POLIOMYELITIS VIRUS IN FLY-CONTAMINATED FOOD COLLECTED AT AN EPIDEMIC[1]

THIS paper is concerned with an experiment on the mechanism of spread of poliomyelitis. Its purpose is to report the detection of poliomyelitis virus in food exposed to flies at an epidemic in the summer of 1944. Much new evidence has accumulated within the past few years which suggests that the human alimentary tract (mouth and pharynx to colon) may be a portal of entry for the virus in human poliomyelitis.[2,3,4] Furthermore the virus has been repeatedly demonstrated in human stools,[5,6] in sewage,[7,8] and in flies,[9,10,11,12] but no direct evidence has yet been produced that fecal material, sewage or contact with flies on the part of the individual, his food or fomites, actually constitute links in the poliomyelitis infection chain.

It would be important therefore if it could be shown that food merely exposed to flies at an epidemic area is infective when ingested. The chimpanzee was selected to receive the exposed food because next to man, this animal appears to be the most natural experimental host. The work of Howe and Bodian has proved not only that poliomyelitis virus given by mouth produces paralytic[4] and non-paralytic[13] infections in the chimpanzee, but also that these animals are susceptible to spontaneous infection.[14] They were therefore ideal test animals for this type of experiment in which as close an approximation to nature as practicable was desired.

Materials and methods: Although material was collected in several epidemic areas (North Carolina and New York) and at two camps in Connecticut during the summer of 1944, it is possible to speak with certainty of the results only after feeding the materials obtained in North Carolina.[15]

Flies were collected and fly-bait and food were exposed at 12, and food alone at an additional 8 homes of poliomyelitis patients within a week of the onset of illness. The food to be exposed was purchased at local stores and consisted of bananas, which were peeled and sliced on the spot to be studied, and sprinkled with a little sugar and water. One or two plates of food were exposed in and about the homes, usually in the kitchen and on the back porch, and the family was admonished not to touch them. The fly-bait, composed also of bananas and sugar plus liver or fish, likewise obtained locally, with added water to prevent desiccation, was placed beneath a fly-trap in the yard or on the back porch. The food and fly-bait were thus exposed for 24 to 48 hours, frozen on dry ice, transported to the laboratory and held in the frozen state until fed to the chimpanzees. In most instances there was gross evidence of fly contamination on both food and fly-bait in the form of vomit and fecal spots, and often many flies[16] were observed to rise from the food at the times of collection.

The chimpanzees were received as new animals shortly before the experiment was begun. In order to prevent cross-contamination not only from outside sources and between the chimpanzees, but also from our local monkey colony to the chimpanzees, two outdoor enclosures were separately screened and one chimpanzee was caged in each. During the first three weeks of the experiment while the first positive chimpanzee stools were being collected, all other primates being used for poliomyelitis were quartered in another building across the street. Furthermore, the chimpanzees were fed and their cages cleaned during this period by a caretaker who had no contact with the monkeys in the poliomyelitis laboratory. No human cases of poliomyelitis, moreover, were reported in

[1] Aided by grants from the National Foundation for Infantile Paralysis, Inc.

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[4] H. A. Howe and D. Bodian, "Neural Mechanisms in Poliomyelitis." New York, The Commonwealth Fund, 1942.

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[7] (a) J. R. Paul, J. D. Trask and S. Gard, *Jour. Exp. Med.*, 71: 765, 1940. (b) J. D. Trask and J. R. Paul, *Jour. Exp. Med.*, 75: 1, 1941.

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[10] (a) A. B. Sabin and R. Ward, SCIENCE, 94: 590, 1941. (b) A. B. Sabin and R. Ward, SCIENCE, 95: 300, 1942.

[11] J. A. Toomey, W. S. Takacs and L. A. Tisher, *Proc. Soc. Exp. Biol. and Med.*, 48: 637, 1941.

[12] (a) J. D. Trask, J. R. Paul and J. L. Melnick, *Jour. Exp. Med.*, 77: 531, 1943. (b) J. D. Trask and J. R. Paul, *Jour. Exp. Med.*, 77: 545, 1943.

[13] D. Bodian and H. A. Howe, *Jour. Exp. Med.*, 81: 255, 1945.

[14] H. A. Howe and D. Bodian, *Jour. Exp. Med.*, 80: 383, 1944.

[15] This epidemic began and reached its peak in June, 1944, in Catawba County, where most of the cases in North Carolina occurred and where almost all the collections were made. The outbreak was rural in character, most of the affected families were in the lower income group, hygienic conditions were poor, outdoor privies the rule, weather hot and flies abundant. Dr. J. S. Gaul, of Charlotte, N. C., Dr. H. C. Whims, Lincoln-Catawba County health officer, and his staff, particularly Mr. Jack Wildey and Miss Frances Allen, R.N., gave valuable assistance in this area.

[16] Tests on flies trapped in North Carolina are as yet incomplete. Two samples consisting of a total of 428 flies were identified by Dr. M. E. Power, of the Osborn Zoological Laboratory, Yale University, as follows: *Musca domestica*, 349; *Fannia* sp., 26; *Phaenicia sericata*, 22; *Ophyra leucostoma*, 11; *Phormia regina*, 9; *Sarcophaga* sp., 5; *Muscina stabulans*, 2; *Cochliomyia macellaria*, 2; *Lucilia illustris*, 1; and *Bufolucilia silvarum*, 1.

New Haven during this period. These circumstances reduced the possibility of accidental contagion.

The food exposed to flies in North Carolina was fed successfully to chimpanzee "Hickory" (female, aet. 2 years) in approximately one-quart amounts daily for 10 days. The fly-bait was fed in similar quantities for 6 days to chimpanzee "Catawba" (female, aet. 16 months) who relished the bananas but refused the meat and fish. Daily rectal temperatures were taken on both animals (Figs. 1 and 2). Although their temperatures rose to 101° F, neither animal at any time showed evidence of paralytic poliomyelitis. In view of the possibility that either nonparalytic poliomyelitis or an asymptomatic carrier state might be the only form of the experimental disease produced in the test animals, daily specimens tested for virus by intracerebral inoculation in rhesus monkeys.

Results: A record of these tests appears in Table 1 and also in Figures 1 and 2. The control, or pre-

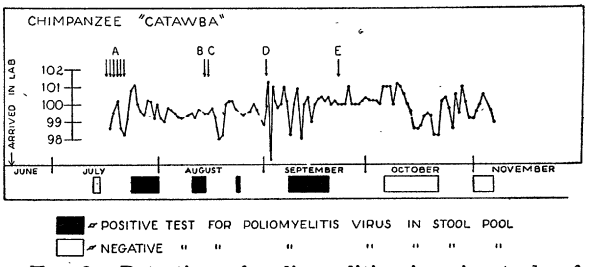

FIG. 2. Detection of poliomyelitis virus in stools of Chimpanzee "Catawba" fed *fly-bait* from North Carolina (A), food from New York (B), fly-bait from Connecticut (C) and from New York (D) and (E).

TABLE 1

RESULTS OF TESTS IN RHESUS MONKEYS FOR VIRUS IN STOOLS OF CHIMPANZEES FED FOOD AND FLY-BAIT

	Material fed to chimpanzee			Test for virus in chimpanzee stools				
						Result of inoculation		
Name	Date	Source	Type	Date of stool pools	Rhesus No.	First day of Fever	First day of Paralysis	Lesions
"CATAWBA"	June 19–July 16	Control period		July 13–15	2945	0	0	0
	July 17–22	North Carolina	Fly-bait	July 24—Aug. 1	2894	0	0	0
				" "	2939	14	16	+
				Aug. 11–15	2895	7	11	+
	Aug. 15	New York	Food					
	Aug. 16	Connecticut	Fly-bait	Aug. 25	2896	0	8	+*
	Sept. 2	New York	Fly-bait	Sept. 8–20	2902	0	0†	+
	Sept. 26	New York	Fly-bait	Oct. 6–22	2825	7	0	0
				Nov. 1–7	2897	0	0	0
"HICKORY"	June 19–July 16	Control period			2953	9	0	0
				July 13–15	2961	0	0	0
					2962	0	0	0
	July 17–27	North Carolina	Food					
				July 18–29	2938	4	8	+*
				July 30—Aug. 10	2906	0	0†	+
	Aug. 15	New York	Food					
	Aug. 19	Connecticut	Food and fly-bait	Aug. 16–24	2956	9	21	+
				Sept. 15–21	2807	0	0	0
				Sept. 22–28	2808	13	0	0
				Oct. 11–25	2890	0	0	0
				Nov. 8–15	2957	13	0	0

* Passage of CNS positive in 1 monkey, negative 10 mice, 3 guinea pigs and 3 rabbits.
† Mild weakness of hind legs.

of stool from these 2 chimpanzees were collected before and after feeding the test materials. These stools were prepared by ultracentrifugation[17] and

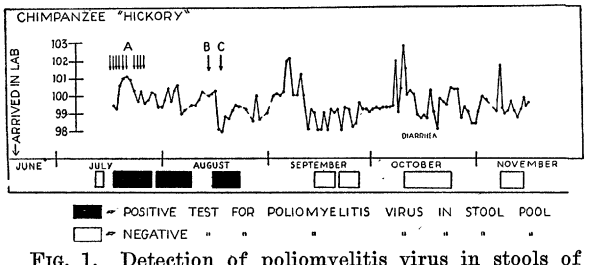

FIG. 1. Detection of poliomyelitis virus in stools of Chimpanzee "Hickory" fed *food* from North Carolina (A), food from New York (B), food and fly-bait from Connecticut (C).

feeding, stool specimens of both chimpanzees gave negative tests for poliomyelitis virus, indicating that neither animal was a carrier at the beginning of the experiment. Seven separate stool pools in the post-feeding period have given positive tests for poliomyelitis virus. Thus chimpanzee "Hickory," who ate North Carolina food from July 17 to 27, passed virus in each of 3 stool pools, July 18–29, July 30–Aug. 10, and Aug. 16–24 (Fig. 1). Likewise "Catawba," who received fly-bait from July 17 to 22, had demonstrable virus in 4 stool pools, July 24–Aug. 1, Aug. 11–15, Aug. 25 (single specimen), and Sept. 8–20 (Fig. 2). Typical poliomyelitis lesions were found in the cord and medulla of each of the infected test monkeys.

[17] J. L. Melnick, *Jour. Exp. Med.*, 77: 195, 1943.

Passage of the strain of virus from two monkeys (representing each chimpanzee, respectively) produced characteristic poliomyelitis in two additional monkeys and was negative in mice, guinea pigs and rabbits. The evidence is clear therefore that poliomyelitis virus appeared in the stools of both chimpanzees in the period immediately after ingestion of food and fly-bait, and also at later periods, 20 days after the last feeding in 1 case, and 3 to 14 days in the other. This suggests the likelihood that the chimpanzees acquired subclinical infections or carrier states and that virus multiplied in them. Although virus was continually eliminated by the chimpanzees for periods of about 1 and 2 months, respectively, it is impossible to state from the present data whether the North Carolina materials were solely responsible or whether materials B, C and D, collected in New York and Connecticut, also contributed.

Discussion: The results of these experiments indicate that food exposed to flies at the homes of poliomyelitis patients in an epidemic area may acquire a quantity of poliomyelitis virus sufficient to produce in chimpanzees by oral administration a non-paralytic infection or asymptomatic carrier state. The question concerning the origin of the virus has three possible answers: the food became contaminated with virus (a) prior to its being exposed at the homes, (b) at the homes by means other than flies or (c) at the homes by flies. With respect to (a), the fact that the bananas were peeled and the skins discarded at the homes makes it unlikely that the bulk of the food was contaminated beforehand, although the sugar and water can not be ruled out. In regard to (b), it is possible that contamination of food occurred by handling or by droplets expelled from an individual's sneezing or coughing, although virus has yet to be demonstrated in human saliva or droplets. On the other hand, the fly-bait, situated beneath a fly-trap which was anchored firmly to the ground, was protected by coarse wire screen and was consequently less accessible to certain other agents such as human beings. The balance of probability would seem to favor fly-contamination at the homes.

The observation that food exposed at infected homes within an epidemic area was found to contain virus, serves as additional evidence to support the following *working hypothesis,* which has been tentatively adopted to guide future investigations along these lines. Human poliomyelitis may be transmitted by a number of different routes. Although the disease may occur at any time of the year the tremendous concentration of cases during the warm season is the result of increased dissemination of virus. This may depend on various factors including something which facilitates the contamination of food by insects such as flies. This is based on the fact that flies have been shown to carry virus and also, as probably indicated in this experiment, to contaminate food.

A further step in the testing of this hypothesis will be to conduct a controlled experimental study on the effect of reducing the number of flies during epidemics of poliomyelitis.

Summary: Poliomyelitis virus has been detected in food exposed to flies at homes of poliomyelitis patients within an epidemic area. This was achieved by feeding such exposed food to chimpanzees which developed subclinical infections or asymptomatic carrier states ascertained by positive stool tests in rhesus monkeys.

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THE EFFECT OF DIETARY PROTEIN INTAKE ON THE XANTHINE OXIDASE ACTIVITY OF RAT LIVER

SARETT *et al.*[1] and Unna *et al.*[2] found that the riboflavin level of rat liver is lowered during low protein ingestion. The latter investigators also showed that the ability of the liver to inactivate estradiol *in vitro* was likewise reduced. We have observed the same phenomena of lowered liver riboflavin level from rats on low protein diet. To study this problem further, we started investigating the effect of dietary protein level on the xanthine oxidase activity of the rat liver when we were forced to discontinue the problem due to the urgency of other work. We believe our results, although very preliminary, are worth reporting.

Rats 10 to 12 weeks old were used. One group was kept on an adequate stock diet, while three other groups were fed diets of the following composition: protein, x per cent.; cerelose, 90-x per cent.; salts, 4 per cent.; hydrogenated cottonseed oil, 4 per cent.; and cellulose (pulverized Cellophane), 2 per cent. A daily supplement of 40 γ each of thiamin, riboflavin and pyridoxine, 100 γ calcium pantothenate, 250 γ nicotinic acid, 6 mg choline and one drop of 1,000A–400D feeding oil was given in supplement cups. All diets were fed *ad libitum.* The total protein content of the four diets was: group 1 (stock), 25 per cent.; group 2, 20 per cent., from casein; group 3, 10 per cent., from soybean oil meal; and group 4, 10 per cent., from corn distillers' solubles.

Xanthine oxidase activity was estimated by the method of Axelrod and Elvehjem[3] adapted to the

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