9. We estimated the initial P present in the young plants by regressing total P on fresh weight for a dozen similar plants collected in the same area; the resulting correlation coefficients (r^2) were greater than .90.
10. The mean available sediment P specific activity was calculated by integration, within the growth period, of the regression best fitting the observed specific activity versus time values. The best-fitting regressions were of the form: $y = \ln(t) + c$, where t is time and c is a constant; r^2 was always greater than .96. This method assumes a constant uptake rate of P with time.
11. R. Carignan and J. Kalff, in preparation. Our results for *Myriophyllum* show that, although macrophyte-excreted P is readily available to epiphytes and algae, excretion rates are very low.
12. Supported by grants from the Québec Department of Education, the Inland Water Directorate of Environment Canada to the Memphremagog Project, and predoctoral scholarships from the Direction Générale de l'Enseignement Supérieur du Québec and the National Sciences and Engineering Research Council of Canada to R.C. We thank Dr. D. Planas for providing SRP data for Rivière-du-Sud.
* Present address: Canada Center for Inland Waters, Burlington, Ontario, Canada L7R 4A6.

27 August 1979; revised 5 November 1979

Carbon Dioxide Sensitivity in Mosquitoes Infected with Sigma, Vesicular Stomatitis, and Other Rhabdoviruses

Abstract. *Carbon dioxide, usually an innocuous narcotic for insects, kills mosquitoes infected with rhabdoviruses. Such toxicity was originally observed in* Drosophila *harboring a hereditary virus, sigma, and has been considered unique to* Drosophila. *The new findings support the possibility that insects with piercing and sucking mouthparts harbor similar hereditary viruses and transmit some of them to vertebrates or plants.*

Approximately 40 years ago it was discovered that a certain laboratory strain of the fruitfly *Drosophila melanogaster* failed to recover as expected from the narcotic effect of CO_2 when returned to air (*1*). It soon was demonstrated that this "sensitivity" to CO_2 in the stock was inherited but, surprisingly, not in a Mendelian manner (*2*). Later it was shown that the CO_2 sensitivity could be transferred to normally "resistant" *D. melanogaster* by transplantation of organs or injection of hemolymph, but was not contagious in the usual sense (*3*). Eventually it was shown by filtration and other techniques that the CO_2 sensitivity was conferred by a replicating particle in the size range of a virus, which was named sigma. Electron microscopy revealed that sigma virus had the bullet-shaped morphology characteristic of rhabdoviruses (*4*). Sigma virus is not cytopathogenic for cell cultures, and viral particles are difficult to demonstrate by electron microscopy. Consequently, the only practical way to detect the virus is by assay in *D. melanogaster*. Transmissible CO_2 sensitivity has been found in wild *D. melanogaster* (*5*) as well as in other species of *Drosophila* (*6*), but never in any other kind of insect. The physiology of the CO_2 toxicity has not been explained, although it has been linked to replication of virus in the thoracic ganglia (*7*).

The recent demonstration of generation to generation (vertical) transmission by mosquitoes (*8*) and sandflies (*9*) of viruses affecting man and domestic animals has directed the attention of epidemiologists to the well-studied sigma-*Drosophila* relationship. Two aspects are of particular interest. The first is the mechanism that assures the survival of sigma virus in *D. melanogaster*. Since the virus is not always transmitted to 100 percent of the next generation, its persistence in nature (and in laboratory colonies) suggests that it confers some advantage on its insect host. The second is the ultra-rho form of sigma virus that is transmitted from generation to generation in *D. melanogaster* in an inapparent form (*10*). It is important to know if such masked forms occur among the viruses transmitted to vertebrates and plants by insects when evaluating the possible role of vertical transmission in their epidemiology.

When injected into certain species of mosquitoes, sigma virus was found to replicate but CO_2 sensitivity was not induced nor was vertical transmission of the virus observed (*11*). (Replication in mosquitoes was demonstrated by assay of their triturated bodies in *D. melanogaster*.) In the work reported here the objective was to compare the vertical transmission of sigma virus with that of viruses transmitted by insects to vertebrates in a common insect host. In the course of the work it was found that sigma virus not only replicated in the mosquito species chosen for study but also rendered them lethally sensitive to CO_2. Mosquitoes then were infected with vesicular stomatitis and other rhabdoviruses since such viruses were known to confer CO_2 sensitivity on *D. melanogaster* (*12*).

The sigma virus was obtained from a laboratory colony of *D. melanogaster* started from a single fertilized female captured in nature in Honolulu, Hawaii, in 1978. The descendants of this fly were uniformly sensitive to CO_2 (and not to N_2) in each generation tested. Their longevity and fertility were not different from those of the CO_2-insensitive *D. melanogaster* captured at the same time. Their CO_2 sensitivity could be transmitted to insensitive *D. melanogaster* by injection. Thus they met the criteria of a line "stabilized" for sigma virus (*6*). A virus-containing suspension was prepared by triturating 1.96 g of pooled flies from the sensitive colony in 10.0 ml of heated (56°C for 30 minutes) bovine serum. The suspension was centrifuged to remove large particulate matter, filtered through a Millipore filter with an average pore diameter of 450 nm, and stored in equal portions at −70°C. Filtered material was inoculated intrathoracically (*13*) in 0.17-μl amounts into *Toxorhynchites amboinensis* mosquitoes from a laboratory colony. These mosquitoes were held at 20°C and different groups were tested at 7, 14, 21, and 28 days after inoculation for CO_2 sensitivity. The mosquitoes were exposed to pure CO_2 for 15 minutes at a temperature of 13° to 15°C and then returned to air at room temperature. The number of mosquitoes that failed to recover was determined several hours later and again the next day. In this experiment the numbers of mosquitoes that were CO_2-sensitive at the four time intervals after inoculation were 3 of 12, 23 of 26, 22 of 24, and 18 of 18, respectively. All of the uninoculated control mosquitoes for the same time periods recovered normally. The sigma virus suspension could be diluted as much as 1000-fold and still induce CO_2 sensitivity in at least one-half of the *T. amboinensis* inoculated. Sensitivity to CO_2 could also be induced by sigma in *Culex quinquefasciatus* mosquitoes from a laboratory colony, but not in colonized *Aedes albopictus* mosquitoes. Both *T. amboinensis* and *C. quinquefasciatus*, when inoculated with the same amount of sigma virus, recovered normally from narcosis with N_2.

The same three species of mosquitoes were inoculated with a variety of other rhabdoviruses and three other mosquito-borne viruses to determine whether CO_2 sensitivity would ensue. Table 1 shows that all rhabdoviruses tested, with the exception of VSV-Indiana (vesicular stomatitis virus), induced such sensitivity in one or more mosquito species. For the tests summarized in Table 1 the mosquitoes were held for 7 or 8 days at 32°C after inoculation. This temperature is

0036-8075/80/0229-0989$00.50/0

Table 1. Lethal sensitivity of *T. amboinensis*, *C. quinquefasciatus*, and *A. albopictus* mosquitoes to CO_2 after intrathoracic inoculation of viruses. All mosquitoes were held at 32°C for 7 or 8 days after inoculation before being tested for CO_2 sensitivity, except as noted. At least six mosquitoes were tested for each mosquito-virus combination. Symbols: +, all mosquitoes tested were sensitive except as indicated in parentheses (number sensitive/number tested); −, all mosquitoes tested were insensitive; NT, not tested. All mosquitoes were from colonized stocks (*23*).

Virus	Toxorhynchites (♀, ♂)	Culex (♀)	Aedes (♀)
Vesicular stomatitis group			
VSV-Indiana	−	−	−
VSV-New Jersey	+	+	+
Chandipura	+	+	+
Cocal	+	+ (8/9)	−
Isfahan	+	+ (4/8)	−
Piry	+	+ (5/10)	+
Other rhabdoviruses			
Flanders	−	+ (10/12)*	−
Joinjakaka	+ (8/9)*	+ (3/8)*	+
Gray Lodge	+	+ (5/6)*	+
Flaviviruses			
Dengue type 2	−†	NT	NT
Japanese encephalitis	−†	NT	NT
St. Louis encephalitis	−†	NT	NT

*All mosquitoes were sensitive in next passage in same mosquito species. †Tested both at 6 and 13 days after inoculation.

considerably higher and the time interval shorter than those found suitable for the demonstration of sigma virus–induced CO_2 sensitivity. When held at 32°C, *T. amboinensis* inoculated with VSV-New Jersey were uniformly sensitive as soon as 4 days after inoculation (the shortest period tested).

Linkage of CO_2 sensitivity with replication of the inoculated virus was demonstrated in two instances as follows. Serial tenfold dilutions of a pool of virus prepared from the second passage of VSV-New Jersey in *T. amboinensis* were inoculated into groups of *T. amboinensis*, and the latter were tested for CO_2 sensitivity after being held for 6 days at 32°C. After the CO_2 test, the squashed heads of all mosquitoes were examined (in a blind test) by an indirect fluorescent antibody technique for the presence of VSV-New Jersey viral antigen (*14*). Complete correlation was found between sensitivity to CO_2 and the presence of viral antigen. Similarly, in the case of Joinjakaka virus, the presence of CO_2 sensitivity in some of the *T. amboinensis* and *C. quinquefasciatus* inoculated initially (shown in Table 1) was correlated with the presence of Joinjakaka viral antigen in squashed heads, and CO_2 resistance with its absence. As with sigma virus. *T. amboinensis* mosquitoes inoculated with the same amount of VSV-New Jersey as those found sensitive to CO_2 recovered normally from N_2 anesthesia.

Some differences in degree of CO_2 sensitivity were observed. For example, *T. amboinensis* mosquitoes inoculated with an undiluted VSV-New Jersey suspension showed no movement whatsoever after removal from the CO_2 atmosphere; the same mosquito species inoculated with the undiluted sigma virus suspension sometimes showed feeble, disoriented movement when returned to air. In both instances, however, the mosquitoes eventually died.

The failure to observe CO_2 sensitivity in mosquitoes with a particular rhabdovirus is probably attributable to poor replication of the virus in the mosquito species. At least some strains of VSV-Indiana are known to replicate poorly in mosquitoes on initial inoculation (*15*). However, the *T. amboinensis* inoculated with the flaviviruses and found insensitive to CO_2 contained large amounts of infectious virus.

The rhabdovirus family includes viruses that multiply in insects, vertebrates, and higher plants (*16*). Some multiply in both insects and vertebrates and some in both insects and plants. None is known to multiply in all three types of host. The demonstration that a variety of rhabdoviruses can induce lethal CO_2 sensitivity in mosquitoes suggests that such sensitivity, heretofore considered unique to *Drosophila*, is probably widespread among other insects infected with similar viruses. Since virus-induced CO_2 sensitivity is not limited to *Drosophila*, it is unlikely that viruses analogous to sigma are limited to this genus of insect. This raises the question of whether any such viruses are among those that can be transmitted by their insect hosts to vertebrates or plants in the course of feeding and, particularly, whether they include viruses that produce disease in economically important vertebrates or plants. If so, insect transmission of the virus to vertebrates or plants could represent a biologic and epidemiologic dead end irrelevant to continued propagation of the agent.

One or more viruses of the vesicular stomatitis group may be candidates for such a life history. The epidemiology of vesicular stomatitis is compatible with insect transmission (*17*), the viruses have been recovered from insects collected in nature (*18*), and the viruses can be transmitted by insects to vertebrates experimentally (*19*). However, vertebrates generally do not manifest viremias high enough to serve as a source of infection for insects (*20*). One virus of the group was shown to be transmitted vertically in some of the sandflies infected in the laboratory (*9*), and it is possible that the virus is maintained exclusively by heredity in its insect host. Many factors affect vertical transmission of sigma in *D. melanogaster* in laboratory studies (*6*). Several rhabdoviruses that produce disease in plants (for example, lettuce necrotic yellows virus and potato yellow dwarf virus) are transmitted to the plants by insects that also transmit the viruses vertically (*21*).

The recognition that CO_2 sensitivity induced by viruses is not limited to *Drosophila* suggests that CO_2 might be useful to detect viruses not easily recognized by other methods. It would be possible to screen large numbers of field-caught or laboratory-reared insects in this way. Although viruses other than rhabdoviruses can induce CO_2 sensitivity in *D. melanogaster* (*22*), their effects are different in that they are lethal in *D. melanogaster* and the sensitivity they induce is not limited to CO_2 (it also occurs with other gases) and is detectable only several days before insect death.

The fact that transmissible CO_2 sensitivity has not been observed (or at least attracted attention) previously in mosquitoes or other insects anesthetized with CO_2 may be explained by the critical importance of temperature. To observe the lethal effect of CO_2 on sensitive insects in an unequivocal manner the temperature at which they are exposed to the gas must be lower than that in most laboratories. The physiology of CO_2 sensitivity in insects is worthy of further study and the large size of *T. amboinensis* compared to *D. melanogaster* should facilitate such investigations.

LEON ROSEN
Pacific Research Unit, Research Corporation of the University of Hawaii, Honolulu 96806

References and Notes

1. P. L'Héritier and G. Teissier, *C. R. Acad. Sci. (Paris)* **205**, 1099 (1937).
2. ———, *ibid.* **206**, 1683 (1938).
3. P. L'Héritier and F. Hugon de Scoeux, *Bull. Biol. Fr. Belg.* **81**, 70 (1947).
4. A. Berkaloff, J. C. Bregliano, A. Ohanessian, *C. R. Acad. Sci. (Paris)* **260**, 5956 (1965).
5. A. Fleuriet, *C. R. Séances Soc. Biol.* **166**, 598 (1972).
6. G. Brun and N. Plus, in *Genetics and Biology of Drosophila*, M. Ashburner and E. Novitski, Eds. (Academic Press, New York, in press), vol. 2d.
7. F. Bussereau, *Ann. Inst. Pasteur* **118** 626 (1970).
8. D. M. Watts, S. Pantuwatana, G. R. DeFoliart, T. M. Yuill, W. H. Thompson, *Science* **182**, 1140 (1973); L. Rosen, R. B. Tesh, J. C. Lien, J. H. Cross, *ibid.* **199**, 909 (1978).
9. R. B. Tesh, B. N. Chaniotis, K. M. Johnson, *ibid.* **175**, 1477 (1972).
10. G. Brun, thesis, University of Paris, Orsay (1963).
11. F. X. Jousset, *C. R. Acad. Sci. (Paris)* **269**, 1035 (1969).
12. F. Bussereau, thesis, University of Paris, Orsay (1973).
13. L. Rosen and D. Gubler, *Am. J. Trop. Med. Hyg.* **23**, 1153 (1974).
14. T. T. Kuberski and L. Rosen, *ibid.* **26**, 533 (1977).
15. R. B. Tesh, personal communication.
16. F. Fenner, *Intervirology* **7**, 41 (1976).
17. R. P. Hanson, *Bacteriol. Rev.* **16**, 179 (1952).
18. P. H. Peralta and A. Shelokov, *Am. J. Trop. Med. Hyg.* **15**, 369 (1966).
19. R. B. Tesh, B. N. Chaniotis, K. M. Johnson, *Am. J. Epidemiol.* **93**, 491 (1971).
20. R. B. Tesh, P. Peralta, K. M. Johnson, *ibid.* **91**, 216 (1970).
21. L. M. Black, *Phytopathology* **43**, 9 (1953); D. B. Boakye and J. W. Randles, *Aust. J. Agric. Res.* **25**, 791 (1974); E. S. Sylvester and E. McClain, *J. Econ. Entomol.* **71**, 17 (1978).
22. F. X. Jousset, *Ann. Inst. Pasteur* **123**, 275 (1972).
23. Information on the origin and passage history of the viruses is available from L.R.

24. I thank P. Kondo for technical assistance; H. Carson for the capture and identification of *D. melanogaster*; A. Fleuriet for the initial CO_2 tests on *D. melanogaster*; R. Tesh for viruses and antiserums of the VSV group; T. Monath for Flanders virus; R. Shope for Joinjakaka virus and antiserum; and J. Hardy for Gray Lodge virus. Supported by NIAID contract No. N01 A1 82560.

12 November 1979

Elevated Superoxide Dismutase in Black Alcoholics

Abstract. *Superoxide dismutase concentrations in lysates of erythrocytes from black alcoholics were higher than those of white alcoholics and of nonalcoholics of both races. Higher concentrations of enzyme protein, as determined by competition radioimmunoassay, correspond to proportionately higher enzyme activity. Elevated superoxide dismutase levels were not related to any other clinical, historical, or demographic variables. Increased superoxide dismutase levels may delay or prevent some of the pathological sequelae of alcoholism and may be a useful biological marker for alcohol abuse.*

Superoxide dismutases (E.C.1.15.1.1) are enzymes that catalyze the conversion of potentially harmful superoxide free radicals to hydrogen peroxide and oxygen (*1*). Several distinct classes of superoxide dismutases have been described, and at least one such enzyme is found in almost every organism that can survive an environment in which oxygen is present. This led McCord *et al.* (*2*) to propose that the physiological function of superoxide dismutases is to protect against the toxic effects of oxygen metabolites.

There are two classes of superoxide dismutase (SOD) in humans (*1*): SOD-1, a copper-zinc enzyme present in the cytoplasm of all cells, and SOD-2, a manganese-containing enzyme found primarily in mitochondria and, therefore, not present in red blood cells (RBC's). These enzymes are the products of distinct genes on chromosomes 21 and 6, respectively (*3*), and have different molecular weights and unique immunologic specificities (*4*).

After determining that the gene for SOD-1 is located on chromosome 21, it was found that the levels of SOD-1 in cells from patients with trisomy 21 were approximately 50 percent greater than those of normal individuals—a phenomenon ascribed to the gene dosage (*5*). The level of SOD-1 in RBC's has been studied in order to evaluate the significance of the enzyme in human metabolism. Alterations of SOD-1 levels in a number of disorders were reported, but in many of these studies, essential supporting data were not given, making them difficult to evaluate (*6*).

Recently, we developed a competition radioimmunoassay for human SOD-1 (*7*) and observed that the SOD-1 level in the RBC's of a putative normal individual was markedly elevated. On discovering that this person was an alcoholic, we hypothesized that elevated SOD-1 in RBC's may be a biological marker for alcohol abuse. To test this hypothesis, we compared SOD-1 levels in RBC's from chronic alcoholics and nonalcoholic individuals. The Michigan alcoholism screening test (MAST) (*8*) and an interview concerning medical history, demographic data, and drinking habits were given to patients admitted to an alcoholism rehabilitation center and to putative normal subjects. Blood was obtained for automated multiple analyses, hematologic studies, and radioimmunoassay for SOD-1. Sixty-two subjects were classified as alcoholics based on their admission to an alcoholism rehabilitation program, a history of alcohol abuse, and a MAST score of 5 or higher. Twenty-seven nonalcoholics, including 18 social drinkers and 9 nondrinkers, served as controls.

Concentrations of SOD-1 in lysates of washed RBC's were determined by competition radioimmunoassay (*7*). Approximately 1 ng of ^{125}I-labeled SOD-1 was incubated with appropriate dilutions of an RBC lysate and antibody to SOD-1. The immune complexes were then precipitated with a *Staphylococcus aureus* immunosorbent. The concentration of SOD-1 was determined by comparing the displacement of ^{125}I-labeled SOD-1 from antibody to SOD-1 with that effected by known amounts of purified SOD-1 (*9*). Hemoglobin concentration was determined with Drabkin's solution (*10*).

The median SOD-1 level for the 27 controls was 815 ng per milligram of hemoglobin, and for the 62 alcoholics, 898 ng/mg (P = .007, Wilcoxon rank sum test). The relation between the amount of enzyme protein and each demographic, medical historical, and laboratory finding was evaluated by Wilcoxon rank sum test, chi-square test, and linear re-

Table 1. Activity of SOD-1 in RBC lysates from black alcoholics and nonalcoholics. Lysates were adjusted to 20 mg of hemoglobin per milliliter and then analyzed for SOD-1 by competition radioimmunoassay (*7*). Samples were extracted with chloroform and ethanol (*15*) and centrifuged; supernatants were assayed for SOD-1 activity by using the pyrogallol method (*11*) and then reassayed for SOD-1 in protein by competition radioimmunoassay. Differences among groups are not statistically significant.

Group	N	Specific activity after extraction*	Recovery after extraction†
Black alcoholics (initial SOD-1 levels > 1000 ng/mg)	12	0.91 ± 0.18	0.85 ± 0.27
Black alcoholics (initial SOD-1 levels < 1000 ng/mg)	6	0.95 ± 0.18	0.88 ± 0.37
Black nonalcoholics (mean initial SOD-1 level, 843 ng/mg)	6	1.01 ± 0.17	0.88 ± 0.40

*Ratio of SOD-1 determined by pyrogallol assay to SOD-1 determined by radioimmunoassay. †Ratio of SOD-1 determined by radioimmunoassay before extraction to SOD-1 determined by radioimmunoassay after extraction.

0036-8075/80/0229-0991$00.50/0