sources during the night in the growing season as 25 to 70 ppm (the latter an extreme) above background. Multiplying the values (Fig. 2) by 8 to account for the difference in mixing depth and then by a factor of 2 or 3 to account for the fact that the Clarke-Faoro values occur only during the growing-season night (while the data in Fig. 2 are annual averages) gives annual background increases due to combustion processes generally larger than these natural source values, at least in the Washington–Boston corridor. The conditions of the two models presented here tend to average out the peak concentrations observed within a particular city.

As in all studies of pollution from a multitude of sources, the two important modeling criteria are the meteorology and intensity, distribution, and characteristics of the source material. The meteorological models used here are of the type intended for use over distances of up to a few tens of kilometers. As such, they ignore systematic variations of wind direction between the source and the computation point, perhaps an important consideration in this megalopolis bounded by a mountain range and an ocean. A number of other simplifications, such as ignoring diurnal changes and correlations between wind direction and mixing depth, probably have some effect on the derived patterns. However, on the regional scale considered here, our knowledge of the meteorology of the situation may be greater than our knowledge of the sources. The variety of the possible pollutants of interest, the location of their production, their varying rates of emission, the physical, chemical, and photochemical reactions between various pollutants and classes of pollutants, and the difficulty of correctly measuring their concentrations are but some of the complications. On the regional scale considered here, source inventory data from both metropolitan and nonmetropolitan locations are needed.

The calculations discussed in this note were performed by a CDC 6600 computer. A complete run for each of the models took about ½ minute and included individual output for two or three different values of wind speed, mixing depth, and half-life.

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Concurrent Isolation from Patient of Two Arboviruses, Chikungunya and Dengue Type 2

Abstract. *Chikungunya virus and dengue type 2 virus were isolated from a single blood specimen taken from a patient in the acute phase of a dengue-like illness seen at Christian Medical College Hospital, Vellore, South India, in October 1964. In serial blood specimens collected from this patient there was an increase in antibody to these same two viruses. The technique for unmasking an agent (such as dengue) with a long incubation period in mice in the presence of an agent with a short incubation period is described.*

Occurrence in humans of dengue-like illnesses caused by chikungunya virus, a Group A arbovirus, and by dengue viruses of Group B has been reported from several regions in the Orient, including India (*1*). The patients' responses have ranged from fever with general malaise to a syndrome marked by hemorrhagic phenomena, shock, and death. The term "hemorrhagic fever" has been applied to several outbreaks. Demonstration of viremia or antibody response, or both, in members of the population at risk has shown that during the outbreaks viruses of groups A and B may be active at the same time and in the same place. Serologic evidence of simultaneous infection by viruses of these groups, presumed to be chikungunya and dengue, has been obtained in a number of instances. To our knowledge, however, no instance of proved dual viremia resulting from naturally acquired arbovirus infections has been reported.

In the latter half of 1964 an epidemic of dengue-like illness, associated in some cases with mild hemorrhagic phenomena, occurred in Vellore, South India. We attempted to isolate virus during this time, and took blood samples from 372 patients of the Student-Staff Health Clinic, Christian Medical College Hospital; chikungunya virus was recovered from 195 of these patients, dengue type 1 virus from one, and dengue type 2 virus from three (*2*). Serologic tests on paired or serial blood specimens from 332 of the patients showed that seven patients responded to infection with a simultaneous increase in antibody to both chikungunya and dengue virus antigens. Two of these patients, from whom blood specimens had been taken on consecutive days, were roommates. Both were febrile when first examined in the clinic, and both reported pain in the joints, a common symptom in cases of chikungunya treated in Vellore (*3*); no unusual features suggestive of dual infection were reported or observed during the illnesses of these individuals. Initial specimen 10939-1 from one of these two patients yielded a strain of dengue type 2 virus; initial specimen 10935-1 from the other yielded a strain of chikungunya virus. In October 1965, we attempted isolation of dengue virus from specimen 10935-1, which had been stored in a Revco freezer at −50°C.

Three different mixtures were prepared in sterile test tubes. The first contained equal amounts (0.15 ml) of serum specimen 10935-1 and mouse antiserum to chikungunya virus; the second, similarly, specimen 10935-1 and phosphate saline containing 0.75 percent bovine albumin (BAPS); the third, similarly, specimen 10935-1 and normal mouse serum. Before they were injected into mice, the mixtures were placed in a 37°C bath for 1 hour. Two litters of infant mice (less than 24 hours old) were inoculated per tube, each mouse receiving 0.02 ml intracerebrally; the mice were then observed daily for signs of illness.

The four litters of inoculated control mice, that is, those injected with a mixture of specimen 10935-1 and either BAPS or normal mouse serum, presented a picture typical of the rapid, overwhelming effect of chikungunya virus infection. All the mice showed signs of illness within 2 to 3 days; some died as early as day 3; none recovered. The brains were shown by complement-fixation test to contain chikungunya virus. One of these strains was designated 10935-1C.

The two mouse litters inoculated with a mixture of specimen 10935-1 and mouse antiserum to chikungunya virus presented an entirely different picture. In only four of the 12 mice

Table 1. Identification of 10935-1D virus by neutralizations tests.

Mouse serum, final dilution 1:2	10935-1D virus	
	Titer*	N.I.†
Normal	5.6*	
Dengue-1 immune	2.4	3.2
Dengue-2 immune	1.3	4.3
Dengue-4 immune	3.3	2.3

* The LD_{50}/0.02 ml (lethal dose, 50 percent effective) is expressed as reciprocal in $\log_{10}$ of virus titer. † N.I., neutralization index; the difference in reciprocal log units.

inoculated were signs of illness detected. Two that showed abnormality (slight ataxia, slight ruffling of hair, nose pawing, and slow circling movements) 11 and 13 days after inoculation were killed, and a suspension of brain from each was passed into two new litters. The second-passage mice likewise showed signs typical of dengue infection by day 10; some were paralyzed by day 11, and some were dead by day 16. At the time of the tenth mouse passage (the incubation period was then 5 days and the mortality was 100 percent by the 9th day after inoculation), a crude brain preparation was used as antigen in the complement-fixation test (*4*), and was identified as a strain of dengue type 2 virus. This strain was designated 10935-1D.

Application of the reverse experimental procedures to specimen 10939-1 taken from the patient in the acute phase of the disease resulted only in reisolation of dengue 2 virus, even though we attempted to isolate chikungunya virus. No evidence of dual infection was detected in the inoculated mice.

Table 2. Demonstration of neutralizing antibody to dengue type 2 and chikungunya viruses in three samples of patient's serum (10935-1, 23 October 1964; 10935-2, 4 November 1964; 10935-3, 12 November 1965).

Serum diluted 1:4	Dengue type 2 virus		Chikungunya virus	
	Titer*	N.I.†	Titer*	N.I.†
	Mouse			
Normal	5.3		6.9	
Dengue-2, immune	1.3	4.0		
Chikungunya, immune			<3.0	>3.9
	Patient			
10935-1	4.3	1.0	7.0	Nil
10935-2	<1.0	>4.3	3.7	3.2
10935-3	2.5	2.8	4.2	2.7

* The LD_{50}/0.02 ml (lethal dose, 50 percent effective) is expressed as reciprocal in $\log_{10}$ of virus titer. † N.I., neutralization index; the difference in reciprocal log units.

Evidently the chikungunya virus component of the 10935-1 serum specimen was neutralized by the addition of mouse antiserum to that virus (CMS), and the dengue virus component was thus able to express itself. The results of neutralization tests with 14th mouse-passage 10935-1D virus confirmed identification of this agent as a strain of dengue type 2 virus (Table 1). The tests were done in infant mice inoculated intracerebrally; virus was used in serial tenfold dilutions, with constant dilutions of mouse antiserums for dengue virus types 1, 2, and 4 (Vellore strains 82-1, 60-1, and 968-1R).

In other neutralization tests, serums from patient 10935 in the convalescent and postconvalescent state showed significant increase in antibody to both dengue 2 and chikungunya viruses as compared with serum obtained during the acute phase of the disease (Table 2). The amount of antibody to dengue 2 virus was determined in tests done in mice, with incorporation of CMS to neutralize the chikungunya virus present. The reverse procedure was used for determination of amount of antibody to chikungunya virus.

The isolation of two antigenically unrelated arboviruses from a single serum specimen from a patient in the acute phase of the disease supports the serologic evidence for simultaneous arbovirus infections encountered at Vellore and elsewhere. The unremarkable nature of this patient's illness and the normal development of antibody indicate that the host response was not appreciably altered by the dual infection. Whether or not man could support dual infection with dengue viruses of differing yet closely related antigenic types is not known. Presumably the viruses would compete for the same sites of replication. Though dengue and chikungunya illnesses may be characterized by signs and symptoms that are similar in many cases, the marked involvement of the joints in chikungunya, with prolonged arthralgia that may persist for several months, seems to be a differentiating feature (*3*). Further study may provide evidence of differing tropisms for chikungunya and the dengue viruses.

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Oxygenation Properties of Snake Hemoglobin

Abstract. Natrix taxispilota *hemoglobin has a very high oxygen affinity which depends upon* pH *in an unusual manner. The oxygen affinity increases slightly upon protein dilution, but the* pK's *of the Bohr groups are unchanged. Oxidation promotes hemoglobin polymerization, which can be inhibited by prior treatment with iodoacetamide. Reaction with iodoacetamide also causes a slight increase in the oxygen affinity, no change in the* pK's *of the Bohr groups, and a drastic reduction in heme-heme interaction.*

Largely as a result of the work of Antonini, Wyman, and their co-workers, the oxygenation properties of human hemoglobin are becoming well defined (*1*). However, the oxygenation properties of few other vertebrate hemoglobins have been described. The available data support the idea that the underlying mechanisms controlling the oxygenation reaction of all vertebrate hemoglobins are the same (*2*). Because hemoglobin is thought to be closely adapted to the respiratory needs of each species, it is possible that broad correlations in both structure and function can be made for major taxonomic groups.

The oxygenation properties of hemoglobin from the water snake *Natrix taxispilota* are of interest for several reasons. Water snakes have habits much like those of aquatic turtles, and their hemoglobins may be similarly adapted (*3*). Essentially no data are available on the oxygenation properties of snake hemoglobins (*4*). Chiancone *et al.* (*5*) have reported that hemoglobins from lower vertebrates dissociate less readily