# Survival of Type 1 and Type 3 Polio Vaccine Virus in Blowflies (*Phaenicia sericata*) at 40°C.* (30114)

KANU H. DAVÉ† AND ROBERT C. WALLIS (Introduced by F. L. Black)
*Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Conn.*

Since Flexner and Clark(2) observed that poliovirus could be recovered from carcasses of houseflies 48 hours after the flies had fed on infected monkey spinal cord, the possibility that these insects play a role in the natural history of poliomyelitis has interested investigators. Paul *et al*(8), Sabin and Ward (10), and Toomey *et al*(11) demonstrated that flies trapped during epidemics of poliomyelitis have been found to harbor poliovirus. Ward, Melnick, and Horstmann(12) found that food exposed to flies in nature during an active epidemic proved infective when fed to chimpanzees. Melnick and Penner(7) fed fecal material from poliomyelitis patients to blowflies (*Phormia regina*) and recovered poliovirus from the flies 2 weeks later. Virus was also recovered from the feces of blowflies similarly fed for a period of 3 weeks. Francis, Brown, and Penner(3) tested the ability of flies of various genera to acquire the virus by feeding on infected human feces. Their results indicate that practically any species of filth-feeding fly may acquire the virus. More recently, Gudnadottir(4) observed a possible limited multiplication of poliovirus in blowflies (*Phormia regina*). Since the introduction of live oral polio vaccine, viruses with genetic markers similar to vaccine strains have been recovered from flies trapped in areas of vaccine trials(9).

The question of genetic stability of oral poliovirus vaccine after repeated human intestinal passages has been raised. This question may also pose a problem if flies are accepted as a possible extrahuman source capable of disseminating poliovirus in a community. Experiments were designed to study the survival period, possible multiplication, and any change in genetic make-up of attenuated vaccine strains of poliovirus in flies exposed to an abnormal environment: *viz.*, warm temperatures which are known to favor growth of virulent strains but not growth of attenuated ones.

*Materials and methods. Viruses.* Sabin's attenuated vaccine strains of poliovirus types 1 and 3 were used. Virus stocks were prepared by making one passage in rhesus monkey kidney tissue cultures using the original seed lot (prepared by Merck, Sharp, & Dohme) as inoculum. The virus fluid thus prepared had a titre of $10^{7.5}$ plaque forming units (P.F.U) per millimeter.

*Flies.* Laboratory-bred flies of the species *Phaenicia sericata* were used; adults of either sex, about one to 2 weeks old, were selected.

*Feeding procedure.* Flies were transferred to half-pint food cartons from the stock cage and screened in with nylon mesh. In each box approximately 10 to 30 flies were starved for a period of 24 hours before virus feeding. A sugar cube 1½″ by 1″ was then placed on the nylon mesh, and approximately $10^{7.5}$ P.F.U. of virus were placed on the sugar with a capillary pipette. The flies were then allowed to feed for 30 minutes at room temperature, then the inoculum was removed and the fly boxes covered with cotton pads soaked in warm water. These pads were used to provide adequate humidity.

*Exposure at 40°C.* Infected flies which were maintained originally at room temperature were then transferred to an incubator at 40°C and kept at this temperature for a period of 5 to 7 hours every day. At the end of the daily exposure at 40°C, the fly boxes were transferred to a refrigerator (4°C) and held there. On the following morning they were brought out and kept at room temperature for approximately 20 minutes. Sick and dead flies were harvested and stored at −20°C. The surviving ones were fed with

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† W.H.O. Fellow in Virology and Epidemiology. Permanent address: Department of Virology, Haffkine Institute, Bombay, India.

sugar and water, and the cycle of incubation and refrigeration repeated. These cycles were continued until all the flies had died.

*Processing of fly carcasses.* Flies harvested from each box on a single day constituted one sample. Carcasses were ground in a sterile mortar, and a suspension was prepared using 0.5 ml of sterile Hanks' balanced salt solution (BSS) per fly. Suspensions were centrifuged at 2,500 rpm for 40 minutes at 4°C. The supernates were treated with penicillin and streptomycin in a concentration of 1,000 units and 1,000 μg per ml respectively. The suspensions thus prepared were stored at —20°C until tested.

*Virological studies.* All the experiments were carried out using primary rhesus monkey kidney monolayer tissue cultures. For virus isolation 0.5 ml of fly suspension was placed in a 3 oz bottle culture and allowed to adsorb for 1½ hours. At the end of this time 8 ml of maintenance medium consisting of 97.5% Earle's balanced salt solution, 2% calf serum, and 0.5% lactalbumin hydrolysate was added. For virus titration of fly suspensions, the agar overlay plaque method of Hsiung and Melnick(5) was used.

*Genetic marker studies.* Viral isolates from flies were tested for genetic markers by 2 methods: (1) the rct/40°C marker carried out according to the modification of Carp and Koprowski(1) using monkey kidney monolayer bottle cultures; and (2) the kinetic neutralization test for intratypic serodifferentiation described by McBride(6).

*Results. Persistence of virus in infected flies.* Virus was recovered from flies given type 1 virus for a period of 15 days after feeding and periodic exposure at 40°C (Table I). In the case of type 3 virus, it was not possible to demonstrate the survival endpoint beyond 13 days since all the flies were dead by that time. However, virus was recovered from samples collected on the 13th day (Table II) of the experiment. In both experiments, virtually every sample collected during the 13 to 15 day post-feeding period was found to yield virus. In the case of type 1, of the 51 samples of flies tested during the first 15 days, 42 were found to be positive, while with type 3 there was not a single sample which did not yield virus (*i.e.*, 36 positives out of 36 samples tested).

*Quantative studies.* Original fly suspensions were titrated in bottle cultures using the agar overlay method. The results are

TABLE I. Cyclic Exposure of *Phaenicia sericata* to Type 1 Poliovirus at 40°C for 5 to 7 Hours Daily for 22 Days.

| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cumulative hr exposure at 40°C | 5½ | 10 | 17½ | 24½ | 30½ | 37½ | 43 | 48½ | 53½ | 58½ | 65½ |
| Virus isolation | | + 8/8† | + 7/7 | + 9/9 | + 8/8 | + 1/1 | + 7/7 | 0 0/3 | + 1/1 | 0 0/5 | |
| PFU/ml | | $10^{3.88}$ | $10^{3.5}$ | $10^{3.2}$ | $10^{2.9}$ | $10^{3.0}$ | $10^{2.55}$ | | $10^{1.1}$ | | |
| $Rct_{40}$ test | | T– | T– | T– | T– | T– | T– | | T– | | |
| McBride test | | $V_R$ | $V_R$ | $V_R$ | $V_R$ | $V_R$ | $V_R$ | | $V_R$ | | |

| Day | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cumulative hr exposure at 40°C | 73 | 79½ | 85½ | 92 | 96 | 101 | 106½ | 114 | 121 | 127½ | 134½ |
| Virus isolation | + 3/3† | + 2/3 | + 3/3 | + 3/3 | 0 0/2 | 0 0/2 | 0 0/1 | 0 0/6 | 0 0/4 | 0 0/4 | 0 0/2 |
| PFU/ml | * | | * | | | | | | | | |
| $Rct_{40}$ test | T– | T– | T– | T– | | | | | | | |
| McBride test | $V_R$ | $V_R$ | $V_R$ | $V_R$ | | | | | | | |

T– = $rct_{40}$ negative. $V_R$ = vaccine related. * = insignificant titre.
† = No. of fly samples positive/No. of fly samples tested (each sample contained from 3 to 10 flies.).

TABLE II. Cyclic Exposure of *Phaenicia sericata* to Sabin Type III poliovirus at 40°C for 5 to 7 Hours Daily for 13 Days.

| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cumulative hr exposure at 40°C | 5 | 10 | 17 | 24½ | 31 | 37 | 43½ | 47½ | 52½ | 58 | 65½ | 72½ | 79 |
| Virus isolation | † | + 3/3 | No deaths in files | + 2/2 | + 2/2 | + 1/1 | + 7/7 | + 5/5 | + 5/5 | + 3/3 | + 4/4 | + 2/2 | + 2/2 |
| PFU/ml | | | | $10^{5.0}$ | $10^{4.5}$ | | $10^{3.35}$ | $10^{2.6}$ | $10^{1.3}$ | $10^{2.8}$ | $10^{1.5}$ | $10^{2.2}$ | $10^{1.7}$ |
| Rct/40°C test | | T– | | T– | T– | T– | T– | T– | T– | T– | T– | T– | T– |
| Antigenic marker test | | $V_R$ | | $V_R$ | $V_R$ | $V_R$ | $V_R$ | $V_R$ | $V_R$ | $V_R$ | $V_R$ | $V_R$ | $V_R$ |

† = No. of fly samples positive/No. of fly samples tested (each sample contained from 3 to 10 flies).
T– = rct/40°C negative. $V_R$ = vaccine related.

expressed in terms of P.F.U. per ml (Tables I and II). It will be observed from the results of titration on serial samples that the viral content of flies showed a steady decline. In the case of type 1 virus, starting with 3.88 logs on the second day, the titre fell to 1.1 logs on the ninth day. Samples collected on days 12 and 13, though containing virus, had titres of less than $10^1$ P.F.U. per ml. In regard to type 3 virus, the results indicate a progressive decline up to the ninth day, after which there was some fluctuation. In any case, no significant rise in viral content was observed.

*Genetic marker tests.* A total of 22 strains (11 of type 1, and 11 of type 3) was tested for genetic stability after passage in flies. In all tests both known virulent and attenuated poliovirus strains were included as controls. All 22 of the isolates from flies were found to have the same characteristics as the attenuated vaccine strains, *i.e.*, rct/40°C negative, and to be antigenically related to vaccine viruses in the McBride intratypic serodifferentiation test.

*Discussion and summary.* Interest in the behavior of polioviruses in flies stems from two factors: (1) the potential importance of these insects as vectors of the virus under certain circumstances; and (2) the possibility that if polioviruses multiply in flies, they may undergo mutation during the process. Investigations of the genetic stability of polioviruses have been carried out largely in connection with the use of attenuated vaccine strains as immunizing agents for humans. It is apparent that wild virulent strains multiply readily at 40°C, while attenuated strains, either naturally occurring or laboratory manipulated vaccine strains, do not. In tropical areas, and in temperate climates during the summer, flies are exposed to high environmental temperatures at least for some time during the day. If this occurs while they are hosts of polioviruses, and if polioviruses undergo multiplication in flies, it is possible that attenuated strains might undergo changes in their genetic characters, with a shift toward the pattern of greater virulence. The results of the present investigation, which was undertaken to explore this possibility, indicate that under the conditions tested there was no evidence of multiplication in type 1 or type 3 attenuated vaccine strains (Sabin) in the fly species *Phaenicia sericata.* Poliovirus was recovered in gradually diminishing quantities for as long as 15 days from flies which had been fed type 1 virus and for at least 13 days from those which had received type 3. When certain genetic characters of the virus strains isolated from 2 to 15 days after virus feeding were compared with those of the type 1 and 3 vaccine strains, they were found to be identical; all isolates tested proved to be rct/40°C negative and vaccine-like in the intratypic serodifferentiation test of McBride. Thus the results do not provide quantitative evidence for poliovirus multiplication in the fly *Phaenicia sericata,* nor was it possible to demonstrate any shift in genetic characters suggesting changes toward a pattern of more virulent strains of virus.

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## Preliminary Communication on the Survival, the Sites Harboring Virus and Genetic Stability of Poliovirus in Cockroaches.* (30115)

KANU H. DAVÉ† AND ROBERT C. WALLIS (Introduced by F. L. Black)
*Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Conn.*

There are more than 3,500 known species of cockroaches(5), and roughly one per cent of these are domiciliary pests(4). So far 18 species have been incriminated, naturally or experimentally, in transmission of pathogenic agents and/or have been known to bite man (4,6). The results of experimental studies on the survival of an attenuated poliovirus vaccine strain in one species of cockroach, *Blaberus discoidalis,* are reported here. The roaches were dissected to localize the sites harboring virus, and measurements were made of the genetic character, temperature stability ($rct_{40}$), of the virus recovered.

Transmission of viral diseases by cockroaches to human beings has not been proved, but available data show that roaches are potential vectors of viruses. Syverton *et al* (7) first demonstrated the presence of a virus in roaches in nature. These workers isolated 4 strains of poliovirus from 4 lots of roaches (*Periplaneta americana*) captured in widely separated areas in the homes of paralytic poliomyelitis patients. Several investigators have induced experimental infections of roaches with different strains of poliovirus. Hurlbut(3) inoculated the Lansing strain of type 2 into the haemocoel of roaches. The virus was recovered after 15 days from the survivors. Hsiang *et al*(2), also working with the Lansing strain, were able to recover the virus from the feces of roaches only on the first day after virus feeding. No virus could be detected in roach tissues 24 hours after the virus meal. Syverton *et al*(7), after a single feeding of Mahoney and Minnesota strains of poliovirus, found that the roaches acquired, maintained, and excreted these viruses over a period of 2 weeks. The present report is concerned with similar experiments using an attenuated vaccine strain rather than a virulent strain of poliovirus.

*Materials and methods. Cockroaches.* Adult laboratory-bred roaches (*Blaberus discoidalis*), 2 to 3 weeks old, were used. These are a large species measuring an average of 4-5 cm in length.

*Virus.* Sabin's attenuated strain of poliovirus type 3 was used. Undiluted tissue cul-

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† W.H.O. Fellow in Virology and Epidemiology. Permanent address: Dept. of Virology, Haffkine Institute, Bombay, India.