the antibodies to albumin absorbed from the gut.

To determine whether the elimination of albumin from the hemolymph disturbs the osmoregulation of the flies, as indicated by the suppression of crop emptying and primary excretion, we analyzed the sodium and potassium concentrations of the hemolyph. Hemolymph from about 20 flies was pooled and analyzed with a spectrophotometer (Eppendorf, FCM 6342); results were compared with those of a control group (Table 1). The concentrations of both sodium and potassium were greatly increased in the experimental flies. Consequently the osmotic pressure of the hemolymph was increased. As these parameters are very constant in tsetse flies, even during rapid diuresis (*9*), which follows immediately after the ingestion of a blood meal, it is apparent that the absence of the albumin fraction harms the osmoregulatory capacities of the flies.

The possibility that the albumin-free antiserum itself had a harmful effect on the flies was tested in a control experiment with flies that had previously been fed on bovine blood. These flies did not show any visible disturbance after engorgement with the antiserum.

These experiments show that it may be possible to use antibodies as a biological insecticide. If a proper antigen is selected for the production of antibodies, a single blood meal is lethal. Although albumin is an easily available antigen, the use of antibodies to albumin is not practical because the antiserum is effective only if its own albumin is removed. If flies are fed directly on the ear of the immunized rabbit, the absorption of antibodies to human albumin can be compensated for by the absorption of rabbit albumin. It may be possible to use antibodies for insect control providing antigens are found that give rise to antibodies that interfere with the metabolism of a target insect and that can be utilized on a large scale.

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Carbon Dioxide Sensitivity of Mosquitoes Infected with California Encephalitis Virus

Abstract. *Four species of mosquitoes became sensitive to carbon dioxide approximately 3 to 4 days after they received intrathoracic injections of California encephalitis virus.* Aedes melanimon *and* Aedes dorsalis *infected orally with California encephalitis virus also became carbon dioxide-sensitive, but mosquitoes infected transovarially did not. Sensitivity to carbon dioxide was inhibited by antiserum to California encephalitis virus. To our knowledge this is the first report of carbon dioxide sensitivity induced in arthropods by a bunyavirus and the first demonstration of this phenomenon by an arbovirus in its proven vector.*

It has long been recognized that fruit flies of the species *Drosophila melanogaster*, when infected with certain viruses, become paralyzed if they are exposed to CO_2 (*1*). The effect caused by sigma virus has been studied the most (*2*); but other rhabdoviruses also induce CO_2 sensitivity—vesicular stomatitis (VS) (*3*), Piry (*4*), Chandipura (*4*), spring viremia of carp (SVC) (*5*), and pike fry rhabdovirus (PFR) (*5*). Iota virus, a member of the family Picornaviridae, also causes CO_2 sensitivity in male *D. melanogaster* (*6*).

In our routine use of CO_2 to anesthetize mosquitoes during arboviral studies we noticed that *Aedes dorsalis* infected with California encephalitis (CE) virus (Bunyaviridae) frequently failed to revive after CO_2 anesthesia. This suggested that CE virus had induced CO_2 sensitivity in mosquitoes and a study of this phenomenon was initiated.

Six isolates of CE virus from *Aedes melanimon* were pooled (each isolate had been passed one time in *A. dorsalis*); this virus is here referred to as CE-WT. Mosquitoes were infected with CE-WT virus by intrathoracic inoculation (*7*), ingestion from gauze pledgets soaked with a suspension of virus in defibrinated rabbit blood and sucrose, or transovarial transmission. After infection or hatching of the eggs all mosquitoes were held in an insectary maintained at 27°C and a relative humidity of 80 percent.

Adult mosquitoes were tested for sensitivity 4 to 10 days after intrathoracic inoculation by introducing CO_2 into the 0.5-liter cardboard holding containers for approximately 20 seconds at ambient room temperature (22°C). The CO_2 was from a pressurized cylinder and was humidified by bubbling through water. Nonsensitive mosquitoes revived within a few minutes after removal from the CO_2 atmosphere, and by 5 to 10 minutes after exposure they showed no noticeable effect. The CO_2-sensitive mosquitoes continued to lie on their backs or sides, occasionally with leg or wing movements, and were unable to right themselves. In some experiments, particularly those done three days or less after inoculation at 15 or more days after infection, some of the mosquitoes were able to get back on their feet and walk but could not fly, or would "hop" and then fall. These mosquitoes were designated questionably sensitive (+/−). The infection status of each mosquito was determined by plaque assay in Vero cells (*8*).

The CE virus produced CO_2 sensitivity in all mosquito species that had been infected by intrathoracic inoculation 4 to 10 days previously. These species included: *A. dorsalis*, *A. melanimon*, *Aedes triseriatus*, and *Culex tarsalis* (Table 1). Sensitivity to CO_2 occurred in both field-collected and laboratory-colonized mosquitoes, indicating that CO_2 sensitivity was not an artifact of laboratory colonization.

Since intrathoracic inoculation is an abnormal route of infection, *A. dorsalis* and *A. melanimon* were infected by al-

Table 1. Carbon dioxide sensitivity in *Aedes* and *Culex* mosquitoes infected with CE virus. Infection status was determined by plaque assay in Vero cells, and CO_2 sensitivity was determined 4 to 10 days after intrathoracic inoculation and 9 to 28 days after pledget feeding. Symbols: +, sensitive; +/−, questionable; −, nonsensitive.

Mosquito species	Infection status	Number of mosquitoes CO_2-sensitive		
		+	+/−	−
Infection by intrathoracic inoculation				
*A. dorsalis**	+	30	0	0
*A. dorsalis**	0	0	0	38
A. dorsalis†	+	11	0	0
A. dorsalis†	0	0	0	20
A. melanimon†	+	8	0	0
A. melanimon†	0	0	0	8
*A. triseriatus**	+	10	0	1
*A. triseriatus**	0	0	0	16
*C. tarsalis**	+	21	0	1
*C. tarsalis**	0	1	0	34
Infection by feeding on gauze pledgets				
*A. dorsalis**	+	10	1	1
*A. dorsalis**	0	0	0	8
A. melanimon†	+	5	2	2
A. melanimon†	0	0	1	37

*Laboratory colony. †Field-collected adults.

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lowing them to ingest CE virus from gauze pledgets. When these mosquitoes were tested for CO_2 sensitivity we found that all the CO_2-sensitive mosquitoes were infected with CE virus; however, both +/− and misclassified mosquitoes were more common than they had been in mosquitoes infected by intrathoracic inoculation (Table 1). One possible explanation for the variation was that the mosquitoes received a relatively low dose of virus from the gauze pledgets. Kramer *et al.* (*9*) have shown that infection with western equine encephalomyelitis virus may be restricted to the midgut in mosquitoes that feed on low infectious doses. Thus, mosquitoes that became infected after ingesting a low viral dose would be nonsensitive to CO_2 if the virus had failed to escape the midgut and infect the organ system responsible for CO_2 sensitivity. Studies on sigma virus have shown that infection of the thoracic ganglion is necessary before CO_2 sensitivity will develop (*10*). A second possible explanation is that CO_2 sensitivity may vary over a range of incubation periods. In the present study incubation periods ranged from 9 to 28 days. Nearly all the mosquitoes inoculated intrathoracically were CO_2-sensitive 4 to 10 days after inoculation, but 9 out of 44 tested at 15 or more days were +/−, and 8 out of 42 tested at 17 or more days were nonsensitive. Thus, the +/− and nonsensitive mosquitoes might have been sensitive if they had been tested earlier.

Bussereau (*4*) found that rhabdovirus infections in *D. melanogaster* fell into two groups with respect to their effect on CO_2 sensitivity. The first group included sigma, SVC, PFR, and Piry virus infections that produced lifelong sensitivity to CO_2; the second group included infections caused by the VS serotype (Argentina, Brazil, Cocal, Indiana, and New Jersey) and Chandipura viruses that produced sensitivity after an appropriate incubation period. However, if the flies infected with VS or Chandipura viruses were held sufficiently long before they were exposed to CO_2, they reverted back to a nonsensitive state. This situation was observed with CE virus.

Sensitivity to CO_2 was not observed in transovarially infected progeny of female *A. dorsalis* or *A. melanimon* that had been infected with CE-WT virus by intrathoracic inoculation. No explanation can be offered for their lack of sensitivity.

It was important to determine if the CO_2 sensitivity was due to infection with CE virus or to the presence of a sigma-like agent in the CE-WT virus suspension. Since it was unlikely that field-collected mosquitoes would all contain the same contaminant, we tested individually the isolates of CE virus from three separate pools of collected *A. melanimon* (each isolate was passed one time in *A. dorsalis*). All of these isolates produced CO_2 sensitivity. As a further test, mouse antiserum to a plaque-purified clone of the BFS 283 strain of CE virus was used to neutralize CE-WT virus. Mosquitoes inoculated with CE-WT virus alone became infected and developed CO_2 sensitivity, while those inoculated with neutralized CE virus or with antiserum alone were neither infected nor CO_2-sensitive (Table 2). Thus, CO_2 sensitivity appears to be related specifically to infection with CE virus.

Table 2. Carbon dioxide sensitivity in *Aedes dorsalis* 7 days after inoculation with CE virus or CE virus plus CE antiserum. Infection status was determined by plaque assay in Vero cells.

Inoculum	Infection status	CO_2-sensitive	
		Yes	No
CE virus	+	10	0
CE virus plus CE antiserum	0	0	15
CE antiserum	0	0	8

When groups of infected and uninfected *A. dorsalis* and *C. tarsalis* were anesthetized with chloroform all the mosquitoes recovered, indicating that the sensitivity produced by CE infection is related to the CO_2.

The following questions remain to be answered. (i) Do other diptera infected with CE virus become CO_2-sensitive? (ii) Does CO_2 sensitivity extend to other members of the CE serogroup and to other bunyaviruses? (iii) Why were transovarially infected mosquitoes not CO_2-sensitive? (iv) What is the mechanism of CO_2 sensitivity in mosquitoes?

While this manuscript was in preparation, Rosen (*11*) reported results which extended the range of arthropods which become CO_2-sensitive following infection with a rhabdovirus from *Drosophila* to mosquitoes. The current study further extends our knowledge of virus-induced CO_2 sensitivity in arthropods by demonstrating that such sensitivity can be produced by a virus of the family Bunyaviridae and can occur in the natural mosquito vectors of CE virus, *A. melanimon* and *A. dorsalis* (*12*).

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Juvocimenes: Potent Juvenile Hormone Mimics from Sweet Basil

Abstract. *Two compounds with highly potent juvenile hormone activity were isolated and identified from the oil of sweet basil,* Ocimum basilicum *L., Labiatae.*

During investigations of plants as sources of molecular models to implement the development of biorational chemicals for insect control we found that a commercial distillate of the herb sweet basil (*1*) possessed juvenile hormone activity. From 150 g of distillate we isolated by high vacuum distillation and subsequent open column and high performance liquid chromatography about 0.75 mg of each of two substances which were active in the milkweed bug juvenile hormone test (*2*) in the picogram range. The mass spectra of the unknown compounds designated **1** and **2** gave molecular ions at mass to charge (*m/e*) 282 and 298, respectively (*3*).

The nuclear magnetic resonance (NMR) spectra of compound **1** exhibited 26 protons and indicated the presence of *p*-methoxycinnamyl, isobutenyl, and methylbutadienyl moieties (*4*). These three visualized fragments appeared to be attached to a single methine carbon atom suggesting the provisional structure **1** shown in Fig. 1 (*5*). The NMR

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