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10. $0.89 \leq R^2 \leq 0.99$, $n = 53$. On the average a fiber was stimulated with 10 to 15 test frequencies; range, 4 to 37. Slope values (rad/Hz) for AP fibers ranged from 0.0228 to 0.0583. To a first approximation, phase lag seems to be a linear function of frequency. To the extent that these functions are linear, $d\theta/2\pi df$ is a measure of the system delay. In mammals, however, the rate of phase accumulation above CF may depend on frequency; hence, mammalian travel times calculated from a regression line through the phase-frequency data may be overestimated. [R. R. Pfeiffer and C. E. Molnar, *Science* **167**, 1614 (1970); M. A. Ruggero, *J. Acoust. Soc. Am.* **67**, 707 (1980); D. D. Greenwood, unpublished]. In 23 percent of the neurons we examined, slight deviations from linearity were detected that reduced the goodness of a single linear fit to these data ($0.89 \leq R^2 \leq 0.97$) when compared with the remainder of the fibers ($0.98 \leq R^2 \leq 0.99$). These neurons are indicated by open circles in Fig. 2, B and C. Most of the "nonlinear" fibers had CF's of less than 0.23 kHz. These units, which derive from hair cells at the rostral end of the papilla beneath the most massive portion of the tectorium, may be subject to more mechanical nonlinearities (edge effects, mechanical reflections). Estimates of delay for low CF units are therefore subject to greater variability. For these cases, the rate of phase accumulation was less at higher stimulus frequencies.
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19. Under the most general conditions, the response time for a tuned filter is inversely proportional to its bandwidth. The bandwidths of AP hair cells are not known, but may be estimated from neural bandwidths at 3 dB above best threshold (BW_{3dB}). Using response time (RT) = $1/BW_{3dB}$, which is related to delay through a tuned filter, we determined the predicted RT's for each unit. These estimates were as much as 19 msec longer than the values we observed. Further, cells with the same bandwidths often had time delays that differed by as much as 3 msec. Such variation would not be predicted if the cells act like simple electrical filters. Although the mean difference between the estimated RT and the observed time delays was 8.6 ± 0.87 msec, the former did show a weak inverse relation to CF. Investigations of the electrical characteristics of AP hair cells are required before we can effectively evaluate whether electrical tuning properties contribute significantly to the observed delays.
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21. The TM is implicated in this system since it may generate and propagate intermodulation distortion products [R. R. Capranica and A. J. M. Moffat, in *Comparative Studies of Hearing in Vertebrates*, A. N. Popper and R. R. Fay, Eds. (Springer-Verlag, New York, 1980), pp. 139–165] and participate in the active process of tone generation associated with spontaneous and evoked acoustic emissions from the ear of *Rana esculenta* [A. R. Palmer and J. P. Wilson, *J. Physiol. (London)* **324**, 66P (1981)].
22. Supported by NIH grants NS07005-02 and NS 19725-01 to C.M.H. and P.M.N., respectively. Special thanks are due R. Dunia for assistance with the experiments, C. B. Martinez for developing the phase analysis program, R. D. Zelick for critical input during all phases of this study, and especially E. R. Lewis and W. G. Sokolich for enlightening discussions and suggestions.

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Enhanced Arboviral Transmission by Mosquitoes That Concurrently Ingested Microfilariae

Abstract. *Infection, dissemination, and transmission of an arbovirus in mosquitoes are enhanced by concurrent ingestion of microfilariae. Ingestion of Rift Valley fever virus alone infected only 64 percent of female* Aedes taeniorhynchus. *Of these, only 5 percent of refeeding mosquitoes actually transmitted virus. In contrast, ingestion of the same amount of virus from concurrently microfilaremic (*Brugia malayi*) gerbils resulted in 88 percent infection and 31 percent transmission. Enhanced transmission of virus may be attributed to increased transit of virus across the midgut wall. Endemic filariasis may promote arbovirus transmission in nature.*

Viral transmission by an arthropod requires that the virus infect cells of the midgut and pass into the hemocoel in order to infect the salivary glands. Failure of western equine encephalomyelitis virus to disseminate across the gut wall in a percentage of *Culex tarsalis* limits this species as a vector of the virus (*1*). Similarly, the failure of virus to disseminate in a percentage of *Culex pipiens* limits this species as a vector of Rift Valley fever (RVF) virus (*2*). Any factor that allows virus to directly enter the hemocoel could result in increased competence of a vector for that virus. For example, Merrill and TenBroek (*3*) found that female *Aedes aegypti* that had ingested eastern equine encephalomyelitis (EEE) virus did not transmit the virus by bite. However, if virus were inoculated, or if the midgut were punctured immediately after ingestion of EEE virus, this species was able to transmit EEE virus by bite. Perhaps the punctures allow virus to disseminate into the hemocoel directly. A similar finding was reported for *Anopheles annulipes* and Murray Valley encephalitis virus (*4*).

Because microfilariae ingested in a blood meal rapidly penetrate the mosquito midgut, they may facilitate viral infection and dissemination to remote organs of the vector. Also, injected virus becomes transmissible more rapidly than does ingested virus (*5*). Thus we hypothesized that mosquitoes fed on a host concurrently viremic and microfilaremic would transmit virus more effectively than mosquitoes fed on a host infected with virus alone. We therefore permitted *Aedes taeniorhynchus* to ingest blood from gerbils (*Meriones unguiculatus*) concurrently infected with *Brugia malayi* and RVF virus. Infection, hemocoelic dissemination, transmission rates, and duration of preinfectious period were compared to those of mosquitoes ingesting virus alone.

In the first experiment (*6*), we compared the rapidity of virus dissemination in the mosquitoes that ingested RVF virus (*7*) alone with that in the mosquitoes ingesting virus and *B. malayi* microfilariae (*8*) concurrently. Each mosquito ingested approximately $10^{3.3}$ plaque-forming units of RVF virus from one of two gerbils. The mosquitoes that fed on the gerbil with concurrent microfilaremia ingested in addition a median of 85 microfilariae. After ingestion of virus alone, 59 percent of 85 *Ae. taeniorhynchus* became infected (Table 1). Of the

Table 1. Dissemination of RVF virus in *Ae. taeniorhynchus* after ingestion of $10^{3.3}$ plaque-forming units of virus from amicrofilaremic or microfilaremic gerbils.

Days after infection	Amicrofilaremic			Microfilaremic		
	Number of mosquitoes sampled	Percentage with virus in whole body	Percentage with virus in legs	Number of mosquitoes sampled	Percentage with virus in whole body	Percentage with virus in legs
4 to 5	22	50	9	20	95	50
12 to 13	40	58	18	30	83	70
18	14	64	36	11	91	82
39	9	78	78			

Table 2. Infection (virus in body), dissemination (virus in legs), and transmission (virus in bitten hamster) of RVF virus in *Ae. taeniorhynchus* after feeding on amicrofilaremic or microfilaremic gerbils. PFU, plaque-forming units.

Virus ingested (PFU)	Virus assayed after (days)	Vector competence criterion	Amicrofilaremic		Microfilaremic	
			Number of mosquitoes	Percent	Number of mosquitoes	Percent
$10^{3.3}$	4 to 13	Infection	62	55	50	88
		Dissemination	62	15	50	62
		Transmission	43	5	29	31
$10^{1.7}$	4 to 7	Infection	50	16	44	64
		Dissemination	50	0	44	32
		Transmission	29	0	22	7

85 mosquitoes, the percentage showing disseminated infection increased with time from 9 percent of the 22 mosquitoes sampled at 4 to 5 days to 78 percent of the 9 mosquitoes sampled at 39 days. Only after about 18 days did half of the infections become disseminated. In contrast, when virus was ingested with microfilariae, 89 percent of 61 mosquitoes became infected and over half of infected mosquitoes had a disseminated infection after only 4 to 5 days. We concluded that concurrent ingestion of microfilariae enhanced viral infection of mosquitoes as well as rapidity of virus dissemination throughout the body of the mosquito.

Vector competence of these two groups of mosquitoes was compared 4 to 13 days after infection (Table 2). Each of the three measures of vector competence used (for example, infection, dissemination, and transmission) was significantly higher ($P < 0.01$, χ^2 test) in the group that had fed on the microfilaremic gerbil. Most striking was the greater than six-fold increase in ability to transmit virus by bite.

In a replicate experiment, each mosquito ingested about $10^{1.7}$ plaque-forming units of RVF virus from a microfilaremic gerbil or an amicrofilaremic gerbil (a median of 13 microfilariae were ingested from the infected gerbil). Although none of the mosquitoes that fed on that amicrofilaremic gerbil developed a disseminated infection by 7 days, 14 of 44 mosquitoes that fed on the microfilaremic gerbil had a disseminated infection by this time (Table 2). Results were similar to those reported in the first experiment, except that lower rates of infection, dissemination, and transmission occurred after mosquitoes ingested the lower dose of virus (Table 2). While in the first experiment ingestion of the microfilariae with virus increased vector competence, in the second, ingestion of microfilariae transformed an incompetent vector into a competent one.

Two factors appear to be responsible for this increase in the ability of *Ae. taeniorhynchus* to transmit RVF virus. First, presence of microfilariae simplified development in the vector by eliminating the requirement for replication in the midgut and for release into the hemocoel; and second, by bypassing the replication cycle in the midgut, the time required before virus could be transmitted was greatly reduced.

The hypothesis that concurrent ingestion of microfilariae enables virus to directly enter the hemocoel is supported by the increase in infection rates and by the rapid appearance of disseminated infections in mosquitoes that ingested both agents. However, the mechanism by which microfilariae enhance dissemination of ingested virus has not been defined. One possibility is that virus may escape into the hemocoel along with a small amount of ingested blood through the hole in the midgut produced by the microfilariae. Alternatively, infectious virus may be adsorbed by the sheath of the microfilariae and carried into the hemocoel. Microfilariae apparently exsheath after midgut penetration (*9*), permitting virus direct access to the hemocoel.

Concurrent microfilarial ingestion allowed virus to disseminate in less than 5 days. In contrast, less that 10 percent of mosquitoes infected by virus alone developed a disseminated infection by this time, and only about half developed a disseminated infection by 18 days of extrinsic incubation. Because females were able to oviposit and refeed by 5 days after the initial blood meal, those mosquitoes infected by virus alone might not be able to transmit virus until the second or even the third refeeding, while most mosquitoes infected by the action of the microfilariae would reach their full vector potential at their first refeeding. These results demonstrate a potential epidemiological interaction between the prevalence of filaria in a vertebrate viral reservoir and the ability of potential vectors to transmit that virus. Both vector competence and longevity relative to extrinsic incubation period would be affected. The former contributes linearly to transmission and the latter exponentially (*10*).

Mellor and Boorman (*11*) recently demonstrated a similar effect of concurrent ingestion on vector competence. They found that the midge *Culicoides nubeculosus* became infected with bluetongue virus when it ingested the virus with microfilariae of *Onchocerca cervicalis*, but did not become infected when virus was ingested alone. Our study extends these findings by demonstrating that concurrent ingestion not only allows increased infection in the mosquito vector but also increased rapidity of viral dissemination and increased transmission rates. The increase in vector competence is probably not limited to the combination of RVF virus and *B. malayi* in *Ae. taeniorhynchus*, and may apply to a broad range of arboviruses, vectors, and filarial infections.

Human filarial infections due to *B. malayi* and *Wuchereria bancrofti* presently are pandemic in many tropical regions. Prevalence frequently exceeds 30 percent, occasionally reaching 70 percent (*12*). Filarial infections are also

prevalent in sylvan animals [for example, over 25 percent of white-tailed deer in the southeastern United States circulate microfilariae of *Setaria yehi* (*13*)]. Because microfilaremias are of long duration in natural vertebrate hosts, concurrent infections should not be uncommon in nature. We suggest that filarial infection may promote transmission of arboviral infection in nature.

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6. In each experiment, paired gerbils (one microfilaremic and one of similar weight but not infected by *B. malayi*) were each inoculated intraperitoneally with $10^{7.0}$ plaque-forming units of virus. One day later, each gerbil was anesthetized with sodium pentobarbital and placed in a cage containing about 250 5- to 8-day-old female *Ae. taeniorhynchus* (Vero Beach strain). The mosquitoes were allowed to feed for 15 minutes. To determine quantity of virus ingested, five freshly engorged mosquitoes were removed from each cage, immediately triturated individually in 1 ml of diluent, and then frozen at −70°C for later analysis by plaque assay for infectious virus in Vero cells [T. P. Gargan, C. L. Bailey, G. A. Higbee, A. Gad, S. El Said, *Am. J. Trop. Med. Hyg.* **32**, 1154 (1983)]. Diluent consisted of Medium 199 with Hanks salts containing 10 percent calf serum, 0.075 percent $NaHCO_3$, and antibiotics [penicillin (100 unit/ml), streptomycin (100 μg/ml), gentamycin (50 μg/ml), and fungizone (5 μg/ml)]. The number of microfilariae ingested was determined from the midgut of ten freshly engorged mosquitoes. The remaining engorged mosquitoes were placed in 3.8-liter cardboard containers with netting over one end and maintained at 26°C and 16 hours of light per day. Apple slices or a 10 percent sucrose solution were provided as nutriment. An oviposition dish was placed in each cage 3 days later, and mosquitoes were removed from each cage for vector competence studies beginning the next day. Transmission trials were conducted by permitting mosquitoes to feed either individually or in pairs on restrained hamsters (T. P. Gargan *et al.*, *ibid.*). After the feeding attempt, each mosquito was chilled and its legs and body were triturated separately in 1 ml of diluent. Presence of virus in legs provided evidence of a disseminated infection. Each mosquito was also examined for evidence of feeding on the hamster. Because RVF viral infection is virtually 100 percent fatal to hamsters (T. P. Gargan *et al.*, *ibid.*), hamster death was used as the criterion for virus transmission. A 20 percent liver suspension from a sample of the dead hamsters was assayed for virus content. Virus identity was confirmed by a standard neutralization test. Virus transmission rate was defined as the number of mosquitoes that refed and transmitted virus divided by the number that refed, regardless of their infection status.
7. The Zagazig Hospital 501 strain of RVF virus was used throughout this study after two passages in fetal rhesus monkey lung cells.
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Isolation, Characterization, and Chromosome Assignment of Mouse N-*ras* Gene from Carcinogen-Induced Thymic Lymphoma

Abstract. *Treatment of mice with the carcinogen* N-*methylnitrosourea results in the development of thymic lymphomas with frequent involvement of the N*-ras *oncogene. The activated mouse N*-ras *gene was isolated from one of these lymphomas and, by transformation in concert with restriction digestion, a map of the gene was prepared and its approximate boundaries were determined. By means of somatic cell hybrids the normal N*-ras *gene was found to be unlinked to other members of the* ras *gene family.*

DNA from several human tumors and tumor cell lines contains oncogenes that can transform NIH 3T3 cells (*1*). Oncogenes can be classified into two functional groups known as the *lym* (*2*) and *ras* (*3*) families. Three members of the *ras* family have been identified: H-, K-, and N-*ras*. The N-*ras* gene is the only member of the *ras* family that has not been found in RNA viruses. Animal models are available in which *ras* genes are associated with tumor development (*4*). When working with a mouse model, we found that treatment with the chemical carcinogen *N*-methylnitrosourea (NMU) or with γ-radiation causes the formation of thymic lymphomas, the DNA of which induces foci in rodent fibroblasts (*4*). We identified the activated oncogenes in these tumors as *ras* family members because the transforming phenotype segregated with extra copies of these oncogenes in isolated foci (*4*).

The activated oncogene was isolated from a rat secondary transformant obtained with DNA originally derived from an NMU-induced mouse thymic lymphoma (the 3T3 primary transformant and this rat secondary transformant were both tumorigenic in nude mice). Use of the rat secondary instead of the NIH 3T3 primary transformant allowed us to distinguish the active oncogene from endogenous homologs, because of the species differences.

First we studied the effect of Eco RI on the transforming activity of the gene and found that this enzyme destroyed its activity (Table 1). It was therefore necessary to isolate a partial product of higher molecular weight in order to obtain a functional gene. We also needed a strategy to distinguish the mouse N-*ras* gene from any rat genes with similar sequences.

When genomic DNA from rat cells and from the secondary transformant was partially digested with Eco RI and sub-

Table 1. Transforming activity of the oncogene present in carcinogen-induced mouse thymic lymphoma. Transformations were performed as described (*17*) with 40 μg of genomic DNA per plate. When the recombinant clone was used, three different doses of 0.1, 1, and 10 ng per plate were used with NIH 3T3 DNA being added to make the 40-μg total. All the experiments were performed at least twice. The digestion with restriction enzymes was carried out in the conditions described by the manufacturers. The DNA's were subsequently extracted once with phenol and once with a mixture of chloroform and isoamyl alcohol (24:1 by volume) and precipitated with ethanol prior to transformation. When the donor DNA was λ3.2 N-*ras*T, the following enzymes, which cut inside the Kpn I–Xho I fragment, inactivated the gene: Hind III, Eco RI, Bam HI, Bgl II, Xba I, Pvu II, and Bal I. The transforming activity of cloned DNA treated with each of these enzymes was less than 12 foci per microgram of DNA. Conversely, with the same λ3.2 N-*ras*T donor DNA, the enzymes Sma I, Sst II, Sal I, Xho I, Kpn I, Pvu I, and Nru I yielded DNA that was still able to induce focus formation at a rate of 1×10^4 to 2×10^4 per microgram of DNA. These enzymes do not digest the insert.

Donor DNA	Transforming activity (foci/μg DNA)
Carcinogen-induced thymoma I	0.05 to 0.15
3T3-TI primary transformant	0.25 to 0.45
Rat 2-TI secondary transformant	0.30 to 0.45
λ3.2 N-*ras*T	1×10^4 to 2×10^4