TABLE II. I^{131} and S^{35} Outputs in 72-Hour Stools in 5 Patients with Ulcerative Colitis Studied during Different Stages of the Disease with Oleic Acid-I^{131} and Methionine-S^{35}.

Patient	Date of study	Stage of disease	% test dose I^{131}	% test dose S^{35}
S.C.	* 1/13/56	Acute	7.6	39.9
	* 3/ 6	Convalesc.	9.6	17.6
	4/15	Remission		13.7
	*11/29	Acute	13.0	64.9
	* 2/26/57	Ileost.-colec.	12.1	17.7
C.P.	* 9/16/56	Mod. activity	6.3	23.6
	*12/ 1	Remission	9.9	12.5
H.J.	* 6/21	Acute	7.8	29.6
	*12/ 1	Remission	5.1	20.0
P.K.	3/12	Acute	20.3	
	7/ 1	"		57.1
	* 1/29/57	Remission	8.8	13.6
J.L.	11/ 2/56	Acute		51.6
	1/31/57	Remission		17.6
	3/ 1	"	10.9	

* In these patients the methionine-S^{35} study was begun on the specified date and the 72-hr study with oleic acid-I^{131} was begun 4 days later.

tients improved clinically. Following administration of methionine-S^{35}, fecal excretion of S^{35} increased during the acute phase of the disease to a greater degree than the excretion of I^{131}. Also, as the patients improved clinically excretion in the feces returned toward control values only slowly, with the result that during the remission stage of the disease excretion of S^{35} was still elevated and it was elevated in all ulcerative colitis patients studied to date including those with ileostomy and colectomy.

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Multiplication of Eastern Equine Encephalitis Virus in Mosquitoes Following Intrathoracic Inoculation.* (23187)

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Infection of mosquitoes with arthropod-borne encephalitis viruses by intrathoracic inoculation has been demonstrated (1,2).† Because minimal concentrations of viruses are sufficient to infect mosquitoes by this route, this method appears to be excellent for studying rates of virus development within mosquitoes, uncomplicated by chemical or physical barriers to infection encountered in the digestive tract.

In the present study the intrathoracic inoculation technic was used to determine rate of multiplication of eastern equine encephalitis (EEE) virus in *Aedes triseriatus* mosquitoes.

Methods. The mosquitoes used were *Aedes triseriatus* 3 to 6 days old. They were anesthetized by 15 to 20 seconds' exposure to carbon dioxide gas immediately prior to inoculation. Method of inoculation was similar

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† L. Whitman, personal communication.

TABLE I. Multiplication of EEE Virus in *A. triseriatus* Mosquitoes following Intrathoracic Inoculation.

Hrs incubation	Mouse i.c. LD_{50} recovered/mosquito following inoc. with: 300 mouse LD_{50}	.3 mouse LD_{50}
0	50 - 300	<30
8	300 - 1,250	<30
18	1,600 -16,000	<30
26	16 - 160*	30 - 800
42	63 - 1,000*	16 - 200*
66	200 - 3,000*	60 - 600*
90	160 - 1,000*	50 -1,600*
168	200 - 1,000*	300 -2,000*

* × 1000.

to that of Whitman.[†] A piece of 4 mm glass tubing was drawn out in a flame, then the drawn section was heated in a tiny flame and drawn out again. The tip was snapped off at approximately 75 μ diameter. A piece of rubber tubing was attached to the undrawn end and infected fluid was drawn into the capillary by sucking on this rubber tubing. The fluid was ejected after insertion of the capillary tip into the side of a mosquito thorax by clamping the end of the rubber tube and squeezing the mid-part of the tube in the hand. The EEE virus strain used had been isolated from wild-caught mosquitoes(3) and had undergone 2 mouse brain, 1 chick embryo, and 7 serial mosquito intrathoracic passages. Individual mosquitoes in 2 groups were inoculated intrathoracicly with approximately 0.0003 ml each of either a 10^{-1} or 10^{-4} dilution of EEE-infected mosquito suspension. The 10^{-1} inoculum contained approximately 300 mouse intracerebral LD_{50}, and the 10^{-4} inoculum, about 0.3 mouse intracerebral LD_{50}. Five mosquitoes of each group were then incubated at 80°F and 75% relative humidity for the following periods: 0 hr, 8 hrs, 18 hrs, 26 hrs, 42 hrs, 66 hrs, 90 hrs, and 168 hrs. After incubation they were killed by freezing, ground individually in 1 ml of 25% normal horse serum in buffered water, pH 7.7, and titrated by intracerebral inoculation of .03 ml amounts into 3-week-old white Swiss mice. The amount of virus in each mosquito was calculated to be 33 times the titer of the 1 ml mosquito suspensions.

Results. The results are given in Table I. Apparently nearly a 10-fold increase of virus occurred each 8 hours. About 8 to 9 eight-hour periods were required for the dilute-inoculum series to reach peak virus levels of approximately 1,000,000 - 2,000,000 mouse LD_{50} units per mosquito, while the concentrated-inoculum series required only about 5 of these periods. The nearly straight-line relationship between rates of virus multiplication in the dilute- and concentrated-inocula groups suggests that no appreciable initial lag period followed inoculation and that multiplication occurred cyclically. Recovery of amounts of virus varying from 50 to 300 mouse LD_{50} from mosquitoes immediately following inoculation of an estimated 300 LD_{50} shows close approximation of dosages given.

Summary. Known amounts of eastern equine encephalomyelitis virus were inoculated into the thorax of *Aedes triseriatus* mosquitoes. Individual mosquitoes were ground and tested quantitatively for virus content after various periods of incubation. Approximately a 10-fold increase in virus occurred every 8 hours until maximum virus levels were attained.

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