and they can be further different in the presence of inhibitors, depending on the inhibition mechanism. The only requirement for the attainment of equilibrium is that the Haldane relation, as expressed by Eqs. 4 and 5, be obeyed.

Y. POCKER
NOBUO TANAKA
Department of Chemistry, University of Washington, Seattle 98195

References and Notes

1. T. H. Maren, C. S. Rayburn, N. E. Liddell, *Science* **191**, 469 (1976).
2. S. H. Koenig and R. D. Brown III, *ibid.* **194**, 745 (1976).
3. T. H. Maren, *ibid.*, p. 746.
4. R. G. Khalifah, *J. Biol. Chem.* **246**, 2561 (1971).
5. H. Gutfreund, *Enzymes: Physical Principles* (Wiley-Interscience, London, 1972), p. 48.
6. Y. Pocker and M. W. Beug, *Biochemistry* **11**, 698 (1972).
7. Although small differences were noted in V_{max} values for different anion concentrations (Fig. 1b), the variation in slope with the change in anion concentration was indicative of competitive inhibition in this system.
8. J. B. S. Handane, *Enzymes* (Longmans, Green, London, 1930).
9. The K_m values for both CO_2 hydration and HCO_3^- dehydration were found to be nearly independent of *p*H in the *p*H range where activity showed a major change. Although K_m was dependent on the buffer concentration, the ratio $K_m^{CO_2}/K_m^{HCO_3^-}$ did not vary significantly with buffer concentration (Y. Pocker and N. Tanaka, in preparation).
10. We thank S. Sarkanen for valuable discussions and D. Moore for his diligent assistance in all aspects of the computer work. Supported by grants from the National Institutes of Health and the National Science Foundation.

25 March 1977; revised 17 October 1977

Transovarial Transmission of Japanese Encephalitis Virus by Mosquitoes

Abstract. *Female* Aedes albopictus *and* Aedes togoi *mosquitoes infected with Japanese encephalitis virus either by intrathoracic inoculation or by ingestion of a virus-sucrose-erythrocyte mixture transmitted the virus to a small percentage of their* F_1 *progeny. Adult* F_1 *female* Aedes albopictus *thus infected transmitted the virus in turn to newly hatched chickens by feeding on them.*

In terms of human morbidity and mortality, Japanese encephalitis (JE) is by far the most important of the mosquito-borne virus encephalitides. Japanese encephalitis virus is found throughout much of eastern Asia, extending from the southeastern Soviet Union in the north to Indonesia in the south and as far as India to the west. Human disease caused by the virus occurs throughout this vast area but is more frequent in the northern part of the range (for instance, there were 2429 deaths in South Korea in 1949). Because of the higher incidence of human disease, the most extensive epidemiologic studies of JE virus have been carried out in northern temperate areas, where mosquitoes are not active in the winter. The virus reappears in the same locality year after year, and consequently considerable effort has been devoted to determining how the virus survives when its vectors are inactive.

One possibility considered in early studies was transovarial transmission of the virus by mosquitoes, with overwintering of the agent in eggs, larvae, or imagoes, depending on the mosquito species involved. In fact, Japanese (*1*), Russian (*2*), and Chinese (*3*) workers reported the recovery of JE virus from adult mosquitoes reared from field-collected larvae and never exposed to JE virus in the laboratory. However, failure to confirm the initial findings for field-collected material and negative experimental results, both with JE (*4–6*) and with the related St. Louis encephalitis (*7*) and West Nile (*8*) viruses, led to the current consensus that transovarial transmission of JE virus does not occur (*9, 10*). Most of the initial positive results were obtained by "blind" passage in laboratory mice and were subsequently attributed to the use of mice already infected with either JE or a mouse encephalitis virus, or to infection of the mice in the course of the experiments by infected wild mosquitoes which gained access to the animal quarters.

Despite various hypotheses and considerable work since JE virus was first isolated in mice in 1935, there is no generally accepted explanation of how the virus survives the winter in the northern part of its range (*9*). The reports of transovarial transmission by mosquitoes of La Crosse virus (*11*), a bunyavirus, and Koutango virus (*12*), a flavivirus, prompted us to reexplore the possibility of transovarial transmission of JE virus by mosquitoes. We report here the demonstration of such transmission by *Aedes albopictus* and *Aedes togoi*.

Two hundred female *A. albopictus*, 3 to 6 days old, from a colony derived from mosquitoes collected on Oahu, Hawaii, were inoculated intrathoracically with JE virus isolated from *Culex annulus* mosquitoes captured in Taiwan, China. The *A. albopictus* had been caged with males and had access to a 10 percent sucrose solution, but not blood, before use. The virus was prepared from a suspension of male *A. albopictus* which had been infected by intrathoracic inoculation from the original *C. annulus* pool.

Six days after inoculation, the female *A. albopictus* were given an opportunity to feed on a normal laboratory mouse, and at least 90 percent did so. An oviposition cup was placed in the cage immediately afterward. Eggs were removed from the cage 4 days later, kept moist for one additional day, and then dried and stored at ambient temperature. The temperature in the room in which the parent mosquitoes and eggs were kept ranged from 25° to 31°C. Exposure to light varied since room lights were often left on at night.

Twenty-four days after removal from the cage, the eggs were immersed in water to hatch and the larvae reared on a diet of Tetramin fish food and Purina guinea-pig chow. The temperature of the room in which these larvae and later F_1 stages were kept was 29° ± 1°C with constant artificial light. Beginning 7 days after the eggs were first immersed and daily thereafter for nine additional days, all pupae were removed from the rearing pans and placed in one or more 4-liter cylindrical cardboard containers with fine nylon mesh covering one end. The F_1 adult mosquitoes were killed 2 to 17 days after emergence for virus assay. Before they were killed, some of the F_1 adult female mosquitoes were tested for ability to transmit JE virus by giving them an opportunity to feed on a single occasion between 9 and 17 days after emergence on newly hatched White Leghorn chicks. Chicks were tested for JE viremia 3 days after exposure to mosquitoes (by inoculation of Vero cell cultures) and for JE hemagglutination-inhibition (HI) antibody 20 days afterward.

When F_1 adult mosquitoes were killed, males and females from each container were pooled separately in groups of 1 to 120 for testing. Each pool was ground in a Ten Broeck tissue grinder in 1.5 to 2.0 ml of diluent (phosphate-buffered saline with 30 percent calf serum) and tested for the presence of JE virus by intrathoracic inoculation of *Toxorhynchites amboinensis* mosquitoes (*13*). The latter were examined by a direct fluorescent antibody procedure analogous to that described for dengue viruses (*14*).

Fourth-instar F_1 larvae were also removed from the rearing pans for virus assay. The larvae were pooled in groups of 100, washed in tap water, and killed by grinding in a tissue grinder with 2.0 ml of diluent. These pools were tested for JE

0036-8075/78/0224-0909$00.50/0

Table 1. Japanese encephalitis (JE) virus in F_1 progeny of infected female *Aedes albopictus* and *Aedes togoi* mosquitoes.

Mosquito species	F_1 progeny tested		Number of pools tested	Number of pools JE-positive	Minimum infection rate
	Number	Form			
	Parent females infected by inoculation of virus				
Aedes albopictus	4000	Fourth instar	40	17	1 in 235
	2653	Adult female	41	7	1 in 379
	1086	Adult male	52	5	1 in 217
Aedes togoi	693	Adult female	12	4	1 in 173
	1224	Adult male	19	8	1 in 153
	Parent females infected by ingestion of virus				
Aedes albopictus	340	Adult female	4	3	1 in 113
	826	Adult male	10	1	1 in 826
Aedes togoi	414	Adult female	6	5	1 in 83
	497	Adult male	7	3	1 in 166

virus by the inoculation of *T. amboinensis* mosquitoes.

The frequency of JE virus recovery from various stages of the *A. albopictus* F_1 generation is shown in Table 1 along with data from a similar experiment carried out with a colonized strain of *A. togoi* from Taiwan. At least five female *A. albopictus* mosquitoes transmitted JE virus to chicks, as indicated by viremia or the appearance of HI antibody, or both. The JE virus was recovered from the pool of female mosquitoes from each of the five containers to which positive chicks had been exposed.

The identity of the virus recovered from mosquitoes and chicks was confirmed by a complement-fixation typing technique similar to that described for dengue viruses (*15*), employing either the infected *T. amboinensis* mosquitoes or Vero cell culture fluids as antigens. In addition, the identity of the original JE virus inoculum and a sample of F_1 isolates was confirmed by plaque reduction neutralization tests with an immune mouse ascitic fluid prepared with the Nakayama strain of JE virus.

In other experiments we demonstrated transovarial transmission of JE virus by colonized *A. albopictus* from Taiwan and by colonized *A. togoi* from Japan, and also of dengue viruses by *A. albopictus* from Hawaii. Moreover, we found that *A. albopictus* and *A. togoi* infected by ingestion of a virus-sucrose-erythrocyte mixture transmitted JE virus transovarially (Table 1). In the latter experiments, the F_1 progeny tested were derived from eggs laid by female mosquitoes fed on a normal laboratory mouse 6 days after they had fed on the virus mixture.

Although we did not demonstrate that JE virus was actually inside the mosquito egg rather than on its surface, the latter possibility seems improbable for two reasons. First, we observed infection in F_1 generation mosquitoes hatched from eggs which had been dried and kept at room temperature as long as 2 months (the longest period tested). It is unlikely that JE virus would remain viable extracellularly under such conditions. Second, we found that few *A. albopictus* larvae become infected when eggs were hatched in a liquid medium containing a large amount of JE virus and the emerging larvae were allowed to remain therein for 21 hours. Only 1 of 64 mosquitoes exposed in this way to a medium containing, per milliliter, at least 10^6 doses of JE virus sufficient to infect 50 percent of mosquitoes intrathoracically was found infected with JE virus when it attained the adult stage.

Previous failure to demonstrate transovarial transmission of JE virus in a convincing manner was probably the result of the rarity of the phenomenon. It has been assumed that if transovarial transmission did occur, infection could be demonstrated in a relatively large percentage of the F_1 generation. Also, it is possible that the strain and passage history of JE virus affects its ability to be transmitted transovarially, and there may be differences in this regard between various species of mosquitoes. In addition, methods of virus assay may have played a role. Under some circumstances, transovarial passage may affect the virulence, but not the infectivity, of JE virus for vertebrates. In previous attempts to demonstrate transovarial transmission of JE virus the appearance of disease in mice was used to detect the agent.

Only field studies can determine whether transovarial transmission is the mechanism for overwintering of JE virus. Although experimental transovarial transmission rates appear low, they may be higher under certain field situations or with other combinations of vectors and virus strains. For example, La Crosse virus was transmitted to approximately 34 percent of the F_1 generation of *Aedes triseriatus* mosquitoes (*11*) but to only about 3 percent of the F_1 generation in *A. albopictus* (*16*). Also, under some conditions of vector and vertebrate host density, a high transovarial infection rate may be disadvantageous for virus survival because it would result in rapid depletion of the supply of susceptible vertebrate hosts. Marchoux and Simond (*17*), who first reported transovarial transmission of a virus by mosquitoes in 1905 (yellow fever virus in *Aedes aegypti*), also noted its relative rarity. Many subsequent investigators failed to confirm their findings for this virus-vector combination (*18*), but in view of our results with JE and dengue viruses, further studies on transovarial transmission of yellow fever virus appear warranted.

In most areas in which JE is endemic *Culex* are now far more important than *Aedes* species in the transmission of the virus to humans, but species of the latter genus may be significant in the basic ecological cycle of JE virus and as vectors to humans in some types of relatively undisturbed natural habitat. For example, although *Culex tritaeniorhynchus* and domestic pigs are now the most important hosts of JE virus in Japan from the point of view of human infection, swine have been reared in significant numbers in Japan only since the opening of that country to contact with the outside world in 1853. The southeastern Soviet Union is the one area where the epidemiology of JE virus has been studied in a setting not greatly changed by humans. Russian investigators consider three species of *Aedes* (*togoi*, *japonicus*, and *koreicus*) as important vectors of JE to humans in some types of habitat in this region (*19*). In fact, the initial Russian report of recovery of JE virus from field-collected larvae concerned *A. togoi* (*2*). Unfortunately, only a single isolate was obtained, and that after six blind passages in mice. Subsequent Russian experimental work on transovarial transmission (in *Culex* mosquitoes) was negative (*5*) and it is understandable that the initial positive report was given little credence. Further field evaluation of transovarial transmission as a possible overwintering mechanism is now indicated, not only for JE virus, but also for the related St. Louis encephalitis, Murray Valley encephalitis, and West Nile viruses.

LEON ROSEN, ROBERT B. TESH
Pacific Research Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, Honolulu, Hawaii 96806

JIH CHING LIEN, JOHN H. CROSS
U.S. Naval Medical Research Unit No. 2, Taipei, Taiwan

References and Notes

1. T. Mitamura, M. Kitaoka, S. Watanabe, T. Hosoi, S. Tenjin, D. Seki, K. Nagahata, K. Jo, T. Shimizu, *Trans. Soc. Pathol. Jpn.* **29**, 92 (1939).
2. K. P. Chagin and I. I. Savoyskiy, *Zh. Mikrobiol. Epidemiol. Immunobiol.* **14**, 44 (1943).
3. C.-J. Wu and S.-Y. Wu, *Acta Microbiol. Sin.* **5**, 27 (1957); C. H. Huang, *Acta Virol.* **1**, 36 (1957).
4. T. Mitamura and M. Kitaoka, *Jpn. Med. J.* **3**, 141 (1950).
5. A. I. Drobyshevskaya and V. A. Korshunova, in *Neurovirus Infections*, A. A. Smorodintsev, Ed. (Medgiz, Leningrad, 1954), p. 192.
6. W. C. Reeves and W. M. Hammon, *J. Exp. Med.* **83**, 185 (1946).
7. R. W. Chamberlain, W. D. Sudia, R. H. Gogel, *Am. J. Hyg.* **80**, 254 (1964).
8. A. M. Davies and Y. Yoshpe-Purer, *Ann. Trop. Med. Parasitol.* **48**, 46 (1954); Y. Nir, *ibid.* **57**, 428 (1963).
9. W. C. Reeves, *Prog. Med. Virol.* **17**, 193 (1974).
10. C. H. Huang, *Acta Microbiol. Sin.* **13**, 200 (1973).
11. D. M. Watts, S. Pantuwatana, G. R. DeFoliart, T. M. Yuill, W. H. Thompson, *Science* **182**, 1140 (1973).
12. J. Coz, M. Valade, M. Cornet, Y. Robin, *C.R. Acad. Sci.* **283**, 109 (1976).
13. L. Rosen and D. Gubler, *Am. J. Trop. Med. Hyg.* **23**, 1153 (1974).
14. T. Kuberski and L. Rosen, *ibid.* **26**, 533 (1977).
15. ———, *ibid.*, p. 538.
16. R. B. Tesh and D. J. Gubler, *ibid.* **24**, 876 (1975).
17. E. Marchoux and P.-L. Simond, *C.R. Seances Soc. Biol. Paris* **59**, 259 (1905); *Ann. Inst. Pasteur (Paris)* **20**, 16 (1906).
18. A. Stokes, J. H. Bauer, N. P. Hudson, *Am. J. Trop. Med.* **8**, 103 (1928); C. B. Philip, *J. Exp. Med.* **50**, 703 (1929); N. C. Davis and R. C. Shannon, *Am. J. Hyg.* **11**, 335 (1930); J. D. Gillett, R. W. Ross, G. W. A. Dick, A. J. Haddow, L. E. Hewitt, *Ann. Trop. Med. Parasitol.* **44**, 342 (1950).
19. P. A. Petrischeva, Ye. N. Levkovich, S. T. Boldyrev, *Japanese Encephalitis* (Medgiz, Moscow, 1963).

20. We thank K. Sorensen for facilitating the work on Taiwan; P. S. Kondo for her excellent assistance in both Taiwan and Honolulu; and M. Lien, C. Mizumoto, A. Rosen, and D. Rosen for their help.

29 September 1977; revised 12 December 1977

Supplemental Lighting Stimulates Growth and Lactation in Cattle

Abstract. *Sixteen hours of light daily (114 to 207 lux) increased weight gains and milk yield 10 to 15 percent in Holstein cattle in comparison with cattle exposed to natural-length photoperiods (39 to 93 lux) of 9 to 12 hours. The weight gain was accomplished without increased consumption of feed. Manipulation of supplemental light may thus cause dramatic increases in food supplies from animals.*

In dairy cattle, 16 hours of light daily increase concentrations of serum prolactin (*1*), an anabolic hormone (*2*) associated with lactational production (*3*). We now report that 16 hours of supplemental lighting stimulated body growth and milk yields of dairy cattle 10 to 15 percent in comparison with natural-length photoperiods of 9 to 12 hours.

Twenty Holstein heifers, 3 to 6.5 months of age at the start of this experiment, were divided according to age and genetic potential for milk yield into two equal groups. Each group was housed unrestrained within separate pens inside a barn, and no supplemental heat was provided. Between 18 November 1975 and 9 March 1976, one group (control) received an average of 9.8 hours of indirect sunlight daily through northern windows (natural photoperiod) (*4*). The second group received the natural photoperiods plus supplemental cool-white fluorescent light between 0600 and 2200 hours (16 hours total light daily). The ration fed daily to each heifer consisted of 2.3 kg of 14 percent protein pelleted concentrate, with alfalfa hay and water freely available. Girth at the level of the heart (heart girth) was measured weekly on each animal with a tape measure to quantify growth. In the control group, heart girth increased from 114 to 139 cm during the 16-week experiment (Fig. 1A). In comparison, the heart girth of heifers receiving 16 hours of light increased from 112 to 141 cm. The difference in the total heart girth gain (24.8 ± 1.1 versus 28.7 ± 1.1 cm) between the two groups of heifers was significant (*t*-test, $P < .02$).

The ability of 16 hours of supplemental lighting to stimulate growth was confirmed in another similarly designed experiment (*5*) conducted between 6 October 1976 and 2 March 1977, except that body weight was quantified instead of heart girth. Weights were measured on one day each week for 22 weeks. Animals were deprived of water for 16 hours before being weighed. Average body weights in control heifers increased from 160 kg at week 1 to 274 kg at week 22, while heifers given supplemental lighting increased from 159 to 285 kg (Fig. 1B). Thus, average daily weight gains of Holstein heifers exposed to natural-length photoperiods ($N = 14$) or supplemental lighting ($N = 14$) were 0.78 ± 0.02 and 0.86 ± 0.02 kg (*t*-test, $P < .05$), respectively. All concentrates fed were consumed completely each day. Daily feed intakes of alfalfa hay by heifers exposed to 16-hour and natural photoperiods averaged 4.6 ± 0.7 and 4.7 ± 0.5 kg, respectively. Thus, supplemental light during the fall and winter in Michigan increased growth rates 10 to 15 percent without requiring additional feed. The increased growth was of similar magnitude to that achieved by steers receiving diethylstilbestrol (*6*).

A third experiment, designed similarly to the second, was conducted between 1 May and 13 August 1976, when the natural photoperiod ranged between 13.6 and 15.3 hours of light. Daily weight gains of 13 Holstein heifers receiving natural-length photoperiods averaged 0.89 kg, not significantly different from that (0.90 kg) of 13 heifers receiving 16 hours of supplemental fluorescent light.

To determine if photoperiod affected milk yields, we exposed 46 lactating Hol-

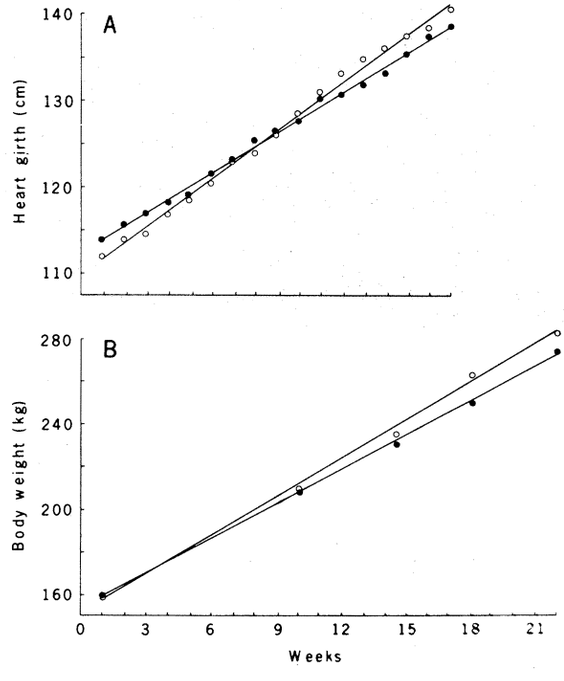

Fig. 1. Growth response of Holstein heifers to 16-hour (○) or natural (●) photoperiods in East Lansing, Michigan. (A) Heart girth, 10 heifers per treatment, 18 November 1975 to 9 March 1976. (B) Body weight, 14 heifers per treatment, 6 October 1976 to 2 March 1977.

0036-8075/78/0224-0911$00.50/0