sulin secretion is correlated with the change in the gap junctions. It is also uncertain whether the increase in the number and size of gap junctions is a cause or a consequence of the modified secretory activity of the B cells. That hormones can modulate the development of gap junctions has been reported for other secretory systems (*13*).

If the gap junctions evaluated between B cells are permeable, the likely functional consequence of their increase is an enhancement of the exchange of ions and small molecules (ionic and metabolic coupling) between adjacent stimulated B cells (*9, 14*). Since there is morphological evidence that the gap junctions of glibenclamid-treated islets are permeable structures (*15*), the correlation observed between the development of gap junctions and the functional state of B cells indicates (*1-3*) that a modulation of direct intercellular communication may participate in the regulatory system by which B cells adjust their level of activity in relation to need.

P. MEDA
Institute of Histology and Embryology, Geneva University School of Medicine, 1211 Geneva 4, Switzerland
P. HALBAN
Institute of Clinical Biochemistry, Geneva University School of Medicine
A. PERRELET
Institute of Histology and Embryology, Geneva University School of Medicine
A. E. RENOLD
Institute of Clinical Biochemistry, Geneva University School of Medicine
L. ORCI
Institute of Histology and Embryology, Geneva University School of Medicine

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6. For each experiment, five batches of ten isolated islets were washed three times with cold Krebs-Ringer bicarbonate buffer containing 0.5 percent bovine serum albumin and were subsequently dispersed into 1 ml of acid ethanol (ethanol, water, and HCl, 140:57:3 by volume) at 4°C. Rat insulin was used as standard for radioimmunoassay of acid ethanol extracts with guinea pig antibody to porcine insulin.
7. For freeze-fracture, batches of 150 to 200 islets were centrifuged into pellets and fixed for 60 minutes in 0.1*M* phosphate-buffered glutaraldehyde (2 percent). After being rinsed several times in phosphate buffer, the pellets were infiltrated for 60 minutes in 30 percent phosphate-buffered glycerol and frozen in Freon 22 that had been cooled with liquid nitrogen. The pellets were subsequently fractured and shadowed in a Balzers BAF 301 apparatus. The replicas obtained were washed in a sodium hypochlorite solution, rinsed in distilled water, and mounted on copper grids. They were examined with a Philips 301 electron microscope whose magnifications were calibrated with a grid containing 2160 lines per millimeter. For each experimental condition, eight to ten islets were randomly selected in at least three replicas, each obtained from a different experiment. The B cells and their gap junctions were identified in the fractured islets by previously discussed criteria (*2*). Gap junctions found on randomly selected P-fracture faces were photographed at a fixed magnification of ×24,400. The number of their constitutive particles was counted on negatives enlarged 4.5 times by projection on a screen. Gap junction areas were estimated by multiplying this number by 10^{-4} μm^2, the average area of a single gap junction particle (*2*). The areas of B cell plasma membranes were measured on ×23,000 enlarged micrographs with a graphics tablet (Tektronix 4953) connected with an IMSAI 8080 microcomputer system (*8*). Gap junction size is expressed in medians because of the highly asymmetric distribution of gap junctions (*2*). Accordingly, statistical comparisons were done using either Student's unpaired *t*-test or the median test.
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10. For each experimental group, dissociated islet cells were prepared by incubating isolated islets of Langerhans for 60 minutes at 37°C in a bicarbonate-buffered Ca^{2+}-free medium containing Pronase (4 μg/ml) and 1 m*M* [ethylenebis-(oxyethylenenitrilo)]tetraacetic acid (EGTA). The diameter of the dissociated spherical cells was measured on ×1125 enlarged phase-contrast photographs of semithin sections, and its corrected value (*11*) was used to estimate the mean B cell surface and volume.
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Specific Antibodies: A Potential Insecticide

Abstract. *When tsetse flies are fed on human blood, the hemolymph of the flies contains human albumin. If the flies then ingest antibodies to human albumin, they die within a short time. The albumin fraction in their hemolymph disappears and osmoregulation is severely disturbed.*

Many insects are able to absorb orally administered antibodies. Antibodies to muscles or nerves of the flesh fly *Sarcophaga falculata* Pand., when fed to the flies, were found to be attached specifically to the tissue that had served as antigen (*1*) and interfered with the growth and function of these tissues (*2*). Antibodies to symbiotic microorganisms of the tsetse fly *Glossina morsitans morsitans* Westwood, when fed to the flies, were absorbed by the mycetocytes, specialized midgut cells, and caused elimination of the symbionts (*3*).

These results suggested the possibility of using antibodies as a potential tool for the control of blood-feeding insects, for example, tsetse flies. Albumin was selected as an antigen for the following reasons. (i) Albumin is a constituent of the diet of tsetse flies that is essential for their reproduction (*4, 5*). (ii) Tsetse flies are able to absorb undigested albumin from the gut into the hemolymph (*6*). Flies fed on human blood contain human albumin in their hemolymph. Human albumin disappears from the hemolymph and is replaced by bovine albumin when the flies are fed on bovine blood. (iii) Albumin is an easily available antigen.

Table 1. Ionic concentrations and osmotic pressure in the hemolymph of experimental and control flies (about 20 flies in each group).

	Experimental flies	Control flies
Sodium (m*M*)	360	150.7
Potassium (m*M*)	80	3.32
Osmotic pressure (mosmole)	620	345

An antiserum was produced by injecting human albumin intramuscularly into rabbits. Injections were repeated at intervals until an antibody titer of 1:40 was achieved (as indicated by Ouchterlony tests). After the antiserum had been withdrawn from the rabbit, the albumin was removed by affinity chromatography with Affigel Blue (Bio-Rad). This albumin-free antiserum was fed to tsetse flies, *G. m. morsitans*, through a membrane feeding system (*7*), with the use of a silicone rubber membrane (*8*). All of the flies that had previously been fed on human blood, and therefore contained a human albumin fraction in their hemolymph, died within 2 hours after becoming engorged with the antiserum. Their metabolism seemed to be severely disturbed, since the transport of the meal from the crop to the midgut was suppressed and no primary excretion could be observed. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis showed that the albumin fraction was eliminated from the flies' hemolymph, apparently as a result of precipitation by

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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.

G. NOGGE
M. GIANNETTI
Institut für Angewandte Zoologie, D 5300 Bonn-1, West Germany

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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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