Lack of Multiplication of Simian Virus 40 (SV40) in Experimentally Infected Mosquitoes.* (32422)

KEERTI V. SHAH, REZA BEHIN, AND LLOYD E. ROZEBOOM
(Introduced by F. B. Bang)
Department of Pathobiology, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Md.

In parts of India where rhesus monkeys live in or around human communities, simian virus 40 (SV40) infection may be transferred from rhesus to man. In a recent study(1), neutralizing antibodies to SV40 were detected in sera of 8.7% of the residents of north India living in areas of high rhesus prevalence, and in 27.0% of the animal handlers who cared for large numbers of rhesus. Infection with SV40 was common in free-living rhesus in these areas(2).

The manner in which infection is transmitted from rhesus to man is not known. One of several possibilities that may be considered is transmission by hematophagous arthropods. In the experimentally infected *Cercopithecus* monkey, viremia occurs between day 2 and day 16 following inoculation of virus(3). Man is susceptible to infection with subcutaneously administered virus(4). It was therefore of interest to know if SV40 multiplied in mosquitoes artificially infected with virus. Mosquitoes were infected either by feeding on virus-erythrocyte suspension or by parenteral injection of the virus into the hemocele, and individuals were tested at various times thereafter for presence and quantity of virus.

Materials and methods. Five species reared in our laboratory colonies were tested. *Aedes aegypti* (L.) was tested both by feeding and by parenteral injection, *Aedes albopictus* (Skuse) by feeding alone, and 3 species, *Aedes taeniorhynchus* (Wiedemann), *Culex molestus* Forsk, and *Armigeres subalbatus* (Coquillett), by parenteral injection only.

Strain A2895 of SV40, isolated originally from a hamster tumor produced by inoculation of rhesus kidney extracts(5), was obtained from Dr. B. Eddy. Prior to use in this study, the virus had undergone at various times one additional passage in hamster tumor, one passage in HeLa cells and 10 passages in *Cecropithecus* kidney cells, either primary cultures or continuous cell line B-SC-1(6). Mosquitoes were injected parenterally with virus prepared in *Cercopithecus* kidney cells, diluted 1:5 in medium 199. An estimate of the amount injected per mosquito was .0003 ml. For feeding mosquitoes, virus was mixed with fresh chicken erythrocytes as follows: a chicken was bled with a syringe wet with heparin and the blood was centrifuged at 2000 rpm for 10 minutes; supernatant serum was removed and replaced with an equal quantity of a 1:3 dilution of the tissue culture virus. Mosquitoes were starved overnight and were fed on the constantly agitated virus-erythrocyte mixture through an animal membrane(7).

Infected mosquitoes were maintained at 28°C and 78% relative humidity. At various intervals after the blood meal or injection, individual mosquitoes were withdrawn, ground up and the suspensions were titrated in B-SC-1 cells or in secondary *Cercopithecus* kidney cells, using 2 tubes per dilution. The inoculated tubes were incubated at 35-36°C in a stationary position and were observed for 21 days for characteristic SV40 cytopathic effect. Cells were maintained on medium 199 with 2% fetal calf serum and antibiotics. At least one isolate from each species was identified as SV40 by a neutralization test.

Virus titers of mosquito suspensions were adjusted to represent the total amount of virus in each individual mosquito. A virus titer of less than $10^{1.7}$ $TCID_{50}$ (tissue culture infective dose, 50% endpoint) per mosquito would be undetected in this system because of dilution factors.

Results. After feeding: The virus-erythro-

* Supported by Grant 9 RO7 TW00141-05 CIC from USPHS to Johns Hopkins Center for Medical Research and Training, Grant E440 from American Cancer Society, and USPHS Grant 5 RO1-AI00351.

TABLE I. Quantity of SV40 in Individual Mosquitoes at Different Intervals After an Infective Meal.

Species	Day after feeding 0	2	5	8	9	15	20	22
Aedes aegypti	4.2, 4.2 3.7	N, N		N, N		N, N		N, N, N
Aedes albopictus	3.7, 4.2 2.7	N, 1.7 N	N, N, N		N, N, N	N, N	N, N, N, N, N	

Figures are positive logarithms of $TCID_{50}$ titers per mosquito.
Each figure represents virus quantity in an individual mosquito.
N = negative for virus = $<10^{1.7}$ $TCID_{50}$/mosquito.

cyte suspension on which *A. aegypti* and *A. albopticus* fed had a $TCID_{50}$ titer of $10^{6.2}$/ml.

Results obtained with the two species were comparable (Table I). Virus titers ranging from $10^{2.7}$ to $10^{4.7}$ $TCID_{50}$ per mosquito, with a mean value of $10^{3.8}$, were obtained on day 0 for mosquitoes which were withdrawn soon after completion of feeding. These values indicate the amount of virus ingested by the mosquitoes. On day 2, only one of 5 mosquitoes yielded virus with a titer of $10^{1.7}$ $TCID_{50}$. From day 5 through day 22, 20 individual mosquitoes were withdrawn and tested; virus was not detected in any.

After injection: The virus dilution used for parenteral inoculation of *A. aegypti, A. taeniorhynchus, C. molestus* and *Armigeres subalbatus* contained $10^{8.7}$ $TCID_{50}$/ml. Titers of individual mosquitoes on day 0 ranged from $10^{3.2}$ to $10^{5.2}$ with a mean value of $10^{4.1}$ (Table II). These titers indicate the amount of virus injected. On day 2, virus was recovered from ten of 12 mosquitoes with titers in positive specimens ranging from $10^{1.2}$ to $10^{4.2}$ and a mean value of $10^{2.1}$ for all 12 specimens. On day 7, three of 10 specimens contained virus in low titers; the mean value for all 10 was $10^{0.4}$. On day 14, none of 21 mosquitoes yielded virus. There was no marked difference between the 4 species as to survival of virus.

Discussion. This exploratory investigation revealed no evidence of multiplication of SV40 in any of the 5 mosquito species. After inoculation or feeding of approximately $10^{4.0}$ $TCID_{50}$ of virus, virus titers decreased with time. Virus survived longer in the mosquito when it was introduced by parenteral injection than by feeding. The species of mosquitoes tested included *Aedes aegypti,* an important vector of human viral infections.

Summary. Five species of mosquitoes were tested as possible vectors of SV40 virus. The insects were infected by imbibing a virus-chicken erythrocyte suspension, or by parenteral injection of the virus. There was no evidence of multiplication of the virus in the mosquito, or that any of the species tested could serve as biological vectors.

TABLE II. Quantity of SV40 in Individual Mosquitoes at Different Intervals After Parenteral Injection.

Species	Day after injection 0	2	7	14
Aedes aegypti	4.7, 4.2, 3.7	2.2, 2.2, 1.7	1.7, 1.2	N, N, N
Aedes taeniorhynchus	3.7, 4.2, 3.2	4.2, 1.2, N	1.2, N	N N N
Culex molestus	4.7, 3.2, 5.2	2.7, 2.7, 3.7	N, N*, N	N N N N N N N N N N
Armigeres subalbatus	4.7, 4.2, 3.7	3.2, N*, 2.2	N N N	NNN, N, N
Mean	4.1	2.2	0.4	N

Figures are positive logarithms of $TCID_{50}$ titers per mosquito.
Each figure represents virus quantity in an individual mosquito.
N = negative for virus = $<10^{1.7}$ $TCID_{50}$/mosquito.
N* = negative for virus = $<10^{2.7}$ $TCID_{50}$/mosquito.

1. Shah, K. V., Proc. Soc. Exp. Biol. & Med., 1966, v121, 303.
2. Shah, K. V., Southwick, C. H., Ind. J. Med. Res., 1965, v53, 488.
3. Meyer, H. M., Jr., Hopps, H. E., Rogers, N. G., Brooks, B. E., Bernheim, B. C., Jones, W. P., Nisalak, A., Douglas, R. D., J. Immunol., 1962, v88, 796.
4. Sweet, B. H., Hilleman, M. R., Proc. Soc. Exp. Biol. & Med., 1960, v105, 420.
5. Eddy, B. E., Borman, G. S., Berkeley, W. H., Young, R. D., ibid., 1961, v107, 191.
6. Hopps, H. E., Bernheim, B. C., Nisalak, A., Tijo, J., Smadel, J., J. Immunol., 1963, v91, 416.
7. Behin, R., Mosquito News, 1967, v27, 87.

Received June 9, 1967. P.S.E.B.M., 1967, v126.

Effect of Isoproterenol on Calcium, Protein, and Electrolytes of Rat Submaxillary Gland.* (32423)

ROBERT H. DREISBACH
Department of Pharmacology, Stanford University School of Medicine, Palo Alto, Calif.

The marked effect of isoproterenol on calcium content of rat submaxillary gland(1) is an opportunity to learn something of the mechanism of storage and release of Ca. Two hypotheses have been considered as mechanisms to explain the high submaxillary gland Ca: (a) a specific one-way transfer mechanism(2) or (b) the presence of a binding substance(3). Loss of Ca after stimulation could occur by reversal of the transfer mechanism, change of pH, or by extrusion of the binding substance.

Methods. Male Long-Evans rats, 100-150 g, were prepared by once daily injection of ascending doses of isoproterenol(1). An injection-free period of 40-48 hours was then allowed before rats were used either as controls or for reinjection of 10 mg/kg isoproterenol. Rats were killed 2 hours after drug injection. Analytical methods were as follows: sialic acid(4), amylase(5), Mg(6), CO_2(7), intracellular pH using inulin as indicator of extracellular space(8), inulin (9), Na and K by direct flame photometry, total P(10), or as described previously(1-3). Statistical summaries are arithmetic mean ± standard error of the mean.

Results. Effect of isoproterenol on gland electrolytes. Reinjection of 10 mg/kg of isoproterenol 40 hours after the last injection of an ascending series of the same drug produced the alterations in electrolytes shown in Table I. Significant changes in addition to the marked loss of Ca previously reported(1) include a fall in Mg and a rise in Na and Cl.

TABLE I. Effect of Isoproterenol on Submaxillary Gland Electrolytes (μM/g water).*

	Ca	Mg	Na	K	Cl	P	CO_2
Control	28.5 ±1.4	27.0 ±1.0	42.8 ± .9	137.5 ±1.2	62.7 ±1.0	152. ±4.	21.8 ±1.2
Isoproterenol	2.3 ± .1	13.1 ± .7	53.0 ±2.9	143.0 ±3.0	72.5 ± .9	144. ±2.	20.4 ±1.3
% Change	−92	−51	+24	+4	+16	−5	−6

* All determinations, except CO_2, made on each gland from 6 rats in control (n—12) and 5 rats in experimental (n—10). CO_2 determinations made on individual glands from additional 16 rats.

Gland protein N, total N, and sialic acid content fall significantly in relation to total P but much less than Ca (Table II). In another experiment, gland amylase was found to be 91 ± 6 units/g wet weight after isoproterenol but only 13 ± 2 units/g in control rats.

TABLE II. Gland N, Protein N, Sialic Acid, and Ca (per mM total P).*

	Total N, mg	Protein N, mg	Sialic acid, μM	Calcium, μm
Control	252 ± 17	144 ± 5	46 ± 4	131 ± 4
Isoproterenol	190 ± 12	102 ± 7	22 ± 2	23 ± 5

* Determinations done on pooled glands from each rat (n=4 for both groups).

* This investigation was supported by USPHS Research Grant DE00794 from Nat. Inst. of Dental Research.