

Laboratory Studies on the Interaction between Dengue Virus and the Aedes aegypti Mosquito Vector

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Experiment No. 1 :

OBJECTIVE : To develop a suitable laboratory method for infecting A. aegypti with dengue virus.

DESCRIPTION : For this experiment we used Dengue 1 virus, New Guinea—C strain (NGC) at suckling mouse brain passage level 28, an outbred strain of 1 day old, albino mice (8 mice/litter), and 4—5 day old Aedes aegypti female mosquitoes (Koh Samui strain) reared through many generations in the laboratory. We inoculated each mouse with dengue virus intracerebrally (10^4 PFU/0.02 ml/mouse), and collected blood daily by decapitation of 5 inoculated mice. The serum was tested for virus by the direct and delayed plaque methods in MK2 cells. One litter per day of inoculated mice was observed for signs of illness. When the mice sickened, uninfected mosquitoes were allowed to feed on them; the engorged mosquitoes were kept for 14 days before virus titration.

PROGRESS : The results are shown in the following table :

	Day after virus inoculation in mice						
	day 0	day 1	day 2	day 3	day 4	day 5	day 6
1. No. mice ill	—	—	—	—	4/8*	8/8	All died
2. Viremia							
a) Direct Plaque	0/5**	0/5	0/5	2/5	5/5		
b) Delayed Plaque	0/5	0/5	0/5	2/5	5/5		

* No. mice sick/No. mice tested. **No. mice viremic/No. mice tested.

Thirty—five mosquitoes fed on sick mice on day 5. The amount of virus recovered from 0.3 ml blood obtained from each mouse ranged from 7—22 PFU. Five engorged mosquitoes were triturated on day 0, and 10 more mosquitoes were triturated on day 14 post—feeding and tested for virus. No virus was recovered from the mosquitoes. The low level viremia probably accounted for the failure to infect mosquitoes.

CONCLUSION : Suckling mice inoculated with dengue virus (New Guinea C strain) intracerebrally developed illness and low level viremia. However, A. aegypti mosquitoes which fed on these viremic mice had no evidence of dengue virus infection.

** Deceased

Experiment No. II:

OBJECTIVE: To measure the amount of virus emitted from the mouthparts of dengue-infected Aedes aegypti.

DESCRIPTION: Mosquitoes were infected by inducing them to feed on infected gibbon blood (2% citrated blood + Dengue 2, NGC) through a Baudruche membrane. Engorged mosquitoes were kept for 14 days in the insectary. Then, each presumably infected A. aegypti was immobilized by taping its wings on adhesive. A sterile capillary tube, filled with sterile 5% glucose and water plus 10% fetal bovine serum, was put over the mouthparts for 15 minutes. The mosquito may feed on this fluid and salivate. The fluid from the capillary tube was collected and tested for virus.

PROGRESS:

Mosquito No.	Virus Titer	
	<u>In Mosquito</u>	<u>In Capillary Fluid</u>
1	1.8×10^3 PFU/ml	32 PFU
2	1.6×10^3 "	0 "
3	1.8×10^2 "	0 "
4	5.7×10^2 "	0 "
5	5.2×10^1 "	0 "
6	(Not done)	0 "
7	(Not done)	0 "
8	(Not done)	0 "

The above table shows that of 5 Aedes aegypti tested, all became infected with dengue virus after feeding on infected blood under the Baudruche membrane. However, only 1 of 8 mosquitoes emitted dengue virus into capillary fluid. The amount of virus one mosquito emitted was 32 PFU.

CONCLUSION: This experiment indicates that dengue-infected mosquitoes can secrete virus during feeding. However, the number of infected mosquitoes secreting virus and the quantity of virus excreted was small.

Experiment No. III:

OBJECTIVE: To determine if small plaque Dengue-2 virus (BKM-551) can infect mosquitoes.

DESCRIPTION: In previous experiments, a large plaque variant of BKM-551 passaged in LLC-MK 2 cells was shown to infect A. aegypti (Koh Samui Strain) mosquitoes using the membrane feeding technique. However, in two similar experiments, A. aegypti mosquitoes were not infected by the small plaque variant of BKM-551. A third attempt was made to infect these mosquitoes with the small plaque variant by the membrane feeding technique. Engorged mosquitoes were collected; mosquitoes were titrated by the direct and delayed plaque method in LLC-MK2 cells on days 0, 3, 7, 10 and 14 after engorgement.

PROGRESS: No virus was recovered.

CONCLUSION: Repeated attempts to demonstrate infection of A. aegypti by small-plaque Dengue 2 virus (BKM-551, SP) were unsuccessful using the membrane feeding technique, whereas large plaque Dengue 2 virus (BKM-551, LP) could infect A. aegypti using the same technique. It thus appears unlikely that small plaque dengue virus can infect mosquitoes and be transmitted to man.