THE DETECTION OF POLIOMYELITIS VIRUS IN FLIES COLLECTED DURING EPIDEMICS OF POLIOMYELITIS*

I. Methods, Results, and Types of Flies Involved

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Concern over the possible insect transmission of poliomyelitis has been aroused more than once, but the work has been confined for the most part to two brief periods. The first of these covers the short space of 5 or 6 years—1911 to 1917. It may be briefly reviewed as follows:—

In 1911 Flexner and Clark (1) allowed flies to feed upon tissue from the spinal cords of paralyzed monkeys, and showed that these insects harbored the virus either as a superficial contaminant, or in the gastro-intestinal tract for at least 48 hours. A year later Howard and Clark (2) demonstrated that the domestic fly could carry the virus for several days upon the surface of its body and for several hours in the intestinal tract. Howard and Clark also experimented with lice (*Pediculus vestimenti* and *Pediculus capitis*) and bed bugs (*Cimex lectularius*), which fed upon human beings or monkeys with poliomyelitis. All of the experiments were negative with the exception of one instance (out of 16), in which the filtrate from a bed bug which had fed upon an infected monkey 7 days previously, produced the experimental disease. Kling, Pettersson, and Wernstedt (3) performed similar experiments with extracts of fleas collected from human patients. Their results were negative.

Next followed a series of provocative experiments on the biting fly (*Stomoxys*). These began in 1912, when Rosenau and Brues (4) reported several instances of the successful experimental transmission of poliomyelitis in "Java" monkeys[1] by means of the biting stable fly (*Stomoxys calcitrans*). This finding was at first quickly confirmed by Anderson and Frost (5), who used 2 *rhesus* and 1 Java monkey in their small series of positive experiments. But a second series of experiments on *rhesus*

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[1] The species of monkeys used in these experiments are described in one of Professor Brues' articles (21) as "monkeys of a small Javan species." From correspondence with both Professors Brues and Rosenau, it would seem, however, that the monkeys used were probably *rhesus* monkeys (*M. mulatta*).

monkeys conducted by Anderson and Frost and published in 1913 (6), were totally negative, as were the experiments in *rhesus* monkeys carried out the same year by Sawyer and Herms (7), and others (8).

Later, in 1917, Noguchi and Kudo (9) carried out experiments on the larvae of mosquitoes (*Culex pipiens*) and of non-biting flies, the house fly (*Musca domestica*), and the blue bottle fly (*Calliphora vomitoria*). These larvae were exposed to poliomyelitis virus, and subsequently some were allowed to hatch. A search for the virus was then made in the pupae and imagoes. Noguchi and Kudo also used the more ordinary methods of allowing mosquitoes to feed upon an infected and subsequently upon a normal monkey. All of these procedures yielded negative results. In the face of these negative experiments, this line of investigation was not pursued further.

Perhaps the main reason that interest in flies (or biting insects) and poliomyelitis lagged for so many years was because of the then current assumption that poliomyelitis seemed to be a "respiratory disease," or at least that the virus entered the body through the olfactory nerves; it thus became "unnecessary" to try to bring insects into the picture. In fact, nothing new was heard on the subject of flies and experimental poliomyelitis until some 20 years later, when E. C. Rosenow and his colleagues (10) made brief mention of the fact that experimental poliomyelitis developed in one out of three monkeys inoculated with filtrates of flies collected during the epidemic of poliomyelitis in Kentucky in 1935. These experiments should be mentioned, but it is difficult to evaluate them, for, besides the monkey, the filtrates also induced paralysis on inoculation into rabbits and mice, and this ready susceptibility of these rodents to this infectious agent would indicate that some filtrable agent other than poliomyelitis virus, or besides poliomyelitis virus, was involved, that is, if we are to follow criteria used in this and other laboratories for the identification of poliomyelitis virus.

In our own laboratory during the period 1931 to 1940, we had made repeated attempts at irregular intervals to isolate poliomyelitis virus from flies, mosquitoes, and other insects collected in the field during at least 8 different epidemics. All of these results were negative. They are listed in Table I. It is not clear whether these negative results were due to the fact that the methods were inadequate, whether the inoculated animals were "resistant" (*rhesus* and green African monkeys were the only animals used), or whether the insects tested either were not harboring or were not contaminated with the virus.

Toomey and associates (11) state that prior to the first positive result from their laboratory in 1941, 20 experiments were carried out on house flies (during the previous 10 years). All of their tests were likewise negative.

By the summer of 1941, however, methods for the detection of poliomyelitis virus in stools and other types of materials had been improved, and not the least among the forward steps was the more general use of the highly susceptible Java (*cynomolgus*) monkey, which became available for this purpose in this country in adequate numbers during part of that year. It is not remarkable therefore that, through the use of various improved methods, three laboratories (12, 13, 11) reported, within the short space of a few weeks, the detection of

virus in samples of flies collected in epidemic areas during the summer and fall of 1941.

Our own experiences in 1941 will be described in two papers: The first, or present paper, deals largely with the technique of demonstrating virus in flies; and the second (14) with clinical and epidemiologic circumstances under which the positive experiments were obtained.

TABLE I

Negative Tests on "Insects" Captured in the Field during 8 Different Epidemics of Poliomyelitis. 1931–1940

Year	Place	Type of material	Monkey Nos.	Species	Result
1931	New Haven, Conn.	Flies (few)	2	*Rhesus*	Neg.
1932	Bryn Mawr, Penn.	Spoiled fruit contaminated by insects	B-20	"	Died. Brain abscess
1937	New Haven, Conn.	Adult midges (*Culicoides*)	7-89, 7-90, 7-91, 7-92, 7-93	"	Neg.
"	Toronto, Canada	Mosquitos (*Culexpipiens*)	7-72	"	"
"	" "	Flies (captured indoors)	7-98, 7-99, 8-00, 8-01, 8-02	"	1 died. Brain abscess 1 died (cause unknown). Others neg.
1939	Columbia, S. C.	" " "	12-20		Neg.
"	Charleston, "	" " outdoors)	12-23, 12-34, 12-37	"	"
"	Frankenmuth, Mich.	" " indoors)	12-21	"	"
1940	Cheshire, Conn.	Many varieties of insects	15-67, 15-68, 15-69, 15-72	Green African	"
"	Waterbury, Conn.	Bot fly larvae (*Gastrophilus*)*	15-88, 15-90	*Rhesus*	"
"	" "	Many insect varieties adult and larval)*	15-91, 17-06	Green African	"
"	Huntington, W. Va.	Mosquitoes (adult and larval)*	16-09, 16-14	" "	"
"	" "	Midges and flies	16-10, 16-16, 16-17	" "	"
"	Logan, W. Va.	Mosquito larvae*	16-54	" "	"
"	" " "	Midge larvae*	16-52, 16-55, 17-02, 17-36	" "	"

* The larvae tested were captured in water thought to be polluted with poliomyelitis virus.

Methods

Fly Trapping.—Non-biting flies were caught in traps (Fig. 1) similar to those recommended to farmers by the United States Department of Agriculture (15). These traps were sterilized by boiling, prior to their use. They were usually baited with fish, and were set out of doors—within the yard, near a privy, or near a house in which one or more cases of poliomyelitis had occurred. If weather conditions were optimal, a sample of flies running into hundreds, or thousands, could be caught in a few hours. Usually, however, the catch was collected at the end of 6 hours (10:00 a.m. to 4:00 p.m.) but occasionally the traps stood overnight, or for a period of 2 days.

Biting insects were collected with butterfly nets. In some instances the catch was made from the back and sides of a cow which had been tethered in the epidemic area for the purpose of attracting these insects.

Preparation of Specimens.—As our methods were more or less exploratory, several variations were introduced, which had to do largely with the transfer of flies from the nets or traps, and their preparation for inoculation into the test animal. In two out of four of the positive tests herein reported, the flies, representing the catch of several days were transferred alive (by means of a butterfly net) from the traps to wide-mouthed jars. These jars were brought immediately to the laboratory and then kept

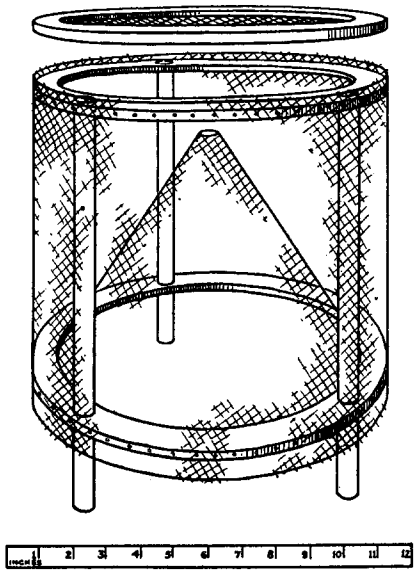

FIG. 1. Type of trap (with dimensions) which was used to collect non-biting flies in these experiments. The bait (usually fish) was placed directly below the center of the cone and the trap was firmly secured with wire to prevent its being overturned by dogs, cats, or barnyard fowls.

at ice box temperature (about 7°C.) for a period of 3 to 7 days. During this time the inside of the jars became coated with fly excrement. Whether this accumulated excrement had anything to do with rendering these samples more satisfactory for the demonstration of the virus is unknown, but it would seem as if this item cannot be ignored.

Aniother method which was used more frequently, and which also yielded two positive tests, was to spray the traps with ether until all flies were dead or anesthetized, and then to transfer the specimens to a jar which was kept at low temperature (for the most part) until the specimens were ready to be tested. One of the posi ive specimens (A-1), see Table II, was collected in this manner in Alabama during August, and shipped by air mail, packed in dry ice. Another positive specimen

(NB-1), was similarly collected in New Brunswick, Canada, and shipped during late September.

The preservation of flies at low temperatures has been found to be important for our purposes. Dead flies disintegrate quickly in a warm environment, and if, in such a sample, there are also any living flies of the types usually caught by these methods, the carcasses may be quickly devoured.

A third method could be used only when a large, low temperature (−70°C.) refrigerator box was available. The procedure was as follows: The whole trap was placed in a low temperature refrigerator box (generally one-third full of dry ice) for a few minutes. This was sufficient to stun or kill all flies within the traps, and they could be transferred readily to suitable small containers and kept in the dry ice refrigerator until ready to be used.

Types of Inocula.—

(*a*) *Fly Washings*: 100 to 600 dead flies were washed in 50 cc. of distilled water, and the same fluid was also used to wash out the inside of the jars in which the flies had been kept. The suspension was then centrifuged at low speed, and from the midlayer one portion was set aside for nasal instillation, while to another portion, usually about 20 cc., 15 per cent ether was added (for bactericidal purposes). The etherized fraction was allowed to stand in the ice box overnight before being injected intra-abdominally.

(*b*) *Fly Emulsions*: Another type of inoculum consisted of an emulsion of disintegrated flies. This was made by mixing in a Waring blendor a sample of 100 to 500 flies in 200 cc. of water. This material was prepared in the same manner as were the washings, for nasal instillation and intra-abdominal injection.

Inoculations.—Each of the 39 tested samples listed in Table II was inoculated into one monkey, in most instances by two routes (*viz.*, 2 cc. intranasally on each of 3 successive days, and 10 to 20 cc. of etherized suspension intra-abdominally as one dose). In four instances (tests 1, 6, 27, and 33) an intracerebral inoculation of 1 cc. of ultracentrifuged material was substituted for the intra-abdominal inoculation.[2]

Monkeys: Java (*M. cynomolgus*), green African (*Cercopithecus aethiops sabaeus*), and *rhesus* (*M. mulatta*) monkeys were used for both the original tests (39 monkeys) and for the passage experiments (8 monkeys). Daily temperature and exercise records were kept on all inoculated monkeys for a period of 4 or 5 weeks, unless it was found advisable to kill them earlier, and also daily exercise records of all monkeys were made as long as the animals were in our possession, both before and after the experiments. If an animal showed signs suggesting experimental poliomyelitis, it was killed at what seemed an appropriate time; histologic sections were examined from the medulla, cervical, thoracic, and lumbar regions of the spinal cord, and, if it seemed indicated, an attempt was made immediately to pass the virus to another monkey, using multiple intracerebral inoculations (acceleration) at intervals of 5 to 7 days, if necessary.

Identification of the Virus.—One of the first questions which invariably arises when claims are made that poliomyelitis virus has been isolated from any unusual (and

[2] The use of the ultracentrifuge in preparing human stools for intracerebral inoculation has been described in a recent paper by one of us (16).

TAB

Record of Tests for Poliomyelitis Virus

Test No.	Sample No.	Date	Site of collection	Method of preparing inoculum*	Days of storage‡
1	L-1‡‡	8/4–9	Camp L, Conn.	Extr. and ultracent.	4–9
2	"	" "	" " "	"	
3	L-2	8/9	" " "	" and washing	3–6
4	"	"	" " "	" " $(NH_4)_2SO_4$ pptn.	5
5	"	"	" " "	"	67–69
6	L-2 and S-2	8/9 and 8/6	Camps L and S, Conn.	" and ultracent.	250–280
7	S-1‡‡	8/6–8	Camp S, Conn.	" " "	4–6
8	"	" "	" " "	"	3–7
9	S-2	" "	" " "	" and washing	4–8
10	"	" "	" " "	" " $(NH_4)_2SO_4$ pptn.	6–8
11	"	" "	" " "	"	32–34
12	"	" "	" " "	"	31–33
13	"	" "	" " "	Washing	"
14	S-3‡‡	8/15–23	" " "	Extr. and washing	11–18
15	S-4	9/8	" " "	" " "	11–14
16	S-5	9/17	" " "	" " "	2–5
17	"	"	" " "	" " "	75–77
18	C-1	8/18–29	Cleveland, Ohio	" " "	2–12
19	"	" "	" "	" " "	80–95
20	A-1	8/24	Alabama	" " "	2–4
21	"	"	"	Washing	52–54
22	"	"	"	Extr.	"
23	A-2	9/19	"	" and washing	3–5
24	"	"	"	" " "	73–75
25	B-1	9/10–18	Bridgeport, Conn.	" " "	1–12
26	" III	" "	" "	" " "	61–64
27	M-1***	9/16–19	Middletown, "	" " ultracent.	138–151
28	Ch-1	9/17–18	Camp Ch, Penn.	" " washing	2–3
29	" III	" "	" " "	" " "	62–65
30	Ch-1 and 2	9/17–10/13	" " "	" " "	1–6
31	NB-1	9/21–22	New Brunswick, Canada	" " "	4–7
32	"	" "	" " "	" " "	57–60
33	"	" "	" " "	" " ultracent.	206
34	F-1	9/18	Fairfield, Conn.	" " washing	1–4
35	No. H-1	10/4	No. Haven, "	" " "	4–17
36	" "	"	" " "	" " "	49–69
37	H-1	10/8–11	Hawleyville, "	" " "	7–13
38	Pool		NB-1; C-1; B-1; F-1; Ch-1	Washing	23–58
39	"		" " " " "	Extr.	"

* Abbreviations in this column are: Extr. = extraction; ultracent. = ultracentrifugation of extract; $(NH_4)_2SO_4$ pptn. = ammonium sulfate precipitation of extract.

‡ This includes the time between collection and inoculation in which whole flies or suspension were in transit; or at − 70°, or at ice box temp.

§ Initials to designate monkey species are: (J) = Java or *M. cynomolgus;* (G) = green or *Cercopithecus aethiops sabaeus;* and (R) = *rhesus* or *M. mulatta.*

LE II

in 19 Samples of Flies Collected in 1941

Monkey No. and species§	Dose equivalent in flies	Result of inoculation				Passage			
		Fever‖	Paralysis	Lesions	Remarks	Monkey No. and species	Results**	Rodents	Results**
16-79(J)	20	—	—	—					
18-85(G)	14	+32	—	—					
16-75(J)	68	+8	—	+		19-96(G)	—§§	6 mice 2 g. pigs	— —
18-38(J)	200	—	—	—	Died 19th day—peritonitis				
20-08(J)	14	+13	+	+		20-21(R)	+	6 mice 2 g. pigs	— —
21-34(R)	700±	—	—	—					
16-77(J)	80	—	—	—					
18-86(G)	5	—	—	—					
16-76(J)	34	+18	+	+	6 mice negative	19-87(G) 19-74(R)	+ +	6 mice	—
18-36(J)	100	—	—	—					
19-63(R)	118	—	—	—					
19-67(R)	12	—	—	—					
19-68(R)	70	—	—	—					
19-32(G)	63	—	—	—					
18-37(J)	35	—	—	—	Pelvic tuberculosis				
19-82(G)	31	—	—	—					
20-38(J)	29	—	—	—					
19-41(R)	650	—	—	—					
20-35(J)	225	—	—	—					
18-40(J)	190	+9	+	+	6 mice negative	19-70(R) 19-75(R) 19-80(G)	+ + +	6 mice	—
20-09(J)	42	+15	—	—					
20-10(J)	24	—	—	—					
19-86(G)	42	+4	—	—					
20-36(J)	30	—	—	—					
19-81(G)	25	+5	—	—					
20-26(J)	60	—	—	—					
20-77(R)	240	—	—	—					
19-84(G)	38	+5	—	—					
20-34(J)	17	—	—	—					
19-85(G)	75	+3	—	—					
19-94(G)	24	+15	—	—					
20-13(J)	40	+13	+	+		20-71(R)	+	6 mice 2 g. pigs	— —
21-33(R)	150	—	—	—					
19-83(G)	45	+5	—	—	Abdominal tuberculosis				
19-32(G)	95	—	—	—	Pelvic tuberculosis				
20-37(J)	46	—	—	—	Died 17th day—colitis				
20-14(J)	345	—	—						
20-11(J)	115	+18	—	—					
20-12(J)	90	—	—	—					

‖ +32 = fever occurred 32 days after inoculation.

** + = experimental poliomyelitis was produced; — = negative result.

‡‡ Sample consisted almost entirely of biting flies.

§§ Died 22nd day—peritonitis.

‖‖ Pooled with sample from F-1.

*** Pooled with sample from Camp P, Conn., and from Monroe, Conn.

particularly from an extrahuman) source is: Are you sure that it is poliomyelitis virus? Criteria on which the answer to this question is based will probably change from year to year, but at present we know of no reason to alter those which we have used in previous experiments on the isolation of poliomyelitis virus from human stools (17) and from sewage (18). They include three, and in many instances four standards which each strain must fulfill: (*a*) The production of a "clinical picture" in the inoculated monkey which is compatible with that of experimental poliomyelitis; *viz.*, after an appropriate incubation period there occurs a train of characteristic symptoms, exemplified usually by some of the following: fever, excitement, tremor, ataxia, weakness, and paralysis of one or more limbs, the latter being generally associated with a fall in temperature. (*b*) When the animal is killed, lesions typical of experimental poliomyelitis must be found in the spinal cord, in lumbar as well as in cervical levels. These lesions should be "unequivocal," and besides presenting evidence of neuronophagia, there must be perivascular infiltrations with mononuclear cells. (*c*) Passage of the strain to a second monkey must be successfully accomplished, in the course of which, criteria (*a*) and (*b*) must again be fulfilled. A fourth criterion, which has been used in most instances, is: (*d*) The suspected material, or strain, when inoculated into other laboratory animals, such as guinea pigs or Swiss mice[3] does not produce an encephalomyelitis in these animals. It has been our practice to observe these smaller animals for a period of 4 weeks; to take daily temperatures on the guinea pigs during this period; to sacrifice all the animals at the end of the 4 week period, and to study the midbrain and three levels of the cord histologically. Such tests should be helpful in differentiating the virus of poliomyelitis from that of lymphocytic choriomeningitis, equine encephalomyelitis, and from other viruses capable of producing encephalitis in these rodents. The extent to which these four criteria have been met appears in Table II.

Identification of Flies.—Genera, and in many instances species, were determined in 14 of the 19 samples of flies which were tested for virus. In all but one of these (Table IV), the identifications were made by a trained entomologist.[4]

If the sample was small (*viz.*, less than 150 flies) the specimens were first reviewed by an entomologist and then tested for virus; if large, the identifications were made on a representative sample of 100 or more, which were subsequently discarded.

RESULTS

The major series of experiments to be reported in this paper appears in Table II. It includes 39 tests performed during the summer and fall of 1941 on 19 different fly samples. Of these 39 tests, 37 may be said to have been satisfactory. In 4 of them the virus of poliomyelitis was definitely isolated; in a fifth (duplicate) test (test 3), it was also probably demonstrated. The positive

[3] For testing fly emulsions and monkey passage material, each mouse was inoculated intracerebrally and intra-abdominally; guinea pigs were inoculated both intracerebrally and subcutaneously.

[4] We are indebted to Dr. R. B. Friend, of the Connecticut Agricultural Experiment Station of New Haven; and to Mr. G. S. Allen, a graduate student in the Department of Zoology, Yale University, for the identification of these specimens.

results, and the "equivocal" result have been analyzed from a number of standpoints, but the series is too small and the variations in technique too few to expect these analyses to yield much pertinent information as to optimal methods. Thus there is no indication that the use of washings of flies or of the inside of the containers in which they had been stored, was superior to the use of emulsions of ground-up flies, as a method of isolating the virus. There was little indication that an inoculum representing large numbers of flies (100 or more) was more apt to yield a positive result than a smaller one; the average number of flies in the inocula of the 4 positive and 1 equivocal result, was about 70, but in one monkey a positive result was achieved in a dose representing approximately only 14 flies.

TABLE III

Tests for Poliomyelitis Virus in Biting Insects and Non-Biting Flies Collected within the Same Epidemic Areas during the Summer of 1941

Biting insects*				Non-biting flies‡		
Area	Date	Sample No.	Result of test for virus	Date	Sample No.	Result of test for virus
Camp L	Epidemic period Aug. 4–9	L-1	–	Aug. 9	L-2	+
Camp S	Epidemic period Aug. 6–8	S-1	–	Aug. 6-8	S-2	+
	Epidemic period Aug. 15–23	S-3	–			
	Post-epidemic period			Sept. 8	S-4	–

* Samples L-1 and S-1 consisted of about 50–100 insects, about half of which were deer flies (*Chrysops*). Sample S-3 consisted largely of mosquitoes, unidentified as to species.

‡ The genera in fly samples L-2, S-2, and S-4 are listed in Table IV.

There is one point, however, which we believe to be important even though the statistical evidence presented in this paper may not be impressive; namely, that all of our positive results were obtained with Java (*cynomolgus*) monkeys. In the 1941 series of 37 satisfactory tests, 20 Java monkeys were used, and of these 5 developed lesions of poliomyelitis, although in but 4 animals were we able to pass the strain to a second monkey. Ten satisfactory tests were performed in green African monkeys, all of which were negative; 6 tests in *rhesus* monkeys were also negative.[5]

[5] A series of similar experiments in which flies were trapped in epidemic areas has also been carried on in our laboratory during the season of 1942. We are not yet ready to report on these results but one pertinent preliminary fact may be mentioned. In the 1942 series, 9 samples were tested in 13 *rhesus* monkeys, all of these tests were negative; 1 pooled sample from the San Antonio epidemic of 1942 was tested in 1 Java monkey with a positive result.

TABLE IV

Different Genera of "Non-Biting" Flies Represented in (A) Four Samples from Which Poliomyelitis Virus Was Isolated; and (B) Ten Samples Which Were Negative

	A. Positive samples				B. Negative samples									
Site of collection	Camp S, Conn.	Camp L, Conn.	Ala.	New Brunswick	Camp S, Conn.	Camp P, Conn.	Middletown, Conn.	Camp S, Conn.	Bridgeport, Conn.	Camp Ch, Penn.	Ala.	No. Haven, Conn.	Hawleyville, Conn.	Camp Ch, Penn.
Sample No.	S-2	L-2	A-1	NB-1	S-4	P-1	M-1	S-5	B-1	Ch-1	A-2	No.H-1	H-1	Ch-2
Date	Aug. 6–8	Aug. 9	Aug. 24	Sept. 21–22	Sept. 8	Sept. 15	Sept. 16–19	Sept. 17	Sept. 18–23	Sept. 17–18	Sept. 19	Oct. 4	Oct. 8–11	Oct. 13
Approx. No. of flies in sample.	1100	2000	200	300	160	23	66	200	500	300	200	200	1500	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Phormia (including *Protophormia*)	30±*	70	30±‡	5		20	8	15	7	28	13	23	25	26
Phaenicia (*Lucilia*)	45±	28	20±	7		20	83	37	79	25	36	19	47	11
Ophyra	5±			18		12	2	5	2	5	5			
Calliphora				8						5	7		14	15
Protocalliphora	5±										1			
Sarcophaga	5±	1				2	2		3	3	13			
Musca	5±		5±		80		3	40	6			50	14	
Muscina	5±	1		6	20	4	2	3		13	5			4
Morellia						40			3					
Fannia				48		2				7				7
Cohliomyia										14				30
Stomoxys	?										10	3		
Others				8										
Cynomyia												3		7

* Percentages are from approximations in this sample.

‡ Percentages are from approximations and identification of this sample was not made by a trained observer.

Types of Flies.—Early in these experiments the attempt was made to collect and test for virus two main groups of insects within a given epidemic area; *viz.*, (*a*) biting flies (including small numbers of mosquitoes), and (*b*) non-biting flies.

(*a*) *Biting Flies:* Only a few representative samples of these were secured, and the opportunity to perform a comparative experiment on biting *vs.* non-biting flies within areas where the virus was known to be harbored by the latter, was presented only twice, namely in two children's camps (Camp L and Camp S). The limited results presented on Table III merely indicate, therefore, that some preliminary observations on this question have been made. They are inadequate in so far as a relative determination of the virus-carrying properties of these two groups of insects is concerned. But as far as they have gone, they failed to reveal the presence of virus in association with small numbers of biting flies caught within the same two epidemic areas, where larger numbers of non-biting flies were known to be harboring poliomyelitis virus.

(*b*) *Non-Biting Flies (Genera Represented):* In Table IV are recorded some of the data which we were able to secure about the genera of the flies represented in the 4 samples which yielded the virus, as compared with 10 samples (secured for the most part later in the season), from which no virus was discovered. There is nothing to indicate that the *positive* samples in our series were unique in their composition. Certain common genera and species are represented in almost all of the 14 samples, notably representatives of the green bottle fly, *Phaenecia* (*Lucilia*),[6] and of blow flies, *Phormia* or *Protophormia*. These are the types of flies which are apt to predominate in summer collections from Connecticut (and many places elsewhere) which have been made in traps of the type herein described, baited in the manner described. These common genera were present in all of the four virus positive samples, as well as the ten negative samples. Two points deserve mention—they are: (*a*) the fact that the common house fly (*Musca domestica*) was found in only two of the four positive specimens and then only in small numbers; and (*b*) that the stable fly (*Stomoxys calcitrans*) was not found with certainty in any of the samples.

COMMENT

These experiments indicate that poliomyelitis virus can be demonstrated either on the surface or within the body of flies collected in the field during epidemics of this disease. This finding has been confirmed in two other

[6] In an earlier communication from this laboratory (12) in which two of the positive results (Samples A-1 and S-3) were recorded, the term *Lucilia* was used to designate certain green bottle flies. As used in the previous paper, this term *Lucilia* is synonymous with that of *Phaenicia*. The latter is now recommended by the Bureau of Insect Identification, Department of Agriculture, Washington, D. C., and will be followed in this and subsequent publications from this laboratory.

laboratories (13, 11), particularly by the work of Sabin and Ward (13), whose approach to this subject, and whose methods, have been quite similar to ours. In their recent series of tests (19), a higher percentage of positive tests were obtained than are recorded in this paper, in that 8 out of their 15 samples of flies, collected during 1941 outbreaks of poliomyelitis in Atlanta and Cleveland, yielded the virus.

Three features with regard to our experiments and to those in the literature, deserve comment: (*a*) the value of Java (*cynomolgus*) monkeys in this type of test; (*b*) the types of flies present in the positive samples; and (*c*) a word of explanation as to why the virus of poliomyelitis was the only infectious agent to be isolated from flies caught under these circumstances.

(*a*) Our positive results were obtained only in *cynomolgus* monkeys, and this was also the experience of Sabin and Ward (13, 19), who believe that the use of this particular species is an important factor in carrying out successful experiments of this type. Most of the monkeys used in our series, however, were *cynomolgi*, and we have no comparative experiments in which actual superiority of this species as a test animal for this particular purpose is demonstrated. But on the basis of other experiences with *cynomolgus*, as compared with *rhesus* monkeys, our findings agree with those of others (20) that this animal is more vulnerable to infection by various routes. However, the use of this species may not be essential for this type of experiment—at least, if the animal is inoculated *intracerebrally*. Toomey and his associates (11) have reported a successful "take" in a *rhesus* monkey inoculated *intracerebrally* with an emulsion of flies trapped near an open sewer during the Cleveland epidemic of 1941.

(*b*) In our own experiments the commonest genera of fly to be represented in the positive samples were the common green bottle flies, *Phaenicia*, (or *Lucilia*), and blow flies. This was also the experience of Sabin and Ward (13, 19), in whose positive Cleveland specimens the large majority were *Phaenicia sericata*. They also state that, "Virus was isolated from one collection of flies in which only *Phaenicia sericata* (green bottle fly), *Protophormia terraenovae* (black blow fly), and *Musca domestica*, were present." In Toomey's positive result (11) the flies are described as "mostly large, blow flies, with an occasional small house fly. . . ." In other words, in all the positive results reported to date, in which identifications have been made, "blow flies" have been present, and in nearly all, the green bottle fly, *Phaenicia* (*Lucilia*), has been present. In many of them, but not all, house flies have been present. In none of them have *Stomoxys* been definitely noted.

(*c*) A third question which merits comment is: Why should poliomyelitis virus have been singled out, as it were, by these procedures, from all the other possible "infectious agents" which emulsions of flies might contain? The number of bacteria in these emulsions is, of course, greatly reduced by one of the steps in our method, *viz.* the addition of ether, but the inoculum for intra-ab-

dominal injection is generally not rendered completely bacteria-free by this procedure. But another feature in the "selectiveness" of the method is the fact that apart from daily temperature records, only the neuromuscular system of the animals was examined systematically, and the central nervous system tissue alone was used for the passage of the strains. Undoubtedly several of our monkeys became ill as a result of being injected with some of the various toxic or infectious agents which emulsions of flies must contain. To this the fairly frequent presence of unexplained fever during the course of the 4 week post-inoculation period may testify. (See Table II.) But unless our inoculated monkeys also developed symptoms pointing to involvement of the central nervous system, fever was not taken seriously. In other words, our methods were designed essentially for the detection of infection by neurotropic viruses, and poliomyelitis virus was the only one of this group which was detected by the method employed. In this connection it may be mentioned that the monkey is not the most susceptible (or the animal of choice) for the demonstration of the virus of lymphocytic choriomeningitis or for certain encephalomyelitis viruses, such as St. Louis encephalitis and the virus of Western equine encephalomyelitis.

SUMMARY

1. A series of 19 different samples of flies collected within epidemic areas during and after the onset of nearby human cases of poliomyelitis have been tested for the virus of poliomyelitis. Four of these samples proved positive.

2. Methods used in collecting the flies, preparing the inocula, and examining the inoculated monkeys (and other animals) are described.

3. All of the positive tests were obtained by the intranasal and intra-abdominal inoculation of Java (*cynomolgus*) monkeys. Green African and *rhesus* monkeys represented the smaller number of other monkeys used in which only negative results were obtained.

4. All of the positive samples (as well as nearly all the negative ones) contained "blow flies," and green bottle flies.

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