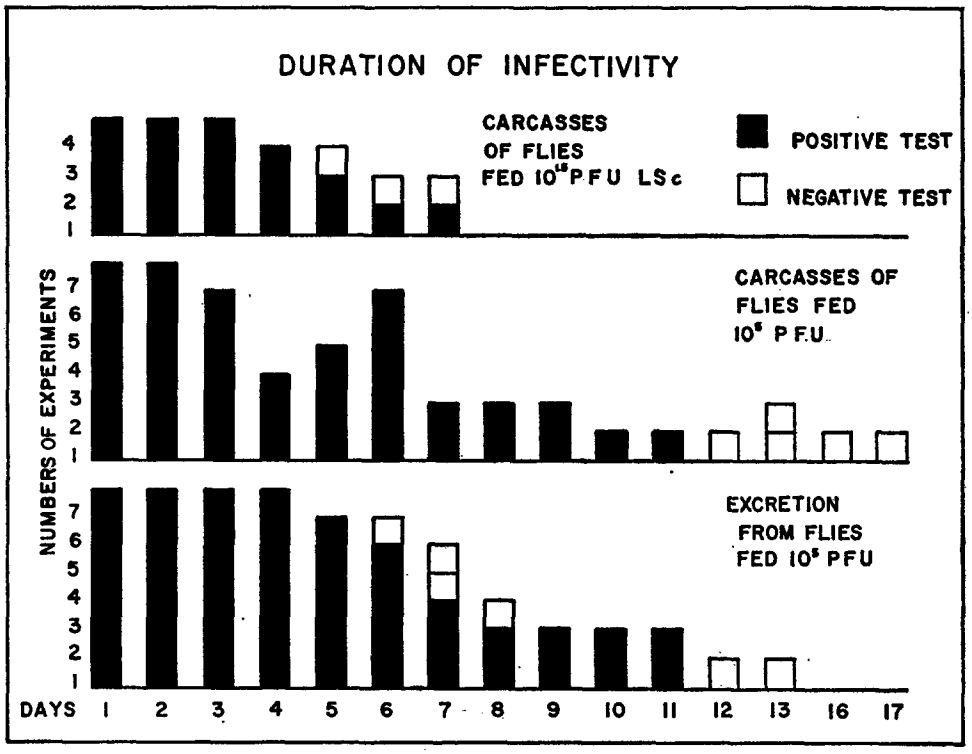

FIG. 2.

2 weeks remained infective for 1 more week when they were transferred to room temperature, and became active.

Common houseflies (*Musca domestica*) were available on one occasion during the course of this work. They were fed 148,000 PFU per fly, in 0.005 ml. and they were found to harbor and excrete virus for 10 days or as long as any flies survived. The houseflies were held at room temperature but cooled for 4 to 6 hours a day at 10°–12°C. in order to prolong their survival. A sample of 4 to 6 flies was taken for testing every day.

The samples all contained virus, but no late increase in titer was seen under these conditions.

Quantitative Studies.—The amount of virus in fly carcasses and fecal material

was determined at different times after a single known virus feeding. Table I gives the results of an experiment in which each of 60 *P. regina* flies was fed 0.01 ml. of a suspension containing 110,000 PFU LSc type 1 poliovirus.

The flies in this experiment were incubated at 36° C. for 2 hours immediately after they ingested the virus and this incubation was repeated daily. The rest of the time the flies were kept at room temperature (23°–24° C.). They were fed 5 per cent sucrose solution daily before incubation. The virus titer per fly on the 1st day was determined in a pool of 6 flies which died within the first 18 hours of the experiment. Similarly a pool of 8 flies which died between 24 and 48 hours after feeding was used to determine the virus in flies on the 2nd day. These had been fed once for maintenance after they ingested the virus and had been reincubated at 36° C. during the 19th and 20th hour of the experiment. The figure for the 3rd day is based on a pool of 8 flies which died 50 to 72 hours after feeding.

Table I shows that the virus titer in the fly carcasses on the 2nd day is approximately 12 times higher than that found in carcasses on the 1st day.

TABLE I

Virus in Phormia regina Carcasses and in Daily Fecal Collection

Time after feeding	Virus in carcasses PFU per fly	Virus in feces PFU per fly
days		
0	110,000	0
1	513	60,000
2	6,425	6,000
3	840	Contaminated
4	74	Not done
6	42	Not done

The virus in flies on the 1st day is also less than the virus in flies which were held for 3 days and fed sucrose twice in the interval.

Fecal material from all flies in this experiment was collected and pooled 18 hours after the virus feeding. This pool was found to contain 60,000 PFU per fly or half of the ingested virus. The high titer in this fecal specimen suggested that most of the virus ingested by the flies had been excreted within the first few hours of the experiment. This may explain the low virus content of the flies that died within the first 18 hours. If the virus recovered from the carcasses on the 1st day gives the correct picture of the virus present in all flies 18 hours after feeding, then the total amount of virus found on the 2nd day both in carcasses and fecal material would indicate that a 24-fold increase in titer occurred from 18 to 48 hours after feeding, a part of this virus being excreted by the flies.

The experiment was repeated with the results shown in Fig. 3. In order to avoid the errors which might be caused by postmortem changes in dead flies and by the selective influence of deaths, 6 female flies were sacrificed to determine each point on the curve.

The flies were incubated as in the previous experiment. The initial feeding to each fly was 0.01 ml. virus suspension containing 400,000 PFU as determined by titration of the material fed. Titration of 6 flies killed immediately after feeding gave a figure of 380,000 PFU per fly. The latter value is plotted in Fig. 3. Twenty two hours after feeding the titer had fallen to 2900 PFU per fly. On the 42nd hour the titer had risen to 7700 PFU per fly and it was 33,000 PFU per fly on the 52nd hour. The flies killed at the 52nd hour of the experiment had been been fed three times and incubated three times at 36° C. for 2 hours, whereas the flies killed at the 22nd hour had been fed once for maintenance and incubated twice at 36° C. Similar changes in titer were observed in 6 out of 6 experiments carried out in this fashion.

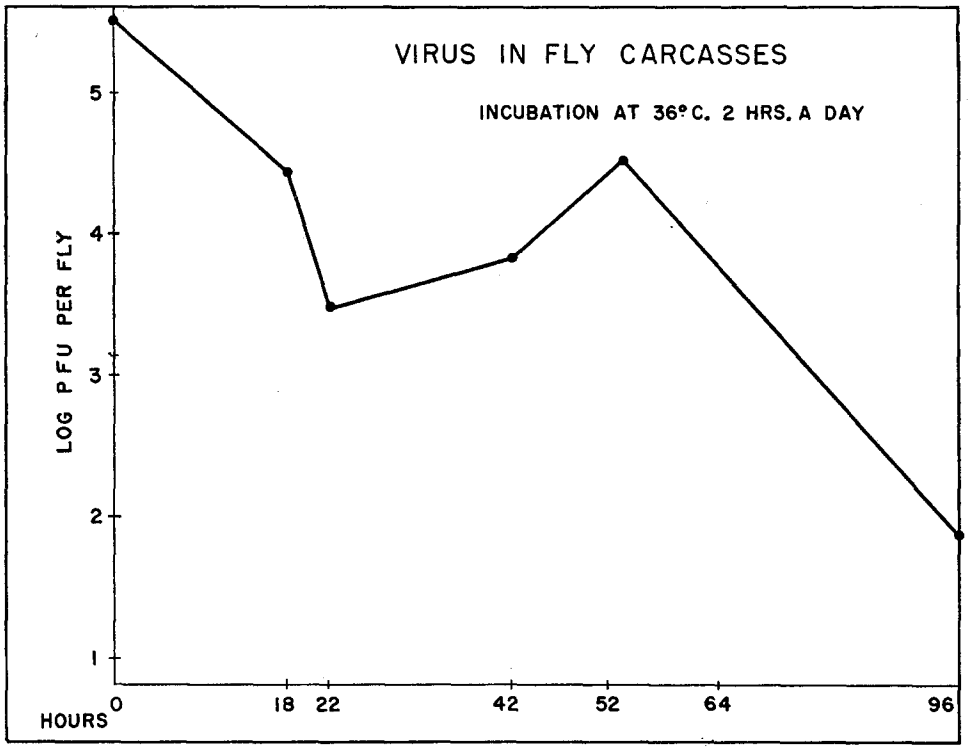

FIG. 3.

The fecal material in these experiments had been pooled each day. Although half of the initial feeding could be accounted for in the first fecal specimen taken 18 hours after feeding, there was no way of telling whether all the flies had excreted their virus at the same rate. Since the later peak titers did not exceed the initial intake, these peaks might have been due to a fraction of the fly population which did not excrete the initial feeding as early as most. Experiments were carried out to compare the amount of virus in fecal material with the virus in carcasses of individual flies, to see whether a high titer in a fly carcass would be accompanied by a low titer in the feces as one might expect if these peaks were only a retention of the initial feeding.

Twenty *P. regina* flies were used in these experiments, 10 males and 10 females. Each fly was fed 0.01 ml. virus suspension containing 670,000 PFU according to the original titration. Two flies killed at the time of feeding and titrated separately had titers of 450,000 PFU and 400,000 PFU respectively. One fly of each sex was sacrificed every 3rd hour. Each fly was ground in 1 ml. BSS and the fecal material was harvested in 1 ml. at the time the insect was killed. All samples were titrated in tissue culture on the same day and under the same condi-

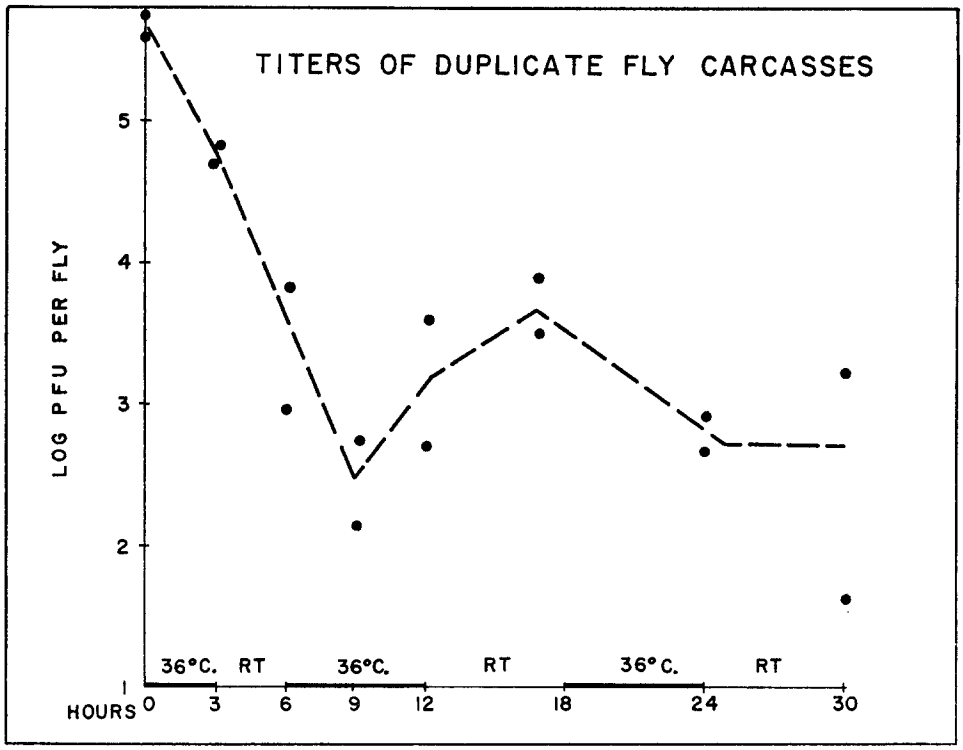

FIG. 4.

tions. The flies in this experiment were exposed to a temperature of 36° C. for longer periods than the flies in the earlier experiments. They had a total of 15 hours' incubation on the 1st day — 9 hours during the first 18 hours — as shown on the abscissae in Fig. 4 and 5, whereas the flies in the earlier experiments were incubated only 6 hours in 2 days. The flies were held longer in the incubator with the thought that if the differences previously observed were caused by an active process in the flies, it might be accelerated by more exposure to higher temperatures. Cyclic incubation was the only way to keep the flies alive more than a day or two while exposed to temperatures about 10° C. above their optimum (27° C.). When flies were kept constantly at 36° C. most of them died in 12 to 24 hours.

This experiment showed no difference in titer between males and females. The titers in carcasses of both are shown in Fig. 4. Titers in carcasses of the

male flies are compared with the titers in their fecal material in Fig. 5. Titers in the feces reached peak values 3 hours after feeding. There was a drop in titer in both carcasses and feces in the 6th and 9th hour samples. Then an increase, as compared with the 9th hour values, occurred in all specimens, both feces and carcasses, collected from 12 to 24 hours after feeding with a

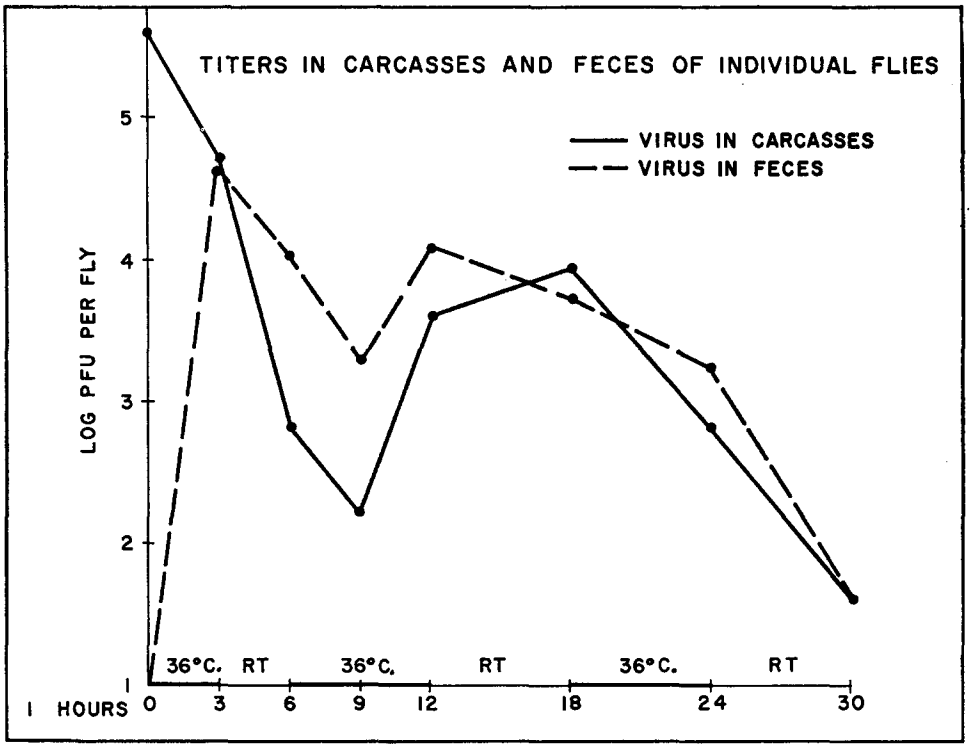

FIG. 5.

peak at the 18th hour. The flies killed 9 hours after feeding contained in their carcasses 150 and 550 PFU respectively. The flies killed at the 18th hour contained 3000 and 8200 PFU. A fourfold increase in titer of the fecal material above the 9th hour value occurred during the same period of time. Nevertheless the total plaque count at the peaks did not exceed the amount of virus found in flies killed at the time of ingestion.

The results of these experiments indicate that it is not possible to explain the high titer in fly carcasses by retention of the initial feeding. A high titer in a fly carcass is accompanied by a relatively high titer in the feces. The peak titers in the fecal material 3 hours after feeding indicate that most of the virus

ingested just passes through the fly, as if it were poured through a leaking container and the effective virus inoculum which remains within the fly thereafter is in fact much less than the amount of virus fed.

Variations of Titers in Individual Flies.—In order to determine the amount of variation from one fly to another in this residual virus, several flies were killed simultaneously at different times after the same feeding and titrated

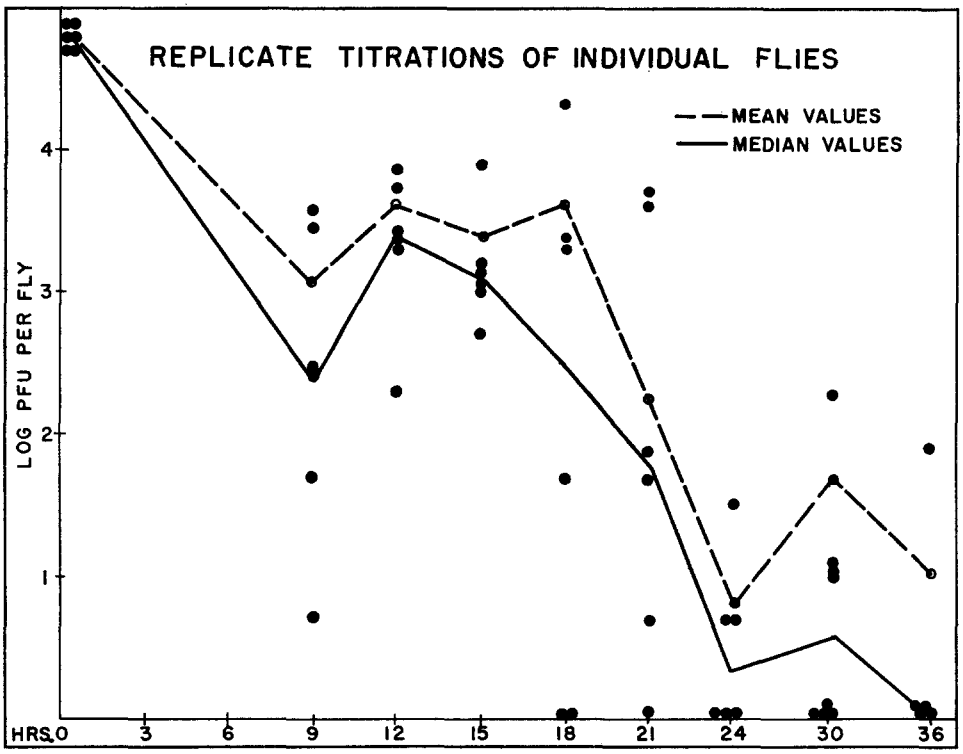

FIG. 6.

individually. The results are shown in Fig. 6. The amount of virus fed was 125,000 PFU per fly as determined by the titration of the suspension. Titers in 6 flies at 0 time varied from 52,000 to 80,000 PFU per fly. The later titers were more variable but both the median value and the mean of all the 6 titers determined at each time show changes similar to those in the earlier experiments.

Table II shows the results of four titrations of material from the same group of flies. 20 flies were ground in 10 ml. BSS and this material was kept in a test tube with a tray of flies that were incubated at 36°C. for 2 hours a day. Samples

were taken daily for titration. The titers showed very little variation over a period of 3 days, indicating that ground fly material does not inactivate poliovirus to an appreciable extent over a 3 day period and that the plaque method gives less than twofold differences in titer if the samples are tested under the same conditions.

Table III shows titers in 12 individual fecal specimens taken 4 and 10 hours after feeding. The fecal titers were subject to more variations than the titers in carcasses and more rapid inactivation of virus may have occurred in this

TABLE II

Virus in Ground Fly Material Held for 3 Days and Incubated at 36°C. for 2 Hours a Day

Hrs.	PFU per fly
0	34,000
24	29,500
48	30,000
72	29,000

TABLE III

Virus in Individual Fecal Specimens at Different Times

	4 hrs.	10 hrs.
Fly 1	6800	500
Fly 2	3300	400
Fly 3	5	340
Fly 4	0	150
Fly 5	5	10
Fly 6	0	5

material, probably due to heat and drying. Since external factors may have had variable influences on the different fecal specimens, it is not possible to determine the errors caused by the methods of harvesting the fecal material. On all occasions the fecal titers have followed patterns similar to the titers in the carcasses.

Effect of Temperature.—Experiments were carried out to investigate the influence of temperature on the virus titer. Table IV compares virus in flies kept at room temperature (23°–24°C.) with titers in flies exposed to cyclic incubation.

Each figure is an average titer of 2 flies. Virus excreted from the 12th hour of the experiment until the flies were killed was added to the virus in carcasses to obtain these figures. The incubated flies were held at 36° C. for 5 hours on the 1st day, 2 hours immediately after feeding, and again between 12 and 15 hours after ingestion of the virus.

From the 18th hour the incubated flies contained more virus than the flies held constantly at room temperature. A drop in titer of the incubated flies occurred during the first 12 hours. Then there was a relative increase in titer in the 18th and 24th hour specimens and possibly a second peak occurred 48 hours after feeding. The titer in the flies at room temperature fell gradually during the first 32 hours, and then a relative increase in titer was found in

TABLE IV

Virus in Phormia regina Carcasses Excreta Collected after 12 Hours

Time after feeding	Virus in flies kept constantly at 23°C. PFU per fly	Virus in flies incubated at 36°C. 5 hrs. a day. PFU per fly
hrs.		
0	35,000	35,000
8	30,000	950
12	750	260
18	560	1,680
24	330	1,525
32	75	460
40	700	670
48	385	1,230

TABLE V

Virus in Phormia regina Carcasses: PFU per Fly

Time after feeding	Experiment 30 Room temperature constantly	Experiment 20*a* Males. 2 hrs. per day at 36°C.	Experiment 20*b* Females. 2 hrs. per day at 36°C.
hrs.			
0	36,000	85,000	85,000
20	260	184	4,000
24	140	6,300	20,000
40	200	13	16,000

the flies killed 40 and 48 hours after feeding. The changes in titer observed in this experiment correlate in time with the changes found in the previous experiments, showing that the increase in titer occurs earlier if the flies are incubated longer. Peak titers occur at 18 hours in the incubated experiment and at 40 hours in the flies at room temperature. The early titers at room temperature show only slight variations They might therefore give an idea of the residual virus when excretion of the initial feeding has reached maximum and serve as a control to compare with the titers in the incubated flies. Fecal specimens taken 12 hours after feeding and titrated separately showed the same trend as the carcasses. 8 to 10 times more virus was excreted by the incubated flies from 0 to 12 hours.

Table V shows the results of 3 experiments, one experiment at room temperature constantly and two experiments at 36°C. for 2 hours a day. The experiments at 36°C. were carried out simultaneously but the experiment at room temperature on a different day. There was less than a threefold difference in the amount of virus fed but later in time the titers varied markedly and 24 hours after feeding, the dead incubated flies were found to have 45 to 140 times more virus than the flies held at room temperature. There may be some question whether these findings have any relationship to the death of the insect or whether they might reflect effect of temperature or other environmental factors on the different experiments. However, flies that died during

TABLE VI

Distribution of Mahoney Recovered from Flies Fed 3200 PFU

Time after feeding	Carcasses	Feces	Total account
hrs.			
0	3000	0	3000
4	1600	0	1600
8	2250	40	2290
12	3200	2275	5475
18	750	1700	2450
22	1150	275	1325
26	0	700	700
34	425	50	475

the course of an experiment were repeatedly found to contain as much or more virus than flies sacrificed for quantitative virus determinations. Mortality rate has been high all through the course of this work, especially in incubated flies.

Comparison of LSc and Mahoney.—All of 17 experiments with the LSc strain in *P. regina* flies exposed to cyclic incubation at 36°C. for 2 hours or more per day showed the relative increases in titers which have been described, but never did the total plaque count per fly exceed the initial feeding. These results were compared with the behavior of a virulent type 1 strain Mahoney in the same species of flies. Table VI shows the plaque count obtained in an experiment with the virulent strain. Two flies were killed and pooled at each time to determine the virus per fly. Little or no virus was found in the fecal material from flies killed 4 and 8 hours after feeding but titers in their carcasses had fallen relative to the initial feeding. The total virus per fly in the 12th hour carcass and fecal samples exceeded the amount of virus fed. The carcasses alone contained about the same amount of virus at 12 hours as at 0 time, but the flies killed at the 12th hour had excreted over 2000 PFU per fly. A twofold increase in titer is not in itself significant but the fact that carcasses

and excreta showed peak titers at the same time is noteworthy. Similar but not greater increases in the virus over the amount fed occurring from 12 to 24 hours after feeding have been observed in 3 similar experiments with Mahoney. As in the experiment shown in Table VI, a high titer in a fly carcass was regularly accompanied by a high fecal titer.

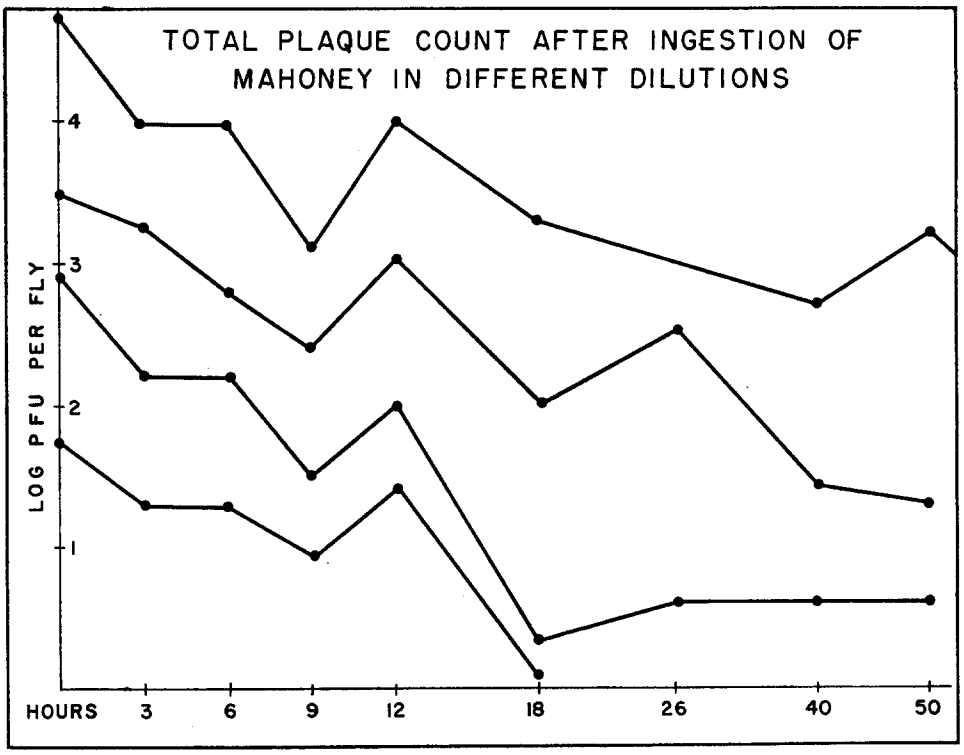

FIG. 7.

Effect of Varying the Initial Dose.—Virus from the 12th hour samples in the foregoing experiment was grown in *rhesus* monkey kidney tissue culture and different dilutions of this passage material were fed to *P. regina* flies, which were incubated for 4 hours immediately after feeding and again from 18 to 22 hours after feeding. The results are shown in Fig. 7. Each curve represents the total plaque count per fly. The changes in titer correlate in time but the amount of virus recovered is proportional to the initial feeding. High titers in carcasses were again accompanied by high fecal titers.

It proved impossible to make quantitative studies on passages straight from fly to fly by the method used. Too little virus was present in 0.01 ml. of the ground fly material to be accurately quantitated when the flies that ingested it were ground and titrated.

Titer in Hibernating Flies.—Titers remained unchanged over a period of 6 hours to 3 months in hibernating flies that were moved to 5°C. immediately after feeding. Flies kept active for a day after feeding and then moved to low temperatures (5°–10°C.) and kept in hibernation 1 to 4 weeks showed only slight variations in titer during the period of hibernation. Titers in individual

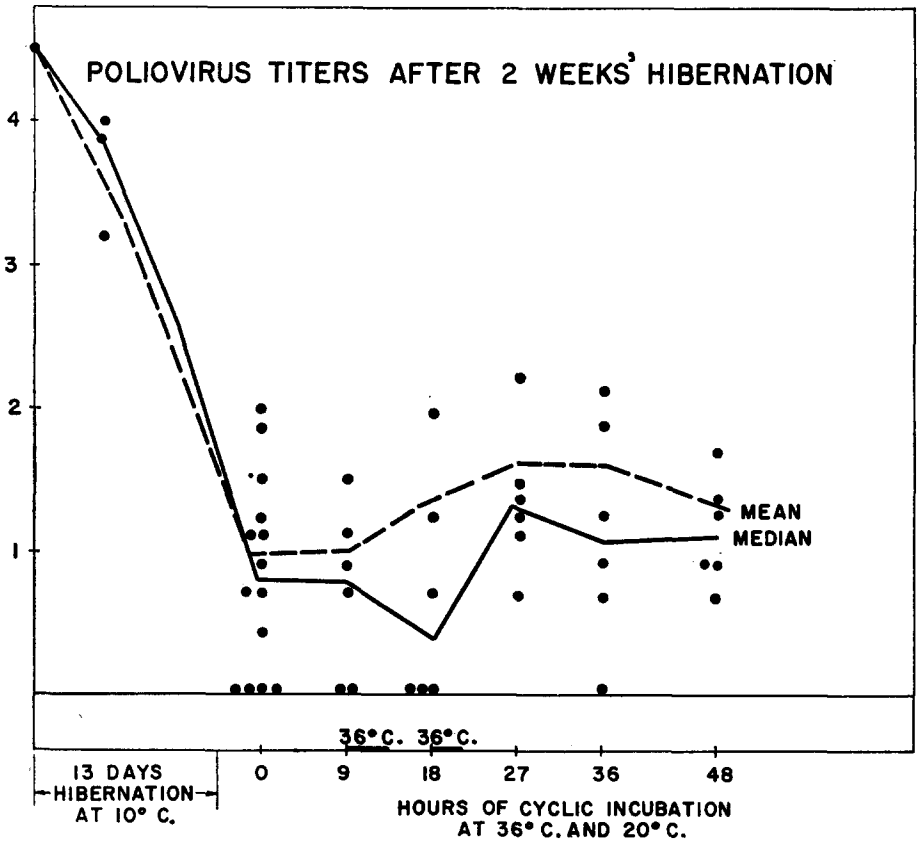

FIG. 8.

flies which, after 2 weeks hibernation, became active on exposure to cyclic incubation are shown in Fig. 8.

DISCUSSION

The results of the present studies on duration of infectivity showed that flies which came in contact with poliovirus harbored it for at least 4 days and most of them for longer periods, regardless of the initial intake. Many flies remained infective through the 2nd week and all flies excreted virus as long as their carcasses contained virus. Even if flies are considered inactive mechani-

cal carriers of these viruses, this relationship and the fact that flies thrive on infected fecal material may help to explain why polioviruses spread so easily in the same season that flies are most prevalent in nature.

Survival of poliovirus in hibernating flies for 3 months without significant drop in titer showed that overwintering of polioviruses in hibernating flies is possible.

As shown in Fig. 6 and Tables II and III, evaluation of the methods used in the quantitative studies indicated that fourfold or greater differences in titers of fly carcasses were not experimental variations caused by the methods but actual differences in the amount of virus harbored in the flies. The simplest explanation of the consistent changes in titer, observed if flies were kept under the same conditions, would be viral multiplication. Multiplication would explain why both carcasses and fecal material were at peak simultaneously and consistently under the same conditions, and why the time the peak occurred was related to the amount of time the flies were exposed to temperature at 36°C. Multiplication would also explain why incubated flies had more virus during the period from 12 to 18 hours than flies kept at room temperature and why feedings did not wash the virus from the fly's gut. Since high titers in fecal material paralleled high titers in a fly carcass the most likely site of multiplication would be the fly's gut or other tissue from which the virus would have an access to the gut. Fig. 7 shows that the virus recovered is proportional to the amount of virus in the initial feeding. The amount of virus ingested might be proportional to the number of cells infected and thus also proportional to the first cycle yield. If after each cycle most newly formed virus is excreted, just as most of the original virus was excreted, then each cycle might be smaller than the preceding one and the total new virus might not exceed the initial dose. The changes occurring during the first 12 hours in all the curves in Fig. 7 might represent one growth cycle of the virus and the second peak at the 26th hour a second cycle. The drop in titer from 6 to 9 hours might represent the eclipse phase of the virus.

The virus recovered did not exceed the virus fed in any of the 37 experiments done with the LSc strain under various conditions. Three experiments with Mahoney, on the other hand, showed approximately twofold increase above the initial feeding in the total count per fly. One can hardly expect to recover every virus particle excreted and heat and drying may inactivate some virus as time passes. The amount of virus excreted must actually have been greater than the amount recovered from the fecal specimens. The initial feeding was in some instances found to be 1000 times larger than the residual titer 9 hours later (Figs. 4 and 5). The residual virus is the only fraction of the initial feeding that can infect the fly and a thousandfold increase in titer would have to occur on such an occasion to allow recovery of the initial titer.

Since the amount of virus recovered is not significantly higher than the

amount fed, other possibilities than multiplication must be considered when one tries to explain the observed changes in titer. Rapid inactivation due to the content of the living fly's gut might cause the early drop titers, even though it was shown that virus titer in ground fly material remained unchanged over a period of 3 days. The ground material was heavily contaminated with growing bacteria and turned quite acid in these 3 days. The cause of the rapid inactivation would therefore have to be something present in live flies. The initial feeding might persist in a masked form for some time and become unmasked later. Inactivation due to heat is more operative at higher temperatures and one would expect to find less virus in the incubated flies if all the virus they contained were derived from the initial feeding.

The results of the quantitative studies show, that *P. regina* flies are not solely mechanical carriers of type 1 poliovirus, but are either capable of supporting multiplication of this virus or of retaining it in a masked form, unmasking it after a few hours.

SUMMARY

Studies on the fate of type 1 polioviruses in two common species of flies were carried out. The amount of virus in carcasses and excreta at different times was determined by the plaque assay method. Flies and their excreta remained infective for 11 days when kept at room temperature or when incubated at 36°C. for 2 hours a day. Flies remained infective for 3 months when kept in hibernation. A relative increase in titer was found to occur between 9 and 18 hours after feeding if the flies were incubated at 36°C. for 5 to 15 hours a day. The peak occurred later, at 40 to 52 hours after feeding if less incubation was used. Titers in excreta were parallel to titers in carcasses. A twofold increase in titer over the initial feeding was observed on 3 occasions with type 1 Mahoney but not with the LSc strain of virus.

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