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Experimental Infection of Flies with Human Poliomyelitis Virus.*

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In previous work from 3 laboratories[1-5] nonbiting flies collected during poliomyelitis epidemics have been found to harbour the virus of poliomyelitis regardless of whether they have been collected at rural, suburban, or urban areas from widely scattered parts of the country. Thus, they have been found positive for virus when collected in 1941 in rural Connecticut and Alabama,[1,2] in semi-rural New Brunswick, Canada,[2] and also in Atlanta, Ga.,[3] and Cleveland, O.[3,4] In 1943, positive tests were obtained from flies trapped in January at San Antonio, Texas[2] and in August at Chicago, Ill.[5] In 1944 virus was isolated from flies collected in rural North Carolina[5] and in 1945, from those trapped in Rockford, Ill.[6]

It would seem that the rôle played by flies in the transmission of this disease has not been determined, and the fact that flies may be "contaminated" with virus in nature, does not tell whether they may act as true hosts for the virus. It is important nevertheless to answer the question of the survival of the virus in the fly especially as it pertains to possible multiplication in this insect.

The first experiments in this connection were carried out by Howard, Clark, and Flexner[7,8] who were able to recover a strain of virus from *Musca domestica* up to 48 hours after being fed on infected monkey cords. This work was confirmed 3 decades later by Bang and Glaser,[9] and by Rendtorff and Francis;[10] both groups of investigators also used *Musca domestica,* which were infected with the Lansing murine-adapted strain. Attempts to recover the Lansing strain from 4 other species of flies were unsuccessful. Bang and Glaser, however, were able to show that the GD VII strain of spontaneous mouse encephalomyelitis virus persisted in house flies in one test for 12 days. The significance of this as far as the human disease is concerned is difficult to evaluate because of the failure to establish any relationship of this virus to human or simian- and murine-adapted strains of poliomyelitis virus.

In selecting the fly species to be used in our experiments, we were influenced by the fact that blow flies *(Phormia regina)* and green bottle flies *(Phaenicia sericata)* have appeared in all the positive batches of flies from epidemic areas tested in our laboratory. Feeding habits of these flies are such that they are attracted to feces as well as common foods.[11,12] Furthermore, in a series of tests for virus carried out on the 4 most prevalent fly species collected at an epidemic in Rockford, Ill., in 1945, only *Phormia regina* were positive, whereas *Phaenicia*

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† Dr. L. R. Penner assisted in these experiments while on temporary leave from the Department of Zoology and Entomology, University of Connecticut, Storrs, Conn.

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sericata, Musca domestica, Ophyra leucostoma gave negative tests.[6]

In addition, the investigations referred to above[7–10] have not, perhaps, been carried out with strains of virus or a form of virus with which flies may come in contact in nature. The strains employed have been either monkey or murine-adapted in the form of central nervous system tissue from infected animals, and in one instance Theiler's virulent strain of mouse encephalomyelitis virus.

Experimental. In the present experiments, an attempt has been made to approach, somewhat, conditions in nature. Thus, studies were designed to "infect" *Phormia regina* by allowing them to feed on virus as naturally present in stools of poliomyelitic patients. As control experiments, this species of fly as well as *Phaenicia sericata* and *Sarcophaga bullata* were infected with Y-SK and Lansing murine-adapted strains of poliomyelitis virus, and with the TO strain of Theiler's spontaneous encephalomyelitis virus of mice as it naturally occurs in the intestinal contents of laboratory mice.

All flies used in the experiments were laboratory bred. To facilitate handling, individual flies were mounted permanently by their thoraces and wings to low melting point paraffin blocks fastened on glass rods (Fig. 1) and kept in specially devised racks that could be transported easily for desirable temperature regulation. These methods are to be discussed in another paper.

Using a specially devised potometer it has been found that under the conditions of these experiments *Phaenicia sericata* average approximately 0.005 cc of fluid although some individuals may take up to 3 times as much (0.015 cc). *Phormia regina* average about twice as much food per meal (0.01 cc) as does *Phaenicia sericata* and some specimens take up to 0.04 cc. *Sarcophaga bullata* may take considerably more than this but it is so temperamental in feeding that accurate measurements are rather difficult to make.

Results. When murine-adapted strains of poliomyelitis virus or the intestinal TO strain of Theiler's virus were fed to flies (*Phaenicia sericata, Phormia regina,* and *Sarcophaga bullata*) virus could often be recovered within 72 hours from the bodies and excreta of the insects. It appeared that virus could be isolated from the abdomens more readily than from the heads and thoraces. Occasional tests from any part of the fly were positive from the 3rd through the 5th days, and none yielded virus thereafter. When a biologically inert substance, such as carmine, was fed, it was found to be excreted in gradually decreasing quantities for 6 days following feeding. Only traces remained in the excreta of the 6th day.

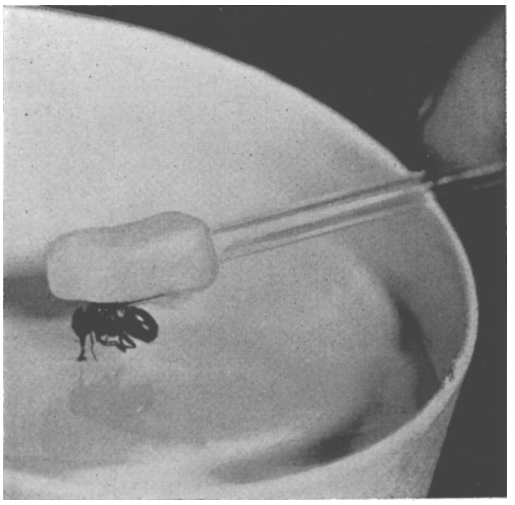

Fig. 1.
Phormia regina permanently mounted on a paraffin block. The fly is shown feeding on a solution of suerose.

These data are presented in Fig. 2 in which the results of 11 experiments on murine viruses are shown. In most of these experiments the flies were kept at night at 10°; every other day they were brought out and held at room temperature (about 25°) for 6 to 8 hours. During their stay at the higher temperature they were fed on molar sucrose solution to which at times autoclaved stool extracts were added to provide additional nutrients. A few experiments were carried out in which the flies were held at a constant temperature of 27°. Such flies were fed daily. Of these 11 experiments, *Phaenicia sericata* were used in 7 (3 with Lansing strain, 1 with Y-SK, and 3 with TO); *Phormia regina* were

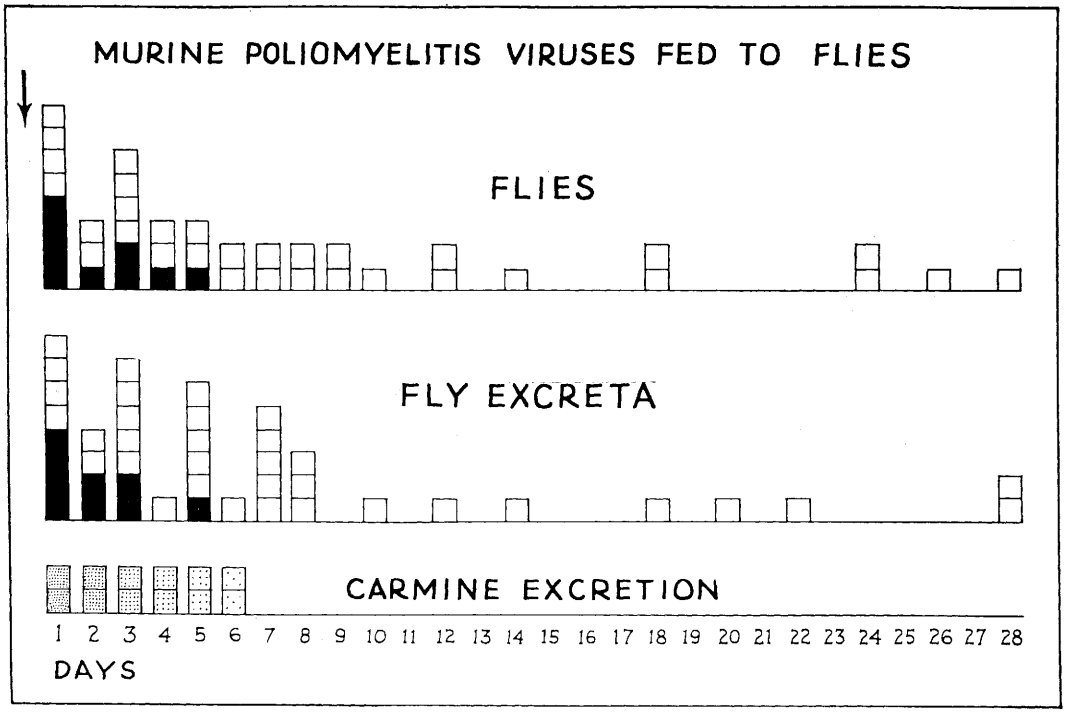

Fig. 2.

Combined results of 11 experiments in which murine poliomyelitis viruses (Lansing, Y-SK and TO) have been fed to *Phormia regina*, *Phaenicia sericacta*, and *Sarcophaga bullata*. White squares indicate negative tests for virus; black squares, positive tests. The relative amount of carmine in the excreta is indicated by the degree of stippling.

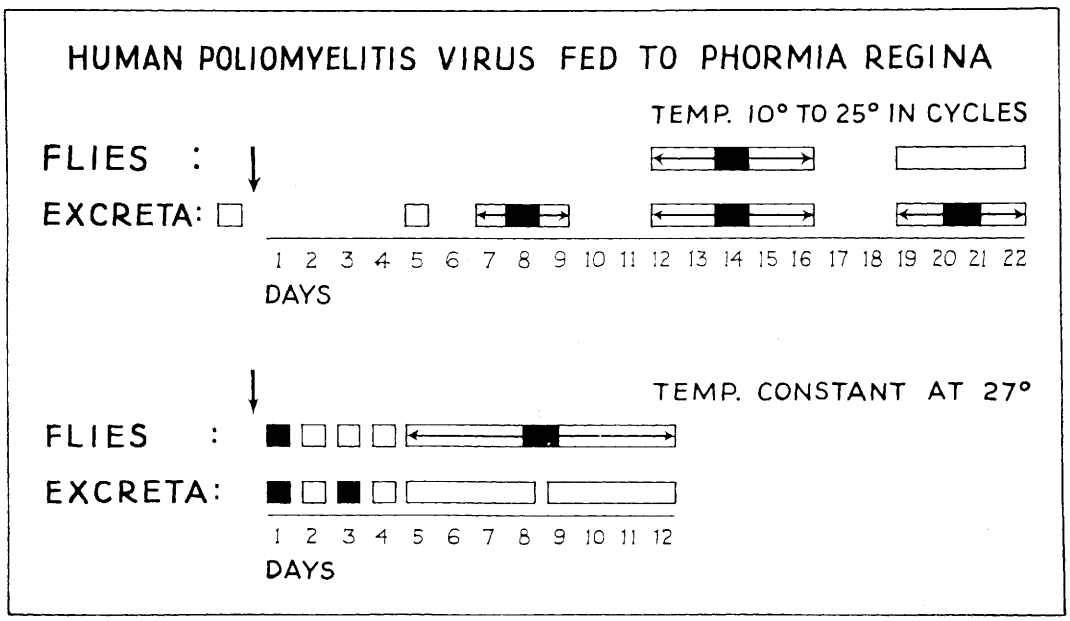

Fig. 3.

Poliomyelitis virus in flies and in their excreta after being fed on stools obtained from patients in the acute stage of the disease. White squares and rectangles indicate negative tests for virus; black areas indicate positive tests. When a pooled sample representing more than one day's collection was used and found positive, the arrows indicate the days covered in the pool.

used in 2 (1 with Y-SK and 1 with TO); and *Sarcophaga bullata* with 2 (both with TO). In 6 of these experiments the flies were fed on virus only once; in 3, they were fed virus for 3 consecutive days, and in 2, for 6 consecutive days. The last day of virus feeding was regarded as the "zero" day. No differences were detected using different species of flies or different strains of virus.

The results were markedly different with human poliomyelitis virus (NYC-44 strain) fed to *Phormia regina,* as shown in Fig. 3. Two experiments were carried out: In the first, 20 *Phormia* were fed once on a virus preparation from human stools from poliomyelitic patients. After the virus feeding they were treated as above with cyclic temperature changes from 10° to 25°. Due to mechanical difficulties the cooler temperature was not always constant, but it was not below 9° nor above 13° except at one period (18th to 22nd days after feeding) when it ranged between 13° and 20°.

Of the 20 flies, 1 died on the 12th and 1 on the 16th day. These 2 flies were pooled with 5 which were removed from the racks on the 14th day; this sample proved positive in a Siamese monkey *(Macaca irus valida);* tests in a rhesus *(M. mulatta)* and 2 Philippine cynomolgus monkeys *(M. cynomolgus)* were negative. Of the remaining 13 flies 1 died on the 19th day, 2 died on the 21st day, 1 died on the 22nd day, and 9 were sacrificed on the 22nd day. These were pooled, and yielded negative tests in 4 monkeys of similar species distribution as those used on the first test.

Excreta collected before the virus feeding and on the 5th day following the feeding were negative. However, samples obtained on the 7th-9th days, 12th-16th days, and 19th-22nd days, all gave positive tests for virus. Only 13 flies contributed to the excreta after the 14th day.

The second experiment was carried out with 200 *Phormia.* These were kept at a constant temperature of 27°. They were fed once on virus and then daily on the sterile nutrient solution. On the first day following the feeding, their excreta were collected and found positive for virus. The excreta collected on the 4th, 5th-8th, and 9th-12th days were negative.

When the flies themselves were tested, a positive test was obtained with a sample of 50 flies sacrificed within 24 hours after feeding on virus. Samples of 16 to 50 flies taken from the 2nd day through the 12th day were negative when tested in rhesus, green, or Siamese monkeys. A third of the sample collected on the 4th day and a third of the sample collected on the 5th-12th days were each tested in a cynomolgus monkey. The monkey receiving the sample from the latter period developed typical experimental poliomyelitis.

Discussion. These experiments illustrate a difference which obtains when human poliomyelitis virus rather than simian- or murine-adapted strains, is used in attempting to evaluate the fly as a potential host of the virus in nature. Whereas murine-adapted viruses or Theiler's TO strain behaved as inert material in the fly, being eliminated in decreasing quantities over a 5-day period, human intestinal virus was found in the fly at the end of 3 weeks.

The difference between the murine and the human strains cannot be explained on the basis of titer of infective material. Thus the Lansing strain preparation, which was fed as a 10% emulsion of infected CNS, could be diluted a further 500 times and still be found infective for mice in 0.03 cc amounts inoculated intracerebrally. In monkeys, furthermore, this strain[13] as well as the Y-SK[6] has a higher titer than in mice (about 100-fold) which may be related to the larger inoculum (1.0 cc) given to monkeys.

Too few test monkeys were used to place significance on the negative tests which were followed by positive ones. This occurred in the first experiment (Fig. 3) when a negative test on the 5th day excreta was followed by positive ones on the excreta of the next 2 weeks. Likewise in the second experiment (Fig. 3) the negative tests in flies from the 2nd through the 4th days were followed by

[13] Bodian, D., and Cumberland, M. C., *Am. J. Hygiene,* 1947, **45**, 226.

a positive one on a later sample.

The result, if any, of the cyclic temperature variation in prolonging the persistence of virus in the fly cannot be evaluated on the basis of the available data. The only thing that can be stated is that under the same conditions of alteration in temperature used with the human strain, murine-adapted strains could not be found beyond the 5th day.

Quantitative virus balance studies were not attempted in these experiments. Without knowledge of the exact amounts of virus taken up by the flies, of the amounts excreted in the first days after the feeding, and the amounts excreted in the 2nd and 3rd weeks following the feeding, it is not possible to answer the important question of whether virus multiplies in the fly. These results however unlike those with the murine-adapted strains, are compatible with the possibility of multiplication.

Summary. Human poliomyelitis virus, as naturally present in stools of poliomyelitic patients has been fed to blow flies, *Phormia regina.* After this feeding, virus was found in the flies for 2 weeks, and in their excreta for 3 weeks.

Results of this type were not encountered when murine-adapted strains of poliomyelitis virus and Theiler's TO strain of spontaneous encephalomyelitis of mice were used. These strains behaved like biologically inert material, such as carmine, in *Phormia regina, Phaenicia sericata* and *Sarcophaga bullata;* following their ingestion they were found in gradually decreasing quantities for a period of 5 days.

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Direct Isolation of Mumps Virus in Chick Embryos.*

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Mumps virus has been adapted to embryonated hen's eggs by the inoculation of relatively large doses of infected monkey parotid by Habel[1] and Levens and Enders[2]. Beveridge, Lind and Anderson[3] using penicillin and sulfonamides as bacteriostatic agents, were able to isolate 4 strains of mumps virus from 12 specimens by direct inoculation of human saliva into the yolk sac of 5 or 6 day embryos. These investigators also isolated one strain by the inoculation of the amniotic sac of 9 or 10 day old embryos. While virus was isolated from 5 of 13 specimens by either yolk sac or amniotic sac inoculation, bacterial contamination was a frequent source of difficulty. Several specimens could not be passed satisfactorily for this reason.

The present report deals with the successful isolation of mumps virus from 8 of 9 patients by the inoculation of saliva into the amniotic sac of 7 or 8 day old chick embryos.

Materials and Methods. Saliva was collected from patients ill with mumps during the acute, inflammatory stage of the disease. This material was emulsified with approximately equal parts of sterile infusion broth. Centrifugation at about 3000 r.p.m. in an angle centrifuge for 15 minutes served to deposit large particles, and presumably, a portion of the bacteria. Penicillin and streptomycin were added to the supernatant to a final concentration of 250 and 2500 units per milliliter, respectively, and the mixture of

* Supported in part by a grant from the Research Grants Division, National Institute of Health, United States Public Health Service.

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