though analogous theories explain altered gating in K^+ channels (*22*), Ca^{2+} binding to the permeation pathway would equilibrate within microseconds of opening (*19*)–too fast to account for slow changes in $P_{oo}(t, t_j)$. A more plausible mechanism might be Ca^{2+} binding to an intracellular site outside the permeation pathway; diffusion (*23*) could then provide the requisite delays. Other possibilities include Ca^{2+} binding to an intrapore site with slow kinetics, or $[Ca^{2+}]_i$-mediated phosphorylation or dephosphorylation (*24*) of the channel or an associated G protein (*25*). In contrast, Ca^{2+} entry also confers some very rapid changes upon gating (Fig. 2E). Since $P_{oo}(t, t_j)$ for both charge-carriers here arises from first openings, comparison of the two traces indicates how quickly Ca^{2+} entry alters gating. The difference trace below shows that changes in gating produced by Ca^{2+} entry reach a quasi-equilibrium within 1 ms, arguing against an exclusive role for the slower mechanisms enumerated above. Diffusion constraints (*23*) suggest that Ca^{2+} inside or near the permeation pathway should be involved in this rapid effect. It will be important to establish whether a common mechanistic paradigm for Ca^{2+}-sensitive modulation of gating is shared among Ca^{2+}-channel relatives: L-type and, perhaps, N-type Ca^{2+} channels of vertebrate neurons are among the prime candidates.

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A New Arbovirus from *Aedes albopictus*, an Asian Mosquito Established in the United States

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Ten strains of a new arbovirus belonging to the Bunyamwera group (Bunyaviridae) were recovered from field-collected *Aedes albopictus* mosquitoes in Potosi, Missouri. This evidence indicates that this species may serve as an arbovirus vector in the United States. The urban-suburban distribution, aggressive biting behavior, and broad viral susceptibility of *Ae. albopictus* may lead to the transmission of viruses of known public health importance and perhaps of viruses hitherto not transmitted to humans because of the feeding pattern of their usual vectors.

AEDES ALBOPICTUS, A MOSQUITO SPECIES native to Asia, was discovered in Houston, Texas, in 1985 (*1*). It has now been found in 160 counties in 18 states. This species has previously been found in used tires entering the United States from Asian ports (*2*). Importation of used tire casings to the United States from Asia, primarily Japan, is a major business (*3*). It was therefore hypothesized that *Ae. albopictus* had come to the United States in used tire casings. This mode of introduction was confirmed in 1986 when larvae of *Ae. albopictus* were found in water-containing tires arriving at the port of Seattle from Tokyo (*4*).

Public health officials are concerned about the establishment of this species in the United States because it is a documented vector of dengue viruses in Asia (*5*). This species has also been shown experimentally to be a vector for a number of arboviruses (*6*); thus it could also become a vector of one or more of the indigenous U.S. arboviruses.

Aedes albopictus is an efficient laboratory vector of La Crosse (LAC) virus (*7*), and vertical transmission of LAC in *Ae. albopictus* has been shown (*8*). LAC virus is endemic in the Great Lakes region and produces encephalitis primarily in children under 15 (*9*). The vector of LAC virus is *Ae. triseriatus*, a mosquito which develops primarily in deciduous tree holes but which readily utilizes water-holding man-made containers as a larval habitat. Increased risk of human LAC encephalitis cases is associated with *Ae. triseriatus* development in man-made containers in and near the premises of case households (*10*) and with the numbers of potential water-holding containers on case premises (*11*). The fact that *Ae. albopictus* and *Ae. triseriatus* use similar larval habitats,

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together with the tire-associated transport of *Ae. albopictus* into areas where LAC virus is endemic (*12*), makes a strong case for the eventual involvement of this newly established mosquito as a vector of LAC virus.

We collected adult *Ae. albopictus* for viral testing from areas where LAC virus is known or believed to occur, by sweeping vegetation (*13*) and by aspirating landing or biting mosquitoes from field workers. Mosquitoes were tested as described in (*14*). In 1988, we tested 6159 *Ae. albopictus* from Illinois, Indiana, Ohio, and Tennessee, and no virus was isolated. In 1989, mosquitoes were collected from Illinois, Indiana, and Missouri.

Aedes albopictus had been found to be extensively distributed throughout a scrap tire yard at Potosi, Missouri, during a survey in 1986 (*15*). The tire yard is approximately 5 km northeast of Potosi, which is located 100 km southwest of St. Louis. Collections were made in the tire yard and surrounding vegetation. The tire pile extended 5 m into a hardwood forest, composed primarily of oak, beech, elm, maple, and hickory. Areas within the woods that had been recently cut were overgrown with scrub oak. Decaying stumps were evident throughout the wooded area, and *Ae. albopictus* larvae were found in stumps containing rainwater. The edge of the woods adjacent to the tire yard was composed primarily of blackberry, sumac, and goldenrod.

Table 1. Mosquito species collected in Illinois, Indiana, and Missouri for virus testing, summarized by state and collection period.

Collection period (1989)	Species	Number of	
		Mosquitoes	Pools*
	Illinois		
August	*Ae. atropalpus*	2	2
	Ae. albopictus	1	1
September	*Ae. triseriatus*	7	1
	Indiana		
July	*Ae. trivittatus*	5	2
	Ae. triseriatus	1,012	86
	Ae. albopictus	72	13
	Ae. vexans	7	2
	Culex salinarius	1	1
	Cx. territans	2	1
	Culex (Cul.) spp.	113	7
	Missouri		
July	*Ae. atropalpus*	62	8
	Ae. vexans	1	1
	Ae. triseriatus	59	7
	Ae. albopictus	4,951	110
	Psorophora ferox	1	1
August	*Ae. albopictus*	6,930	194
September	*Ae. triseriatus*	12	1
	Ae. albopictus	787	32
Totals		14,025	470

*Pools consisted of 1 to 82 mosquitoes combined by collection site and date.

Table 2. Results of serum-dilution plaque-reduction neutralization tests (PRNT) identifying two virus isolates (89-3380 and 89-3470) recovered from *Ae. albopictus* mosquitoes collected in Potosi, Missouri, in 1989; –, <20.

Virus	Strain	90% PRNT titer of antibody to						
		89-3380	89-3470	MD	LOK	CV	TEN	NOR
	89-3380	2560	2560	20	–	–	80	–
	89-3470	5120	2560	20	40	–	160	–
MD	BFS 5015	–	–	320				
LOK	FMS 4332	–	–		>5120			
CV	6V633	–	–			160		
TEN	A9-171B	20	40				1280	
NOR	0234	–	–					1280

The number and species of female mosquitoes tested for virus are summarized in Table 1. From *Ae. albopictus* collected during August at the Potosi tire yard site, three virus strains were recovered. Collections from the same site in September yielded seven virus strains from the same species. No blood-engorged specimens were in pools from which virus was isolated. The minimal infection rate of 8.9 per 1000 mosquitoes for September was significantly higher than the 0.4 per 1000 mosquitoes for the August collection (Poisson distribution, $P < 0.001$).

All of the virus strains were initially screened by indirect immunofluorescence against NIH grouping antibody preparations, and all reacted only with the Bunyamwera grouping fluid. Two newly isolated virus strains were selected for further identification. We prepared hyperimmune mouse sera to these two strains and known Bunyamwera group viruses, using four weekly injections of undiluted live virus according to the method of Brandt *et al.* (*16*). Results, summarized in Table 2, demonstrate that the two representative strains, 89-3380 and 89-3470, are identical and are distantly related to Tensaw (TEN) virus. The other eight strains were tested in a one-way neutralization test with antibody to the two representative strains. All were neutralized to homologous titer and are thus identical or very close to the two representative strains.

One of two representative strains was compared by serum-dilution plaque-reduction neutralization tests with Batai, the only known Asian Bunyamwera group virus, and nine Bunyamwera group viruses occurring south of the United States in the Western Hemisphere (*17*). Only one neutralized the new virus to near homologous titer and five of ten not at all. Kairi virus antibody, which had a low homologous titer (1:40), neutralized the new virus to a titer of 1:20; however, the reciprocal cross demonstrated a 128-fold difference. Hemagglutinins, successfully prepared in baby hamster kidney (BHK-21) but not Vero cells, were tested against a number of arbovirus grouping fluids, and only Bunyamwera group and homologous serum inhibited hemagglutination. Thus, the newly recovered viruses are distinct from any of the Western Hemisphere Bunyamwera group viruses and from the only known Asian member of this group. We propose the name Potosi for this new virus.

Other U.S. members of this serogroup include Cache Valley (CV), Northway (NOR), Main Drain (MD), and Lokern (LOK). In domestic livestock, antibody to CV virus has been shown to be highly prevalent (*18*). CV virus was also isolated from blood taken from an ill horse and from blood and brain tissue from a caribou that died in Wisconsin (*19*). More recently, CV virus has been associated with arthrogryposis-hydranencephaly complex in newborn lambs in Texas (*20*). MD virus has been recovered from the brain tissue of a dead horse with clinically diagnosed encephalitis (*21*).

A single human illness attributed to TEN virus infection has been reported, and apparent TEN antibody has been demonstrated in Florida residents (*22*). The other Bunyamwera group viruses from the Western Hemisphere known to be associated with illness in humans are the Fort Sherman virus from Panama and the Xingu virus from Brazil (*23*).

There are three possible explanations for the presence of the virus strains recovered from *Ae. albopictus* in Potosi. The first, and most likely, is that this virus is endemic in the Potosi area and *Ae. albopictus* became infected by feeding on local mammalian hosts. Recent laboratory studies have failed to demonstrate vertical transmission of Potosi virus (*24*). This evidence confutes the other hypotheses, namely, that the virus was introduced via infected eggs from sites in the United States or Asia. Further, there is only a distant antigenic relationship between the new virus and Batai virus from Malaysia, the only known Asian representative of the Bunyamwera group.

These findings represent direct evidence that *Ae. albopictus* may be an arbovirus vector in the United States. Recovery of the virus from mosquitoes does not establish this species as a vector in the United States; however, laboratory studies have demonstrated that *Ae. albopictus* can transmit Potosi virus (*24*). At the same time, experiments demonstrating that the virus is not vertically transmitted by *Ae. albopictus* provide evidence that this species is unlikely to serve as a reservoir and overwintering host for the virus. Vertebrates that may serve as hosts for Potosi virus, or other mosquito species that may serve as natural vectors, are currently unknown.

The important question of whether the virus will infect humans remains to be addressed. Preliminary testing of field workers exposed during mosquito collection showed that 1 of 16 tested had neutralizing antibody to the new virus. It is unclear whether this person was infected during field studies in Potosi or was previously infected with a cross-reactive virus.

Although a major concern has been the involvement of *Ae. albopictus* as a vector of LAC virus to humans, the possibility remains that other viruses, which have heretofore not infected humans to any appreciable degree because of the nonhuman feeding habits of their arthropod vectors, may be transmitted to humans by *Ae. albopictus* as a result of the aggressive feeding of this species on humans as well as on other mammalian hosts.

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Regulation of Progesterone Receptor-Mediated Transcription by Phosphorylation

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The progesterone receptor (PR) in the chicken oviduct is a phosphoprotein that regulates gene transcription in the presence of progesterone. Treatment with progesterone in vivo stimulates phosphorylation of the progesterone receptor. With transient transfection assays, the present work has tested whether phosphorylation participates in the regulation of PR-mediated transcription. Treatment with 8-bromo-cyclic adenosine monophosphate (8-Br cAMP), a stimulator of cAMP-dependent protein kinase [protein kinase A (PKA)], mimicked progesterone-dependent, receptor-mediated transcription in the absence of progesterone. Inhibition of PKA blocked hormone action. Treatment with okadaic acid, an inhibitor of protein phosphatases 1 and 2A, stimulated transcription in a manner similar to that of progesterone. These observations suggest that phosphorylation of the PR or other proteins in the transcription complex can modulate PR-mediated transcription in vivo.

THE STEROID HORMONE RECEPTORS are members of a superfamily of transcriptional regulatory factors (*1*). These receptors are phosphoproteins (*2–4*) that transduce the signal carried by their cognate hormone, resulting in regulation of gene expression. The general organization of the PRs is shown in Fig. 1. Receptors isolated from chicks treated with progesterone show increased phosphorylation, decreased mobility on SDS-polyacrylamide gels, decreased interaction with heat shock protein 90 (hsp90) in vitro, and increased DNA-binding activity (*2, 3*). The PR has three hormonally regulated sites, two in the NH_2-terminal domain and one in the COOH-terminal transactivation domain (*5*).

The present studies assess the function of phosphorylation in regulation of PR-mediated transcription in vivo. Because progesterone stimulates phosphorylation of the PR, we tested whether stimulation of protein kinases could regulate progesterone-dependent, receptor-mediated transcription of target genes. Monkey kidney CV1 cells, which do not express an endogenous PR, were cotransfected with a PR_A expression plasmid and a reporter gene that contained the progesterone response element (PRE) upstream of the chloramphenicol acetyltransferase (*CAT*) gene (*6*). Treatment of cells with progesterone resulted in a marked induction of PR_A-mediated transcription (Fig. 2A). Treatment with progesterone and 8-bromo-cyclic adenosine monophosphate (8-Br cAMP), an activator of protein kinase A (PKA), resulted in twice as much transcription. In the absence of progesterone, 8-Br cAMP caused an induction similar to that elicited by progesterone (about 20-fold).

Since others have shown that 8-Br cAMP elevates glucocorticoid receptor concentrations about twofold in hepatoma cells (*7*), we evaluated the effects of 8-Br cAMP on PR protein. Immunodots (Fig. 2C) showed that PR abundance was decreased about 60%, not increased, by 8-Br cAMP. Immunoblots (Fig. 2B) were performed to test the effects of 8-Br cAMP on receptor proteolysis. Because the amounts of PR produced in

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