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1. Carp, R., Koprowski, H., Virology, 1962, v16, 71.
2. Flexner, S., Clark, P. F., J. Am. Med. Assn., 1911, v56, 1717.
3. Francis, T., Jr., Brown, G. C., Penner, L. R., ibid., 1948, v136, 1088.
4. Gudnadottir, M. G., J. Exp. Med., 1961, v113, 159.
5. Hsiung, G. D., Melnick, J. L., J. Immunol., 1957, v78, 128.
6. McBride, W. D., Virology, 1959, v7, 45.
7. Melnick, J. L., Penner, L. R., Proc. Soc. Exp. Biol. and Med., 1947, v65, 324.
8. Paul, J. R., Trask, J. D., Bishop, M. B., Melnick, J. L., Dasey, A. E., Science, 1941, v94, 395.
9. Paul, J. R., Horstmann, D. M., Riordan, J. T., Opton, E. M., Niederman, J. C., Isacson, E. P., Green, R. H., Bull. Wld. Hlth. Org., 1962, v26, 311.
10. Sabin, A. B., Ward, R., Science, 1941, v94, 590.
11. Toomey, J. A., Takacs, W. S., Tischer, L. S., Proc. Soc. Exp. Biol. and Med., 1941, v48, 637.
12. Ward, R., Melnick, J. L., Horstmann, D. M., Science, 1945, v101, 491.

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

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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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TABLE I. Survival of Sabin Type 3 Poliovirus in Cockroaches, *Blaberus discoidalis.*

Day after feeding	6 days	18 days	27 days	37 days	44 days	51 days	58 days	65 days
Whole roach	5/5*	5/5	3/3	2/3	1/3	1/3	0/3	0/5
PFU/ml	$10^{2.9}$		$10^{2.0}$	$10^{1.0}$				
Nerve trunk			0/2	0/2	0/2	0/2	0/2	0/5
G.I.T.			1/2	1/2	0/2	0/2	0/2	0/5
Body			0/2	0/2	0/2	0/2	0/2	0/5
rct_{40} marker	T–		T–	T–	T–			

* Denominator equals number of roaches tested; numerator equals number shown to harbor poliovirus.
G.I.T. = Gastrointestinal tract.
T– = rct_{40} negative.

ture fluid with a titre of $10^{7.5}$ plaque forming units per milliliter (P.F.U./ml) was fed to the roaches.

Feeding procedure. The roaches were kept in boxes and pre-starved for a period of 24 hours. Virus fluid mixed with ground meat was placed in petri dishes, and the dishes then transferred to the boxes. The dosage provided each roach was approximately 0.1 ml or $10^{6.5}$ P.F.U.

Maintenance and harvesting of infected roaches. Following virus feeding, roaches were kept in an incubator at 28°C. They were maintained on a diet of commercially prepared rabbit pellets. At intervals of one week, 5 roaches were removed at random from incubation and immediately frozen at —20°C. At the time of processing (usually 6 hours later) they were thawed and dissected under aseptic conditions. For the first 2 batches the whole roach (minus cuticle and appendages) was used for virus isolation. From the 27th day each roach was dissected into 3 components, *viz.*, the gastrointestinal tract, the ventral nerve trunk, and the rest of the body. Each of these components was processed separately.

Virus isolation. One milliliter of 10% to 15% suspension of the roach tissues prepared in Hanks' balanced salt solution was inoculated into 3 oz bottle cultures of primary rhesus monkey kidney monolayers. The inoculum was allowed to adsorb for 1½ hours, at the end of which maintenance medium (M + Earle's with 0.5% lactalbumin and 2% calf serum) was added. Inoculated cultures were incubated at 36°C and observed for a period of 14 days for cytopathogenic effect. Blind passages were made in case of doubtful cytopathogenic effect. To determine the virus content of roach tissues, titrations were carried out on original suspensions using the plaque technique.

Marker test. Using Carp and Koprowski's modification of the temperature marker determination(1), tests were carried out on isolates obtained at different intervals.

Results. Overall results appear in Table I. When dissection of the individual roaches was carried out (beginning 27 days after feeding), it was possible to recover virus only from the gastrointestinal tract. On days 27 and 37, one out of 2 samples of the gastrointestinal tract showed the presence of virus, but beyond this period all samples were found to be negative. All of the samples of nerve trunk and body were found to be negative: no virus isolations were made at any time from these sources.

In those specimens which were positive for virus, CPE appeared around the fifth day in cases of samples obtained up to 18 days, while with samples obtained on days 27 and 37 it was delayed until 7 days. With the sample obtained on day 44, it was possible to detect CPE only after one blind passage. The titres of virus present were low. Six days after infection it was $10^{2.9}$ P.F.U./ml but dropped to an insignificant level by day 37 (10^{1} P.F.U./ml).

Temperature marker tests carried out on isolates obtained on days 6, 27, 37, and 44 did not show any change in rct_{40} character from the original vaccine virus: *i.e.*, all were found to be rct_{40} negative.

Discussion. These results confirm the previous observation that poliovirus persists in the cockroach for some time. The few studies

carried out earlier, using mice as the test system, indicated that virus persisted in the body of the roach for at least 2 weeks; but by utilizing the more sensitive tissue culture techniques in this study it was demonstrated that survival was of longer duration in the roach: *viz.*, up to 51 days.

The results obtained also indicate that although virus was recovered for as long as 51 days, limited investigations did not disclose evidence of its multiplication. Rather, the pattern seemed to be one of simple mechanical carriage with a gradual decline in virus titre. The peculiar anatomy of the roach probably is a factor favoring sequestration and persistence of virus. Thus, at the junction of the stomach and the intestine, there are present in the roach tubular appendages known as gastric caecae. In these hollow structures it is quite possible that the ingested virus becomes trapped and is protected from the flushing action of the intestinal tract. The significance of the persistence of virus is apparent in that roaches, because of their demonstrated habits and anatomy, may be carriers of poliovirus for weeks and may serve as potential vectors for dissemination in certain communities.

Summary. Sabin's attenuated strain of poliovirus type 3 persisted in cockroaches (*Blaberus discoidalis*) after experimental infection for as long as 51 days. No evidence was obtained indicating multiplication of the virus. Rather, the pattern was one of mechanical carriage of the virus with a gradual decline in titre. It was possible to recover virus only from the gastrointestinal tract of roaches dissected 27 days after infection but not from nerve trunk or other parts of the body of the roaches.

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1. Carp, R., Koprowski, H., Virology, 1962, v16, 71.

2. Hsiang, C. M., Pollard, M., Micks, D. W., Texas Rep. Biol. Med., 1952, v10, 329.

3. Hurlbut, H. S., J. Infect. Dis., 1950, v86, 103.

4. Rehn, J. A. G., Sci. Month., 1945, v61, 265.

5. Rehn, J. W. H., Classification of the Blattaria as indicated by their wings (orthoptera). Mem. Am. Ent. Soc., No. 14, 1951.

6. Sailer, R. I., Common names of insects approved by Entomological Society of America. Bull. Ent. Soc. Am., 1955, v1 (4), 1.

7. Syverton, J. T., Fischer, R. G., Smith, S. A., Dow, R. P., Schoof, H. F., Fed. Proc., 1952, v11, 483.

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Uptake, Hydrolysis and Synthesis of Cholesterol Esters by a Transplantable Adrenal Cortical Tumor.* (30116)

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Cholesterol is stored principally as esters in the mammalian adrenal gland(1-3). The uptake of these esters from plasma by the gland and their hydrolysis and formation in the gland have been under investigation in this laboratory. The adrenal gland of the rat derives cholesterol, in part at least, from cholesterol esters of the very low-density chyle lipoproteins(4). Several tissues, including the adrenal gland, were shown to be capable of hydrolyzing chyle cholesterol esters(5). But the manner in which the rat adrenal gland metabolized cholesterol esters is unique(4). Twenty-four hours after an injection of C^{14}-cholesterol-labeled chyle lipoproteins in which about 75% of the sterol C^{14} was in the esterified form, approximately

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