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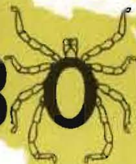

ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

RoH

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IMPORTANT NOTICE: This exchange is issued for the sole purpose of timely exchange of information among investigators of arthropod-borne viruses. It contains reports, summaries, observations, and comments submitted voluntarily by qualified agencies and investigators. The appearance of any information, data, opinions, or views in this exchange does not constitute formal publication. Any reference to or quotation of any part of this exchange must be authorized directly by the person or agency which submitted the text.

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13TH ANNUAL OPEN MEETING OF THE ACAV WITH THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

The worldwide arbovirus community is urged to attend the forthcoming joint meeting of the American Society of Tropical Medicine and Hygiene and the American Society of Parasitologists to be held at the Deauville Hotel, Miami Beach, Florida, November 6-10, 1972.

This is the 13th year that an open meeting of the American Committee on Arthropod-borne Viruses (ACAV) has been held under the auspices of the American Society of Tropical Medicine and Hygiene, at first as a relatively unknown but interested affiliate, now as an integral part. All persons interested in arbovirology are urged to attend the entire Open Meeting, both the initial business session and the outstanding scientific session which follows. As many of you already know, your attendance at an open meeting automatically makes you a member-at-large of this unique committee and entitles you to voting rights for the next three years.

The Open Meeting of the ACAV will be held in Napoleon Room No. 3, Deauville Hotel, Tuesday, November 7, from 8:30-12:00 A.M. The program is as follows:

A. Business Session (please come) 8:30-9:30
   Philip K. Russell, Chairman

B. Scientific Session 9:30-12:00
   Philip K. Russell, Chairman

   1. APPLICATION OF MOLECULAR VIROLOGY TECHNIQUES TO ARBOVIROLOGY

      a. Radioimmune precipitation analysis of subunits of Group A arboviruses. Joel M. Dalrymple, Walter Reed Army Institute of Research, Washington, D.C.

      b. Antigenic composition and morphogenesis of Group B arboviruses. Robert M. Cardiff, University of California, Davis, California 95616.

      c. Structural relationships in the Bunyamwera Supergroup. Frederick A. Murphy, Center for Disease Control, Atlanta, Georgia 30333.

   2. IDENTIFICATION OF ARTHROPOD BLOOD MEALS

      a. Preparation of specific antisera. Constantine H. Tempelis, University of California, School of Public Health, Berkeley, California 94720.

      b. Field application of the microplate precipitin test. Robert B. Tesh, Middle America Research Unit, Balboa Heights, Canal Zone.

In addition, there are many other notable presentations on arboviruses in other portions of the ASTM&H program. All will want to hear the 37th Annual Charles Franklin Craig Lecture, "Epidemiological Experiences in Over-developed Sub-countries" by Dr. Carlos Sanmartin, Cali, Colombia. It is certain that Dr. Sanmartin, one of the world's outstanding arbovirologists, will in part draw upon his long experience in arbovirology. Another must is a 2-hour session, chaired by Dr. Karl M. Johnson, comprised of 8 papers on VEE, EEE and California Group viruses. Another 2-hour session is chaired by Drs. Charles H. Calisher and Robert E. Shope, with 8 papers on Japanese encephalitis, dengue and Ingwavuma viruses. A third session, presided over by Drs. Scott B. Halstead and Leon Rosen, include many papers on subjects of interest to arbovirologists, including Lassa Fever, Machupo virus, the ecology of viruses from phebotomine sandflies and IGM measurements of CSF in encephalitis and meningitis.

Also to be presented are two movies on Venezuelan equine encephalitis, illustrative of the magnificent camera skill of Dr. Telford H. Work. The first is a 30 minute film, "Venezuelan Equine Encephalomyelitis in Mexico," presented by Dr. Hector Campos Lopez, Director for Animal Health, Secretariat of Agriculture and Livestock, Mexico. The second film, entitled "Brote en Nyarit (VEE Reaches Pacific Coast of Mexico in 1972)," narrated by Dr. Luis Fernandez Zorilla of the Equine Health Division, Mexico, and Dr. Telford Work, lasts approximately one hour.

Subcommittee for Arthropod-borne Virus Information Exchange

Report of the Chairman

Work on the third revision of the working Catalogue of viruses is continuing with new registration cards being issued in quarterly installments as they can be completed. While responses have generally been good to requests for up-dated information on previously registered viruses, no replies have been received from a number of individuals responsible for initial registration or re-registration of some viruses in the second edition. With the 3rd Quarter 1972 shipment of Catalogue materials, two installments comprising 181 revised registration cards have been issued.

The Subcommittee on Evaluation of Arthropod-borne Status (SEAS) examined all available data on 270 previously registered viruses in connection with the revision of the working Catalogue. The SEAS, as reported elsewhere in this issue, developed a schema for placing all registered viruses in one of 5 categories. 124 (46%) of the viruses reviewed were rated as arboviruses (82) or probable arboviruses (42), Categories 1 and 2. These are being printed on white paper stock in the revised Catalogue. 126 (47%) were rated as possible arboviruses, Category 3, and these are being printed on gray paper. The 20 remaining viruses (7%) were considered to fall in Category 4, probably not arboviruses (6), or Category 5, not arboviruses (14). Rust-colored paper stock is being utilized for revised registration cards for these agents.

New virus registrations have been submitted at a good rate. During the first three Quarters of 1972, 32 viruses were registered in the Catalogue and one (Uruma) withdrawn. As of this time, a total of 305 viruses are described in the working Catalogue cards. Three new antigenic groups have been added to accommodate newly registered viruses, bringing the total of such serological groups to 42 (including the non-arthropod-borne Tacaribe group). Antigenic group No. 40, Dera Ghazi Khan, includes the tick viruses DGK from Pakistan, Kao Shuan from Taiwan, and Pathum Thani from Thailand. Group No. 41, Warrego, includes the newly registered viruses from Australia, Warrego and Mitchell River. Group No. 42, Boteke, consists of the newly registered Boteke virus and an unregistered virus Dak Ar B 1245, both isolated in the Central African Republic.

Report of the Subcommittee on Evaluation of Arthropod-borne Status[1] (SEAS)

The Subcommittee was requested by the American Committee on Arthropod-borne Viruses to review 270 viruses listed in the published Catalogue of the Arthropod-borne Viruses of the World, published Supplements to the Catalogue, and the Working Card Catalogue to determine which were truly arboviruses. SEAS members decided to classify the viruses primarily on epidemiological and biological evidence and adopted the WHO definition of an Arbovirus[2] as a basis for their classification:

> "Arboviruses are viruses which are maintained in nature principally, or to an important extent, through biological transmission between susceptible vertebrate hosts by haematophagous arthropods; they multiply and produce viraemia in the vertebrates, multiply in the tissues of arthropods, and are passed on to new vertebrates by the bites of arthropods after a period of extrinsic incubation."

The review utilized data from the above three sources of catalogue information, abstracts, published papers, letters from directors of arbovirus laboratories, registrants of viruses, records available at the Yale Arbovirus Research Unit, and research records of SEAS members. Each Committee member was assigned a block of viruses to review, and his summary of data for each agent served as the primary basis for committee decision. Only one member of SEAS was absent at its meeting when all compiled data were reviewed and the classifications reported in the accompanying table were made. The evidence used to classify each virus is indicated by an X in each of the appropriate columns. Additional extensive details are available on the sources, quantity, and quality of data for many viruses. Registrants of any virus may acquire such information by a request to the SEAS Chairman (Dr. W. McD. Hammon). The Committee believes most of the data are accurate. It is anticipated that further research will soon allow many viruses to be reclassified that were placed in an indefinite category.

Five classifications are given in the table, and these are reduced to the following three categories in the new edition of the Working Catalogue:

1. Arbovirus (Column 20), or Probable Arbovirus (Column 21)
2. Possible Arbovirus (Column 22)
3. Not Arbovirus (Column 24), or Probably Not (Column 23)

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[1] Subcommittee on Information Exchange. 1971. Catalogue of Arthropod-borne and Selected Vertebrate Viruses of the World. Amer. J. Trop. Med. & Hyg., 20, p. 1019.

[2] Arboviruses and Human Disease, Report of a WHO Scientific Group, Wld. Hlth. Org. Techn. Rep. Ser. 1967, 369, p. 9.

Categories 1 and 3, although subdivided, include viruses so adequately supported by experimental or epidemiological data that their status is beyond reasonable doubt. The cards of those failing to meet the most rigid criteria described below carry an indication that they are still in a "probable" or "probably not" category. Category 2 (Possible Arbovirus) includes those viruses for which supportive data are still too meager for firm judgment as to whether they should be placed in categories 1 or 3.

Criteria for classification are as follows:

1. Arbovirus or Probable Arbovirus

This classification was applied to all those viruses for which there were many findings supporting principal maintenance in nature by biological arthropod transmission. To be accepted definitely as arthropod-borne (not probably), a virus must have been isolated at least once, usually many times, from an arthropod not believed to have fed on blood for several days; have been transmitted to a vertebrate by means of the bite of an experimentally or naturally infected arthropod (preferably by infection acquired through feeding and not by injection); and a specific viremia must have been demonstrated in some suitable vertebrate host. An occasional acceptable substitute in part for transmission in the laboratory by an arthropod which had acquired infection by feeding has been the isolation of a virus from an exposed sentinel animal, providing: (1) the animal came from a colony well protected from arthropods, and (2) the sentinels were protected from droppings and direct contact with other animals while exposed to arthropods. However, in such an instance, transmission of the virus in the laboratory by an arthropod infected by injection would probably have been required in place of one infected by feeding. Study and observation over a period of many years establishing many epidemiologic associations was also accepted in some instances to substitute for one of the above criteria. Less conclusive evidence of actual arthropod transmission, such as injecting an arthropod and after an extrinsic incubation period reisolating the virus from the salivary glands, or possibly making several such passages, places the virus in the probable category. In many such instances, members of the Committee had no doubt regarding the method of transmission but felt that a critical transmission experiment still should be performed. The number of isolations from an arthropod, the group classification of the virus and closeness of relationship to other accepted arboviruses, etc., also played a role in the judgment decision made by the Committee.

2. Possible Arbovirus

This classification was assigned to viruses that were first isolated from an arthropod or were first isolated from man or an animal under circumstances epidemiologically compatible with arthropod transmission. Accumulated evidence was insufficient for further classification than "Possible". Some such viruses had been isolated only once and this during a project devoted to a search for arboviruses.

3. Not an Arbovirus or Probably Not

A virus was classified as not an arbovirus when evidence indicated clearly that the most likely and most common means of maintenance in nature is not through biological transmission by an arthropod vector. Usually there was good evidence for another effective method of transmission, directly from one vertebrate to another by bite, ingestion or inhalation of excreta, or in a few instances by mechanical transmission through an arthropod. When somewhat less conclusive evidence was available, the virus was classified as probably not an arbovirus.

SEAS will welcome comments from readers. New information, unpublished or unrecorded old information, and constructive arguments will be particularly useful for future re-evaluation of arthropod-borne status.

William McD. Hammon, Chairman
Thomas H. G. Aitken
Roy W. Chamberlain
Donald M. McLean
William C. Reeves
Albert Rudnick

CLASSIFICATION OF ARTHROPOD-BORNE STATUS

Name of Virus	Isolation from naturally infected arthropod			Biological Arthropod transmission demonstrated				Arthropod infected by injection		Phleb. or Tick only	Vertebrate viremia demonstrated			Relation-ship	Epidemiology	Negative Information				Classification					Remarks
	1 Once only	2 2-9 times	3 ≥10 times	4 With arthropod infected by feeding	5 "Isolated" sentinel animal infected	6 With wild caught naturally infected arthropod	7 With arthropod infected by injection	8 Isolation from salivary glands	9 Isolation from whole arthropod	10 Transtadial or transovarian infection demonstrated	11 Experimentally infected animal or man	12 "Isolated" sentinel (col. 5)	13 Wild caught or man other than by laboratory infection	14 Antigenic relationship to a proven arbovirus	15 Strong associations	16 Physical, chemical or biologic characteristics unusual	17 Failure to replicate or pass serially in suitable arthropod	18 Isolation from salivary gland, urine or feces of vertebrate	19 Failure to pass transtadially in tick if isolated from tick	20 Arbovirus	21 Probable arbovirus	22 Possible arbovirus	23 Probably not arbovirus	24 Not arbovirus	
Absettarov			X							X	X		X	X	X					X					Assuming prob. RSSE
Acado	X																					X			
Acara		X			X			X					X	X							X				
African horsesickness			X	X							X		X		X					X					
Aino		X												X								X			
Akabane		X							X		X			X								X			
Alfuy	X							X	X				X	X							X				
Amapari		X									X		X					X						X	Arenavirus
Anhanga														X								X			
Anhembi		X													X							X			
Anopheles A			X					X	X		X										X				
Anopheles B		X						X														X			
Apeu		X			X			X			X	X	X	X	X						X				
Apoi													X									X			
Arkonam		X							X													X			
Aruac			X					X			X										X				
Arumowot	X													X		±						X			
Aura		X												X								X			
Bahig													X									X			
Bakau			X										X									X			
Bandia			X										X									X			
Banzi		X		X							X		X	X						X					
Batai		X							X					X								X			
Bebaru		X												X								X			
Bertioga					X							X										X			
Bhanja			X								X											X			
Bimiti			X		X						X	X	X	X	X						X				
Bluetongue			X	X	X		X		X		X		X		X					X					
Bocas	X																					X			
Boracea		X									X											X			
Bouboui		X																				X			
Bujaru													X	X								X			

CLASSIFICATION OF ARTHROPOD-BORNE STATUS

Name of Virus	Isolation from naturally infected arthropod			Biological Arthropod transmission demonstrated				Arthropod infected by injection		Phleb. or Tick only	Vertebrate viremia demonstrated			Relationship	Epidemiology	Negative Information				Classification					Remarks
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Bunyamwera			X	X			X	X	X		X		X	X						X					
Burg El Arab													X									X			
Bushbush		X			X	X		X						X						X					
Bussuquara			X	X	X	X		X			X	X	X	X	X					X					
Buttonwillow			X	X					X		X		X	X	X					X					
Bwamba		X						X	X		X		X	X							X				
Cache Valley			X	X							X			X						X					
California Encephalitis			X	X			X	X	X		X		X	X	X					X					
Calovo		X									X			X	X							X			
Candiru								0(mosq)					X	X			X					X			
Capim			X		X	X	X					X	X	X						X					
Caraparu			X		X	X		X	X		X	X	X	X	X					X					
Catu			X	X	X			X			X	X	X	X	X					X					
Chaco								X	X													X			
Chagres	?												X	X								X			
Chandipura	X			X							X		X	X	X						X				
Changuinola			X										X	X	X	±					X				
Chenuda			X						X													X			
Chikungunya			X	X				X	X		X		X	X	X					X					
Cocal		X		X	X		X	X	X		X		X	X	X					X					
Colorado Tick Fever			X	X						X	X		X		X					X					
Congo			X				X		X	X	X		X							X					
Corriparta		X						X	X				X								X				
Cotia		X			?			0(mosq)			X	X			X	X	X							X	Poxvirus
Cowbone Ridge													X	X									X		Probably Modoc-like
Dakar Bat														X				X						X	
Dengue 1			X	X							X		X	X	X					X					
Dengue 2			X	X							X		X	X	X					X					
Dengue 3			X	X				X	X				X	X	X					X					
Dengue 4			X						X				X	X	X					X					
Dera Ghazi Khan	X																					X			
Dhori		X																				X			
Dugbe			X						X				X									X			
Edge Hill		X					X		X					X							X				
Eastern Equine Enceph.			X	X	X		X	X	X		X	X	X	X	X					X					
Episootic Hem. Dis.	X							X	X		X		X									X			
Entebbe Bat														X				X						X	
Eubenangee	X							X	X												X				
Flanders			X			X							X							X					
Gamboa		X												X								X			
Ganjam			X						X		X		X									X			
Germiston			X						X		X		X	X							X				
Getah			X								X			X	X						X				
Gossas																		X					X		
Grand Arbaud		X		X							X									X					

CLASSIFICATION OF ARTHROPOD-BORNE STATUS

Name of Virus	Isolation from naturally infected arthropod			Biological Arthropod transmission demonstrated				Arthropod infected by injection		Phleb. or Tick only	Vertebrate viremia demonstrated			Relationship	Epidemiology	Negative Information				Classification					Remarks
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Guajara			X		X	X		X			X	X	X	X						X					
Guama			X	X	X	X		X			X	X	X	X	X					X					
Guaratuba		X			X										X							X			
Guaroa			X					X	X		X		X	X	X						X				
Gumbo Limbo			X								X		X	X	X							X			
Hanzalova		X									X		?	X						X					Assuming prob. RSSE
Hart Park			X				X	X	X		X		X								X				
Hazara	X																					X			
Hughes			X						X	X			X								X				
Hypr			X	X						X	X		X	X	X					X					Assuming prob. RSSE
Icoaraci			X					O(mosq)			X		X	X			X					X			
Ieri		X						X			X											X			
Ilesha	X						X		X		X		X	X							X				
Ilheus			X	X	X		X	X	X		X	X	X	X	X					X					
Ingwavuma		X		X			X		X					X						X					
Irituia													X									X			
Israel Turkey							X				X			X								X			
Itaporanga		X			X								X	X							X				
Itaqui			X		X							X	X	X	X						X				
Jamestown Canyon			X	X					X					X						X					
Japanese B Encephalitis			X	X	X		X	X	X		X	X	X	X	X					X					
Jerry Slough		X		X							X			X	X					X					
Johnston Atoll	X									X	X			X								X			
Juan Diaz					X									X								X			
Junin		?									X		X				?	X						X	Arenavirus
Jurona	X																					X			
Kairi			X		X		X	X	X		X	X		X							X				
Kaisodi			X										X									X			
Kamese	X																					X			
Kammavanpettai											X		X									X			
Kannamangalam													X									X			
Karimabad	X													X								X			
Kasba	X								X							±						X			
Kemerovo			X								X		X								X				
Kern Canyon																X	X	X					X		
Ketapang	X							X			X											X			
Keuraliba													X									X			
Keystone			X	X	X						X	X	X	X	X					X					
Kokobera		X					X		X					X							X				
Koongol		X							X												X				
Koutango													X	X								X			
Kowanyama		X							X													X			
Kumlinge			X										?	X	X					X					Same as RSSE
Kunjin			X	X					X		X			X						X					
Kwatta	X																					X			

CLASSIFICATION OF ARTHROPOD-BORNE STATUS

Name of Virus	Isolation from naturally infected arthropod			Biological Arthropod transmission demonstrated				Arthropod infected by injection		Phleb. or Tick only	Vertebrate viremia demonstrated			Relation-ship	Epidem-iology	Negative Information				Classification					Remarks
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Kyasanur Forest Disease			X	X					X	X	X		X	X	X					X					
La Crosse			X	X	X						X	X	X	X	X					X					
Lagos bat																	X	X						X	Rabies related
La Joya	X																					X			
Langat		X		X						X	X			X						X					
Lanjan		X																				X			
Lassa													X					X						X	Arenavirus
Le Dantec													X									X			
Lipovnik		X																				X			
Lokern			X	X							X		X	X	XX					X					
Lone Star	X										X											X			
Louping ill			X	X		X				X	X		X	X	X					X					
Lukuni		X						X			X											X			
Machupo											X		X			X	X	X						X	Arenavirus
Madrid		X			X	X							X	X						X					
Maguari			X	X			X	X	X		X		X	X						X					
Mahogany Hammock			X		X						X		X	X	X							X			
Main Drain			X	X							X		X	X	X					X					
Manawa			X																			X			
Manzanilla								X	X				X	X	?							X			
Mapputta		X					X	X	X												X				
Marco								X														X			
Marituba		X			X	X		X	X		X	X	X	X	X					X					
Matariya													X									X			
Matruh													X			±						X			
Matucare		X																				X			
Mayaro			X			X	X	X	X		X		X	X	X					X					
Melao		X						X	X		X			X	X						X				
Mermet													X	X								X			
Middelburg			X	X							X			X	X					X					
Minatitlan					X									?								X			
Minnal	X								X													X			
Mirim		X			X	X		X				X	X							X					
Montana Myotis Leuko.														X				X						X	
Modoc											X			X			X	X						X	
Moju			X		X						X	X	X	X	X						X				
Moriche	X													X								X			
Mossuril		X																				X			
Mount Elgon bat									X									X					X		
M'Poko		X												X								X			
Mucambo			X		X	X		X	X		X	X	X	X	X					X					
Murutucu			X		X			X			X	X	X	X	X						X				
Murray Valley Enceph.		X		X			X	X	X		X		X	X	X					X					
Nairobi Sheep Disease			X	X							X				X					X					
Nariva																							X		Probably paramyxoviru

CLASSIFICATION OF ARTHROPOD-BORNE VIRUSES

Name of Virus	Isolation from naturally infected arthropod			Biological Arthropod transmission demonstrated				Arthropod infected by injection		Phleb. or Tick only	Vertebrate viremia demonstrated			Relation-ship	Epidemiology	Negative Information				Classification					Remarks
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Navarro								X														X			
Ndumu		X			X						X		X	X							X				
Negishi											X		X	X								X			
Nepuyo		X			X	X		X			X		X	X						X					
Nkolbisson		X																				X			
Nodamura	X			?	?				X							X							X		Like Picornavirus by EM
Ntaya		X					X	X	X		X			X							X				
Nyamanini			X								X		X		X						X				
Nyando		X																				X			
Okola	X																					X			
Omsk Hemorrhagic fev.			X	X						X	X		X	X	X					X					
O'Nyong Nyong			X	X				X	X		X	X	X	X	X					X					
Oriboca			X	X	X	X		X	X		X	X	X	X	X					X					
Oropouche		X						X	X		X		X	X	X						X				
Ossa		X				X							X	X						X					
Pacora		X																				X			
Pacui			X		±			0(mosq)			X		X				X				X				
Pahayokee			X								X			X	X							X			
Palyam	X								X							±						X			
Patois		X			X	X							X	X						X					
Pichinde			X										X			X		X						X	Arenavirus
Piry								X			X		X	X								X			
Pixuna		X											X	X								X			
Pongola			X	X							X									X					
Ponteves	X																					X			
Powassan			X	X						X	X		X	X	X					X					
Punta Toro													X	X								X			
Qalyub		X							X													X			
Quaranfil			X	X						X					X					X					
Restan		X		X							X		X	X						X					
Rift Valley fever			X	X							X		X		X					X					
Rio Bravo											X			X			X	X						X	
Ross River		X					X		X		X		X	X	X					X					
Royal Farm		X												X								X			
Russian Spring Summer Enc			X	X						X	X		X	X	X					X					
Sabo	X												X	X								X			
Saboya													X	X								X			
Sagiyama			X		X						X			X	X						X				
Salehabad	X													X								X			
San Angelo		X												X								X			
Sandfly fever (Naples)		X		X									X	X	X					X					
Sandfly fever (Sicilian)			X	X						X			X	X	X					X					
Sango	X												X	X								X			
Sathuperi		X												X								X			
Sawgrass			X																			X			

CLASSIFICATION OF ARTHROPOD-BORNE VIRUSES

Name of Virus	Isolation from naturally infected arthropod			Biological Arthropod transmission demonstrated				Arthropod infected by injection		Phleb. or Tick only	Vertebrate viremia demonstrated			Relation-ship	Epidem-iology	Negative Information				Classification					Remarks
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Sembalam													X									X			
Semliki Forest		X		X	X		X	X	X		X			X						X					
Shamonda	X													X	X							X			
Shark River			X								X		X	X	X							X			
Shuni		X											X	X								X			
Silverwater		X									X		X									X			
Simbu		X						X	X		X			X							X				
Sindbis			X	X	X						X		X	X	X					X					
St. Louis encephalitis			X	X	X	X	X	X	X		X	X	X	X	X					X					
Soldado		X																				X			
Sororoca		X												X								X			
Spondweni			X	X							X		X	X						X					
Stratford		X							X					X								X			
Tacaiuma		X			X			X			X	X	X								X				
Tacaribe	X?							0	0?		X						X	X						X	Arenavirus
Tahyna			X	X							X		X	X						X					
Tamiami													X											X	Arenavirus
Tanga	X								X													X			
Tataguine		X											X									X			
Tembe		X																				X			
Tembusu			X											X	X							X			
Tensaw			X	X							X		X	X	X					X					
Tete									X													X			
Thimiri													X									X			
Thogoto			X										X									X			
Thottapalayam																						X			
Timbo																						X			
Tlacotalpan		X												X								X			
Toure																						X			
Tribec		X											X									X			
Triniti	X						X	X	X		?										X				
Trivittatus			X		X						X	X	X	X	X						X				
Trubanaman	X								X													X			
Turlock			X	X	X	X		X	X		X	X	X		X					X					
Uganda S	X			X			X	X	X		X			X	X					X					
Umbre			X					X			?		X	X							X				
Una			X		X						X			X							X				
Upolu	X																					X			
Usutu		X												X								X			
Uukuniemi			X								X		X		X							X			
Vellore	X															±						X			
Venezuelan Equine enceph.			X	X	X	X			X		X	X	X	X	X					X					
Venkatapuram	X								X													X			
VSV-Indiana		X		X	X		X	X	X	X	X		X	X	X					X					
VSV-New Jersey								X	X					X								X			

CLASSIFICATION OF ARTHROPOD-BORNE STATUS

	Isolation from naturally infected arthropod			Biological Arthropod transmission demonstrated				Arthropod infected by injection		Phleb. or Tick only	Vertebrate viremia demonstrated			Relation-ship	Epidemiology	Negative Information				Classification					Remarks
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Wad Medani		X							X	X												X			
Wanowrie		X									X											X			
Western Equine enceph.			X	X	X	X	X	X	X		X	X	X	X	X					X					
Wesselsbron			X	X				X			X		X	X	X					X					
West Nile			X	X			X	X	X	X	X		X	X	X					X					
Whataroa		X					X	X	X		X			X	X					X					
Witwatersrand		X						X	X		X		X									X			
Wongal		X							X												X				
Wyeomyia			X					X			X		X	X							X				
Yellow fever			X	X	X						X	X	X	X	X					X					
Yogue																						X			
Zegla					X								X	X								X			
Zika			X	X	X			X	X		X	X	X	X	X					X					

REPORT FROM THE DIVISION OF MEDICAL MICROBIOLOGY
UNIVERSITY OF BRITISH COLUMBIA,
VANCOUVER, CANADA

From 14 May to 7 August 1972 sera was collected from 1498 small ground mammals in the Yukon Territory of Canada, principally within 60 miles of Whitehorse (61°N, 135°W) but extending northwards beyond Dawson City (64°N, 139°W) to the Arctic Circle. Of the 1241 sera collected to 15 July which have been examined for neutralizing antibody to a Yukon 1971 mosquito isolate of California encephalitis (CE) virus (Montana snowshoe hare subtype), the 176 positive reactors included 116 of 286 snowshoe hares (*Lepus americanus*) and 59 of 943 ground squirrels (*Citellus undulatus*). Although the highest proportion (47%) of CE reactors among snowshoe hares occurred 20 to 30 miles east of Whitehorse, where most of the 1971 mosquito isolates of CE virus were achieved, 48% positive reactors were also found at 64°N. Sera from 51 hares and 5 squirrels with CE neutralizing antibody also had complement fixing antibody.

Of the 30 pools of Yukon mosquitoes which have been tested to date, CE virus has been isolated from one pool of 72 *Aedes communis* collected about 30 miles east of Whitehorse on 1st August 1972.

Arising from the observation that *Culiseta inornata* mosquitoes bit the writer and his associates during evening collections in the Yukon at atmospheric temperatures around 32°F, attempts were made to propagate Yukon CE virus isolates in *Culiseta inornata* at temperatures ranging from 30 to 80°F. The minimum infective dose of CE virus for this species which was held at 50°F for 30 days after intrathoracic injection was 0.1 mouse LD_{50} - the same as for *Aedes aegypti* which were held at 80°F for three days after injection. Virus replication was also detected after mosquitoes were held at 80° and 40°F, but viral persistence only was noted after incubation at 30°F. Virus proliferation has also been demonstrated in *Aedes communis* which were held at 80° and 50°F after intrathoracic injection.

(Donald M. McLean)

REPORT FROM THE VIRAL AND RICKETTSIAL DISEASE LABORATORY,
CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH,
BERKELEY, CALIFORNIA

During 1971, 620 ill persons were tested serologically for arbovirus infection. There were 3 confirmed cases of WEE, and 2 confirmed cases of SLE. One suspect case of SLE (compatible illness, high stationary antibody titers) could not be confirmed conclusively. Several cases of dengue (probably type 2) were diagnosed in travelers returning from Tahiti and Somoa during the summer of 1971, and one case occurred in January, 1972, in a Californian returning from Haiti, W.I.

One case of Venezuelan equine encephalitis was detected retrospectively in an entomologist who became ill in Monterey County September 26, 1971, with fever, nausea, chills, malaise, leucopenia, and possible mild encephalitis. Complement-fixation tests were negative for VEE, WEE, SLE, CE, and herpes. At the end of the year, the sera were selected along with 154 other serum pairs for arbovirus HAI tests in a cooperative study with the School of Public Health, U.C., Berkeley. Rising antibody titers (<1:10 to ≥1:160) for VEE were shown, and subsequently confirmed by indirect fluorescent antibody tests (<1:8 to 1:128), cell culture neutralization tests (<1:8 to 1:1024), and mouse neutralization index tests (0.4 to 5.2). A repeat CF test, using an improved VEE antigen prepared in BHK cell cultures with the TC 83 strain of virus showed a transient rise in titer of the sera from <1:8 to 1:16, then a fall to <1:8 again 8 months later. The patient had been working in Mexico shortly before onset of illness, in Culiacan, Sinaloa State, Mexico, and was heavily exposed to mosquitoes. The patient's wife was tested June 9, 1972, and had no VEE antibody, During 1971, there were no other cases of F.U.O., influenza-like disease, or encephalitis reported from the patient's home area, and no cases of encephalitis were reported in horses from the county. The patient was not bitten by mosquitoes after returning home. Light trap studies in August, September, and October, 1971, showed less than 1 mosquito per trap night from the 8 traps in the mosquito abatement district. Thus, no evidence could be found of virus spread from this introduced case.

There were 145 suspect encephalitis cases in equines reported to the Department during 1971, and blood samples were obtained from 88 for serologic tests. There were 16 positive or presumptive-positive cases of WEE in 10 counties from June to September. Blood or tissue specimens from 79 equines were tested for virus, and 6 isolations of VEE virus (vaccine strain) were made from recently vaccinated equines. There was no evidence of wild VEE virus introduction into the equine population.

There were 195 tissue specimens from wildlife species tested for arboviruses, yielding 1 isolate of lymphocytic choriomeningitis virus from a _Mus musculus_, and 6 isolates of Rio Bravo virus from _Tadarida mexicana brasiliensis_ bats.

There were 60,545 mosquitoes or other Diptera (1,784 pools) tested for virus, and 65 viral isolates were obtained, including 20 Turlock, 16 WEE, 6 SLE, 2 CE-group, and 21 unidentifiable viruses. Isolates were found from June through September in 14 counties in the San Joaquin, Sacramento and Imperial Valleys.

Thus far in 1972 (to August 31) no human cases of encephalitis due to arboviruses have been confirmed in California. Human surveillance has concentrated particularly on travelers returning from Mexico with febrile illnesses, but this has not revealed any VEE cases.

There have been no laboratory-confirmed cases of arbovirus encephalitis in equines as yet this year, although 42 equines have been reported as clinically suspicious for encephalitis, from Central Valley counties. Sera for laboratory tests were either not obtainable, were negative for WEE and VEE antibodies, or in a few instances showed only stationary WEE antibody titers consistent with past infection or immunization. Interpretation of serologic results this year is made very difficult by the WEE and VEE immunization programs which have been intensified in the past 2 years. Brain or tissue samples from 18 equines have been tested in suckling mice, but yielded no viruses.

The State Virus Laboratory has tested over 68,500 mosquitoes (2,459 pools) thus far in 1972. There have been 18 isolates of Turlock virus, 21 isolates of SLE virus, 16 isolates of WEE virus, and 4 unidentifiable viruses. The WEE and SLE viruses have come almost entirely from the Imperial Valley. Eleven sentinel hamsters were tested from study sites in Imperial County after they were found dead, most likely from the heat. No viruses were isolated from the tissues.

Serologic testing of 109 rodents of various species trapped in San Diego, Los Angeles, and Imperial Counties from January through the middle of June has been done by the metabolic inhibition test for VEE and WEE neutralizing antibodies. Low titers (1:4 to 1:8) of WEE antibody were detected in 2 *Neotoma lepida* from San Diego County collected May 11 and June 14, and in 6 *Sigmodon hispidus* collected June 14-16 from Yuma, Arizona, and Imperial County, California. None of the rodents had VEE neutralizing antibody.

There have been at least 5 human cases of Colorado tick fever acquired from tick bite thus far in 1972. All recovered completely. Fluorescent antibody staining of blood smears continues to be a useful method for rapidly identifying cases. A paper reporting on the intraerythrocytic location of the virus in blood of human cases and experimentally-infected animals is being published in the Journal of General Virology.

Samples of Ixodes uriae ticks collected by Dr. Harald N. Johnson from Flat Iron Rock off the coast of Humboldt County, California, June 22, 1972, have yielded 3 viral agents suspected to be in serogroup B or the Kemerovo-Chenuda-Mono Lake group of arboviruses. Further characterization is in progress.

(Dr. Richard W. Emmons)

REPORT FROM THE ARBOVIRUS RESEARCH UNIT, SCHOOL OF PUBLIC HEALTH, UNIVERSITY OF CALIFORNIA, BERKELEY

Ecology of arboviruses in California

The purpose of this study is to develop a comprehensive knowledge of ecologic factors basic to perpetuation of arboviruses in a temperature climate. Aims are: to identify vector population thresholds essential to maintain basic viral transmission cycles and to extend viral transmission to susceptible human, equine and wildlife populations; to develop laboratory techniques and models to elucidate field data on overwintering mechanisms of arboviruses; and to evaluate the impact of desert conversion to irrigated farming on the arbovirus ecosystem.

A third year of data was collected on vector populations; arbovirus infection in mosquitoes and wild vertebrate hosts; and clinical disease in man, horses and wildlife in the Sacramento Valley. Study areas included foothill, suburban, rural community and rural agricultural environments; the areas were subject to intensive, minimal or no mosquito control. Viral tests of 75,777 mosquitoes of 16 species yielded: WEE viral isolates, 23 from Aedes melanimon, 1 from Anopheles freeborni, and 23 from Culex tarsalis; SLE viral isolates, 2 from Culex peus and 26 from C. tarsalis; Turlock viral isolates, 2 from C. peus and 12 from C. tarsalis; and California serogroup isolates, 9 from Ae. melanimon and 1 from An. freeborni. Rio Bravo virus was isolated from 5 organ pools of Mexican free-tailed bats. Hemagglutination-inhibiting (HI) tests were done on sera from 323 wild mammals and 586 wild birds. Antibodies for WEE virus were most prevalent in jackrabbits (55%), gray squirrels (29%), and pheasants (17%); for SLE virus, in gray squirrels (50%), pheasants (17%), and jackrabbits (15%); for Turlock virus, in house finches (20%) and pheasants (17%); for California virus, in jackrabbits (60%); for Rio Bravo virus, in Mexican free-tailed bats (75%); and for Buttonwillow virus, in jackrabbits (24%). Antibodies to Modoc, Powassan, Lokern and Main Drain viruses were rarely detected.

Arbovirus activity in the Sacramento Valley in 1971 was essentially similar to 1970 except that WEE virus was widely active after a virtual two-year absence. Arbovirus activity was highest in rural communities and agricultural areas adjacent to the Sacramento River. Viral activity correlated with lack of mosquito control and high mosquito vector populations. WEE, SLE, and/or Turlock viral activity occurred at most locations, including suburban Chico, where an average of five or more female C. tarsalis were collected per light trap night. There was one confirmed case of WEE in an 11-year-old boy in Glenn County. There were three confirmed and two presumptive cases of WEE in horses in Butte and Glenn Counties.

WEE viral infection rates in Ae. melanimon and C. tarsalis were similar and virus was in both species in the same time period. High WEE infection rates in Ae. melanimon were unexpected, based on previous observations, but are explained partially by a high prevalence of WEE virus antibodies in jackrabbits (indicating that infection rates of jackrabbits had been high) and frequent feeding of Ae. melanimon on jackrabbits in the Sacramento Valley.

We studied the role of gray squirrels in the ecology of WEE virus because this virus has been found in the brain of sick squirrels from Butte County. Following experimental subcutaneous inoculation with two strains of WEE virus, 50 to 100 percent of animals died within 5 to 14 days. These animals had high titer viremias and could have served as a source of vector infection. We have not detected WEE infection in gray squirrels during the period when the virus generally was absent from this area and conclude that gray squirrels are aberrant tangentially infected hosts of WEE virus.

In the Kern County study area there was no evidence that arboviruses other than Turlock virus were active.

A study was made to determine if autogeny in C. tarsalis will dampen the arbovirus transmission cycle in the Sacremento Valley. Autogeny rates were 0 to 20% in the spring, about 95% in July and August, and decreased to 0 to 13% in the fall. Autogenous females in the field rarely sought a blood meal as evidenced by their failure to be attracted by CO_2 but would feed under laboratory conditions.

Modoc virus, a group B arbovirus apparently not vector transmitted, produces chronic infections in deer mice and hamsters. A study was made of vertical and horizontal viral transmission in these hosts. Virus was recovered from lungs of deer mice for up to six months and from urine of hamsters for at least three months after infection. There was no evidence of vertical or horizontal transmission.

We have hypothesized that widespread development of organophosphorous (OP) resistance in C. tarsalis has made them less effective viral vectors. We have now evaluated the susceptibility to WEE virus of field-collected and laboratory reared populations of C. tarsalis. Field populations generally are equally susceptible by intrathoracic inoculation but vary as much as 10,000 fold in susceptibility by pledget feeding. Some populations appear to be composed of mixed virus susceptible and resistant individuals. If vector competence is genetically controlled, then this adds a new perspective to arbovirology. It is significant that nonpressured but OP resistant C. tarsalis were as susceptible as OP nonresistant C. tarsalis. The most OP resistant field population was the most susceptible when fed WEE virus. However, an OP resistant colony of C. tarsalis that was reared in the presence of Baytex was more resistant to infection with WEE virus than the nonpressured counterpart when adult viral susceptibility was tested by intrathoracic inoculation.

The objectives of this program during the coming year will be the same as those outlined above. However, our knowledge on arbovirus activity in Butte and Glenn Counties is sufficient now to focus our attention on one study site along the Sacramento River but we will continue to monitor arbovirus activity at the original 12 study sites. Our study on the susceptibility of field populations of C. tarsalis to WEE virus will be intensified and extended to include SLE and Turlock viruses. Efforts will be made to detect the introduction of VEE into California.

(Dr. William C. Reeves)

REPORT FROM THE ARBOVIRAL DISEASE SECTION,
ECOLOGICAL INVESTIGATIONS PROGRAM, CDC, USPHS,
FORT COLLINS, COLORADO

Investigations of Venezuelan equine encephalitis (VEE) virus were the principal field activities during the summers of 1971 and 1972. Following the confirmation of equine and human VEE cases in the Lower Rio Grande Valley of Texas in early July, 1971, 160 bird sera (34 species) and 283 mammal sera (11 species) were obtained from wild vertebrates at three study sites in Cameron and Hidalgo Counties to determine VEE antibody prevalence. Serologic evidence that rabbits had been infected with VEE virus was obtained. Although the sample sizes were small, both black-tailed jackrabbits (*Lepus californicus*) and desert cottontails (*Sylvilagus audoboni*) were shown to be positive for hemagglutination-inhibition (HI) and serum neutralizing (SN) VEE antibody (Table 1). A subsequent survey of dogs at veterinary clinics and pounds in the aforementioned counties revealed 2/99 (2%) positive for VEE antibody in plaque reduction neutralization tests and 9/172 (5%) positive in HI tests.

A surveillance network for VEE and other arboviruses was established during the spring of 1972 in cooperation with the U.S. Department of Agriculture along 10 major river routes in Texas, Louisiana, Oklahoma, Colorado, New Mexico, and Arizona. Through August 1972 there were isolations of 140 viral agents from more than 86,000 mosquitoes, but none was VEE virus. The arbovirus isolates by mosquito species are listed in Table 2, and the isolates obtained by state are listed in Table 3.

Tests of approximately 100 serial serum samples of sentinel burros located at surveillance sites also had not yielded evidence of VEE virus infection.

Differences in susceptibility to infection with western equine encephalitis (WEE) virus between *Culex tarsalis* from different geographic areas could be an important factor relating to different levels of virus transmission and arboviral disease in epidemic and non-epidemic areas.

Studies of the susceptibility of *C. tarsalis* were done with the Fort Collins colony strain and wild mosquito strains from Colorado and Texas. The mosquitoes were reared from 4th instar larva or pupa collected during May, July, August, and September 1971; in May they were reared from eggs of naturally hibernated adults. The reared females were allowed to feed on pledgets containing WEE virus diluted in defibrinated blood and to incubate virus. Results throughout the season consistently showed resistance on the part of the colony *C. tarsalis* strain when compared to the Colorado and Texas wild strains. A summation of four experiments is given in Table 4.

Studies on the ecology of WEE virus in Hale County, West Texas have repeatedly shown high WEE virus antibody prevalence in adult house sparrows (<u>Passer domesticus</u>). During the spring and early summer seasons numerous isolations ~~isolations~~ of WEE virus were also made from viremic nestling house sparrows. Available literature suggested that newly hatched birds were protected by transovarian passage of maternal antibody. This did not seem compatible with the field observations and a study was initiated to investigate the influence of maternal WEE antibody on susceptibility of nestling birds. Two breeding colonies of house sparrows were established. In one aviary, all birds had WEE HI antibody titers >1:80. The other aviary was stocked with birds having no demonstrable WEE HI antibody. These HI results were later confirmed by neutralization tests. Aviaries were screened to exclude mosquitoes. Twenty-five nestling birds from each flock ranging in age from 4 to 16 days old were inoculated subcutaneously with either an average 250 PFU of WEE virus or PBS diluent. An additional 5 and 7 nestlings from the non-immune and immune flocks, respectively, were inoculated with PBS. Nestling birds were alternately bled by jugular venipuncture and throat scarification with blood collected on a swab that was put into 0.5 ml of diluent. Flocks of immune and non-immune leghorn chickens were also established and 10 day old chicks from both groups were inoculated with the same WEE virus strain. All testing of nestling and chick blood samples was done by plaque assay in primary duck embryo cell culture. There was excessive mortality among nestling birds due to handling trauma. The average survival time for WEE-inoculated nestlings was 2.7 and 4.9 days, respectively, for the progeny of non-immune and immune parents. Average survival for PBS inoculated birds was 4.0 days.

These results suggest a decreased survival for nestlings from the non-immune flock although nestling numbers available for testing after 1 day were not sufficient to allow valid statistical comparison. Generally, WEE viremia exceeded $10^{5.0}$/0.2 ml blood in both groups for 3 days following inoculation and declined to undetectable levels by day 5. A single nestling from immune parents had viremia for 7 consecutive days following inoculation. Not tested on days 8 and 10, this nestling had demonstrable viremia on day 9 and none on day 11. The ratio of viremic nestlings was very similar in the groups from immune and non-immune parents with 18/22 and 18/20 positive respectively. None of the PBS inoculated nestmates became infected throughout the study. Daily viremic patterns in the 2 groups of nestlings are summarized in Table 5.

In contrast to results obtained with house sparrow nestlings, none of 30 chicks from the WEE-immune flock had detectable viremia at 24, 48, 72, or 96 hours post-inoculation. All 24 chicks from the non-immune flock were viremic by 48 hours post-inoculation with viremia persisting through 72 hours in 12 of 23 chicks. None had demonstrable viremia at 96 hours post-inoculation.

A similar experiment was carried out with nestlings reared from flocks of SLE-immune and nonimmune house sparrows. Tabulation of results has not been completed; however, 11/18 (61%) and 30/40 (75%) inoculated nestlings from immune and nonimmune parents, respectively, had detectable viremia on 1 or more days. The viremia profile in the two groups was markedly different. The mean viremia titer for all positive nestlings from immune parents was $10^{1.6}$ and $10^{1.9}$ PFU/1.0 ml blood on days 2 and 3 post-inoculation, respectively, and less than $10^{1.0}$ PFU on all other days through day 8, which was the longest period viremia was demonstrable. The mean viremia titer in nestlings from nonimmune parents was approximately $10^{4.0}$ PFU/1.0 ml blood on days 2, 3, and 4 post-inoculation and $10^{2.6}$ and $10^{2.4}$ PFU on days 5 and 6, respectively. Results from SLE-inoculated chicks reared from immune leghorn hens were similar to those with WEE virus in which viremia was apparently suppressed by transovarian antibody.

The results suggest that house sparrows and chickens are different in regard to the efficiency of suppression of WEE and SLE virus infection as mediated by transovarian transferred maternal antibody. Suppression of SLE viremia in house sparrow nestlings from SLE-immune parents was greater than in those nestlings reared from WEE-immune parents and inoculated with that virus. The apparent effect of SLE maternal antibody in suppressing SLE viremia in nestling house sparrows appears great enough to reduce or eliminate their potential for infecting feeding mosquitoes. This was not the case with the WEE virus experiment in which the nestlings from WEE-immune parents circulated adequate amounts of WEE virus to infected mosquitoes for several days following inoculation.

(R.O. Hayes and D.B. Francy)

Table 1. VEE ANTIBODY IN RABBIT SERUM
CAMERON COUNTY, TEXAS - JULY, 1971

SPECIES	POSITIVE	
	HI	SN
Sylvilagus audoboni	3/9	3/4
Lepus californicus	17/18	15/16

ADS 9/7/72

Table 2. ARBOVIRUS ISOLATES FROM MOSQUITOES DURING VEE SURVEILLANCE THROUGH AUGUST 1972

Species	Collected		Tested		Virus		
	Number	(Pools)	Number	(Pools)	WEE	Tur.	Other*
Aedes dorsalis	8445	(180)	8093	(164)	2		1
Culex erraticus	1295	(49)	911	(41)	1 N.M.		1
C. erythrothorax	669	(9)	669	(9)			2
C. salinarius	10,670	(163)	10,880	(155)		1	2
C. tarsalis	18,945	(496)	15,805	(400)	50	7	64
Psorophora confinnis	20,334	(260)	20,044	(250)			4
P. signipennis	2490	(110)	2412	(98)			5
Totals	62,848	(1267)	58,314	(1117)	53	8	79

* Not Group A arboviruses; identification in progress.

ADS 9/7/72

Table 3. ARBOVIRUS ISOLATES FROM MOSQUITOES BY STATES DURING VEE SURVEILLANCE THROUGH AUGUST, 1972

State	Collected		Tested		WEE	Tur.	Other*
	Number	(Pools)	Number	(Pools)			
Arizona	16,076	282	16,076	282	10	3	15
Colorado	6,707	258	5,191	202	8		7
Lousiana	1,457	28	1,457	28			1
New Mexico	24,826	606	20,901	500	23	4	35
Oklahoma	1,996	95	1,140	66			1
Texas	43,490	950	41,643	853	12	1	19
Totals	94,552	2,219	86,408	1,931	53	8	78

* Not Group A arboviruses; identification in progress

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Table 4. SUSCEPTIBILITY TO WEE VIRUS OF THREE *CULEX TARSALIS* STRAINS DURING MAY, JULY, AUGUST, AND SEPTEMBER 1971.

Amount of Virus[a]	Colony	Infection Rate[b] Colorado Wild	Texas Wild (August only)
7.2-7.3	11/50 (22)	26/33 (79)	
6.2-6.6	11/76 (14)	27/52 (52)	27/42 (64)
5.0-5.3	47/65 (6)	21/49 (43)	7/22 (32)
4.2-4.3	0/68 (0)	1/46 (2)	0/20 (0)

a/ Log_{10}PFU/0.1 ml of suspension

b/ Specimens positive/specimens tested (%)

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Table 5. DAILY VIREMIC PATTERNS IN NESTLING HOUSE SPARROWS AFTER INOCULATION WITH WESTERN EQUINE ENCEPHALITIS (WEE) VIRUS.

Day Post Inocu-lation	Immune Flock			Nonimmune Flock		
	Ratio Viremic	Viremic Titers *		Ratio Viremic	Viremic Titers	
		Range	Mean		Range	Mean
1	18/22	1.4-8.6	5.3	18/20	3.4-9.7	7.8
2	15/17	3.7-7.4	5.7	5/ 5	6.2-8.6	7.1
3	13/15	4.4-7.6	5.9	2/ 2	5.1-5.4	5.3
4	7/ 8	1.7-6.1	3.9	1/ 1	3.4	3.4
5	3/ 6	1.0-5.1	2.4	1/ 1	2.5	2.5
6	1/ 6	2.3	2.3	NT**	-	-
7	1/ 6	2.0	2.0	NT	-	-
8	NT	-	-	NT	-	-
9	1/ 4	1.0	1.0	0/1	<0.7	<0.7
10	NT	-	-	NT	-	-
11	0/ 4	<0.7	<0.7	0/ 1	<0.7	<0.7

* Titers expressed as $\log_{10}$ plaque forming units per 0.2 ml whole blood

** Not Tested

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REPORT FROM THE DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASES,
SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION
SAN ANTONIO, TEXAS

Examples of replication in arthropods of picornaviruses (nodamura), orbiviruses (blue tongue) and rhabdoviruses (cocal) suggest that the potential for replication of viruses in arthropods may be more widespread than is ordinarily supposed. A few exploratory tests have been made.

Since arthropods have long been suspected as possible vectors for the transmission of polio, this was one of the viruses used in the study. Parenteral injection of the black carpet beetle larvae with undiluted polio II virus and incubated at 30° C for seven days resulted in a drop in titer of two logs. On second and third passage, no virus was recovered ($<10^{-1.6}$). So far in passage of polio II in the common house fly (Musca domestica), first passage resulted in a titer drop of three logs. Further passages have not been made. We also tested snails (Biomphalaria glabrata, Puerto Rico strain). A drop in titer of 4-1/2 logs occurred after first passage and further passages have not been made.

Since there is evidence that influenza may pass through hosts other than man and might give rise to the origin of pandemics, this virus was also used. Parenteral injection of the black carpet beetle larvae with undiluted influenza A2/Hong Kong/8/68 resulted in a drop in titer of two logs. Second passage was negative.

As mentioned in the last report, a serological survey of nonhuman primate sera for the presence of antibodies to group A, group B, California group, Bunyamwera, Turlock and Buttonwillow viruses was in progress. This has now been completed. 1,135 sera from Old and New World monkeys and apes were tested, using 15 different virus antigens, with the following results:

Group A: Chikungunya, Semliki Forest, Sindbis, W.E.E. and E.E.E. viruses were used in the survey. The highest prevalence for group A antibodies occurred in the apes. The highest antibody titers with the viruses used was found against Chikungunya. Evidently these primates are highly susceptible to infection with this or a closely related virus. All animals from various parts of the world, for the most part, had highest antibody titers to Chikungunya, even the New World monkeys, but possibly this was due to infection with Mayaro virus.

Group B: Uganda S, S.L.E., West Nile, yellow fever, Dengue I and II viruses made up the antigen battery for this group. Again, the apes had the highest percent positive. Yellow fever antibodies were the most prevalent but this may have been due to vaccination of these animals. The Asian monkeys had the highest titer to the Dengue viruses. Of all the New World monkeys tested, only 6.7% had antibodies to one or more of the viruses from this group.

California Group Viruses: LaCrosse and Tahyna viruses were used in the survey for this group. More New World monkeys, the marmosets in particular (43% of those tested), were serologically positive to this group than any other primate from any other part of the world. Surprisingly, most of the species animals from all parts of the world had antibodies to one or both of these viruses.

Bunyamwera: Very few animals were seropositive to this virus. Antibodies ranged from 4% for African monkeys to 0.5% for Asian monkeys.

Buttonwillow and Turlock: Only the rhesus monkeys had any significant positives and these positives were to Buttonwillow. This may be due to a related virus in the Simbu group.

(J. Feild and S.S. Kalter)

REPORT FROM THE HORMEL INSTITUTE, UNIVERSITY OF MINNESOTA, AUSTIN, MINNESOTA

Lipid analytical studies of arbovirus vectors (*Aedes* species)

A comparison was made of the lipid composition of established ovarian cells of *Aedes aegypti* and *Aedes albopictus* cultivated in the same medium in spinner culture. The medium had a distinct lipid profile distinguishing it from the two insect cells. The two types of cells could be distinguished from each other based on lipid chemical profiles. The age of the cell was pertinent to these studies since major lipid changes were observed as cells aged. The differences in lipid composition of these cells may correlate with differences in susceptibility to arbovirus infection.

(H.M. Jenkin, L. Anderson, and T.K. Yang)

Japanese encephalitis virus (JEV) growth in rat liver cells

Controlled growth of JEV in rat liver cells was accomplished using various lipid compounds. Comparison of growth of JEV in BHK-21 cells vs. rat liver cells showed sufficient differences in growth patterns, virus yield and lipid composition to suggest that antigenic variation occurred relative to the host employed.

(H.M. Jenkin and William Steele)

REPORT FROM THE VIROLOGY LABORATORY, BIOLOGY DEPARTMENT, BOWLING GREEN STATE UNIVERSITY, BOWLING GREEN, OHIO

Sequential development of California encephalitis virus (CEV) in VERO cells

The development of CEV antigen in VERO cells was studied by inoculating confluent monolayers on coverslips with 10^3TCID$_{50}$ doses of CEV (BFS-283). At intervals coverslips were removed, fixed with cold acetone and stained by the indirect fluorescent antibody (FA) technique. Results are shown in Table 1.

We found that diffused fluorescent particles were observed as early as 16 hours after inoculation. The size of the fluorescent particles increased and the brightness of the fluorescence also increased with an increase in time. The viral antigens were detected only in the cytoplasm. Nuclear fluorescence was not seen even during the advanced stage of infection. No fluorescence was detected in uninfected cells.

Detection of specific antigen in the CEV complex by the FA technique

Table 2 summarized the results of FA staining in the CEV complex. CEV-BFS-283, LaCrosse, Keystone, San Angelo, Trivittatus virus were able to be identified by staining with specific antiserum. Snowshoe Hare, Jerry Slough could not be detected by this method. Jamestown Canyon could not be detected by its specific antiserum, but with anti-BFS-283 serum it showed strong fluorescence. Specific antiserum, although it may have a high HI titer may not be usable for fluorescent staining.

The neutralizing ability of our anti-BFS-283 serum was measured in a cross neutralization test in VERO cells. After incubation at 37 C for 30 minutes serum-virus mixtures were inoculated and after 48 hours cells examined by FA for signs of viral growth. Anti-BFS-283 serum neutralized most of the previously listed viruses within the CEV complex. These studies suggest the existence of a common neutralizing antibody not measured by other means.

This study of the development of BFS-283 antigen in VERO cells indicated that CEV is only present in the cytoplasm of the infected cells, thus in agreement with the electron microscope study done by Dr. Atchison at Pittsburgh. Anti-BFS-283 serum will neutralize most of the viruses in the CEV group, but it is not useful for broad-spectrum FA staining of all viruses in the group.

Table 1. Development of California Encephalitis Virus[1] in Vero Cells

Hrs. Post	CPE	Viral Antigen by FA	
		Appearance in cytoplasm	% of cells
8	-	-	0
16	-	Diffused	2
24	-	Fine granules	15
32	±	Small aggregates	20
40	++	Brilliant solid masses	30
48	++	Brilliant solid masses	45
56	+++	Brilliant particulate chunks	60
64	+++	Brilliant particulate chunks	75
72	++++	Brilliant large solid masses	95

1. BFS-283 strain.

Table 2. Detection of CE group viruses in Vero cells by FA staining

Virus	CPE at 48 hrs	Antiserum specific by HI							
		CE 283	LAC	KEY	SA	TVT	SNH	JS	JC
CE 283	++	+++	-	-	-	-	-	-	-
CE 395	-	+++	-						
LAC	++	++	++						
KEY	-*	-	-	+					
SA	++	-	-		+				
TVT	++	-	-			+			
SHN	++	-	-				-		
JS	++	-	-					-	
JC	++	+++	-						-

*CPE - at 72 hr w/10X virus inoculation

Abbreviations:

CE: California encephalitis
LAC: LaCrosse
KEY: Keystone
SA: San Angelo
TVT: Trivittatus
SNH: Snowshoe Hare
JS: Jerry Slough
JC: Jamestown Canyon

(W.D. Hann)

REPORT FROM THE YALE ARBOVIRUS RESEARCH UNIT,
YALE UNIVERSITY SCHOOL OF MEDICINE,
NEW HAVEN, CONNECTICUT

Studies with rabies-related viruses

The rabies-related viruses include the following: Lagos Bat virus, Mokola virus (IbAn 27377) from Nigerian shrews, Kotonkan virus (IbAr 23380) from Nigerian Culicoides midges and Obodhiang virus (SudAr 1154-64) from Sudan Mansonia mosquitoes. See Shope et al. for a discussion of the first two agents, J. Virol., 6(5):690, 1970, and Kemp et al. for Mokola, Am. J. Trop. Med. & Hyg., 21(3):356, 1972.

1. Circulating virus studies (W.G. Downs)

One-day-old mice were inoculated intracerebrally (i.c.) with 10^{-2} suspension of virus, and some 20 mice were exsanguinated daily thereafter. The blood was frozen and thawed three times and the resulting liquid was inoculated i.c. into one-day old mice. Sick or dying mice were examined by CF to confirm presence of virus. Results are as follows:

Virus	Titer of inoculum	Mouse mortality, post-inoculation day:									
		1	2	3	4	5	6	7	8	9	10
Mokola	$10^{-5.5}$	Neg	Neg	3/9	Neg	Terminated, no live mice remaining					
Kotonkan	$10^{-3.1}$	-	-	Neg	-	Neg	8/11	Neg	-	Neg	Neg
Obodhiang	$10^{-3.8}$	-	-	2/8	Neg	Terminated, no live mice remaining					

Note: This experiment is continuing, and will include studies on Lagos Bat virus and one or more selected rabies strains.

2. Mosquito susceptibility studies (T.H.G. Aitken)

A. Mokola virus

8th passage baby mouse brain suspension titering $10^{-4.8}$LD50, i.c. in baby mice, was inoculated parenterally (undiluted) into batches of 30 female Aedes aegypti and Culex quinquefasciatus. Inoculated mosquitoes were held at 80°F (26.7°C). At 7 and 14 days of incubation, 2 individual mosquitoes of each species were tested for virus and found negative.

After 4 weeks incubation, 2 pools (each of 10 Aedes) were positive for Mokola virus (confirmed by CF). One pool of 10 Culex was also positive and the second negative.

At 34 days post-inoculation (p.i.), 6 females of each species were allowed to bite individual day-old mice. No obvious transmission took place. These mosquitoes have not yet been tested for virus.

At 37 days p.i., 5 pairs of salivary glands of both species were negative for virus.

B. Kotonkan virus

10th passage baby mouse brain suspension titering $10^{-3.8}$LD50, i.c., in baby mice, was inoculated parenterally into batches of 30 female Aedes, Culex and Anopheles quadrimaculatus. When it became apparent that virus (confirmed by CF) was present in triturated whole mosquitoes after 7 days' incubation, salivary gland passage of virus was instituted. This consisted of dissecting paired salivary glands from 5 previously inoculated mosquitoes, crushing them under a cover glass and then suspending them in 1 ml. of diluent. This material was inoculated into a fresh group of 30 female mosquitoes and constituted one MIP (mosquito inoculation passage). At the same time a group of day-old mice was inoculated with this material to demonstrate the presence of virus.

Originally the virus passage interval between mosquitoes was scheduled for 7 days, but it was found that mosquitoes either contained no virus or very little. Subsequently the interval was lengthened and salivary gland virus now has been passaged successfully three times at 14 day intervals through Aedes, Culex and Anopheles, i.e., the virus has been maintained serially in mosquitoes for at least 43 days.

First and second MIP Aedes, when exposed to susceptible day-old mice after 7 and 21 days incubation, apparently failed to transmit virus by bite (i.e., no sickness was observed, but mice will be tested for antibody) despite the fact that at least 8/14 mosquitoes contained virus. Similar findings were experienced with Culex and Anopheles.

C. Obodhiang virus

4th passage baby mouse brain suspension titering $10^{-4.1}$ LD50, i.c., in baby mice was inoculated parenterally into batches of 30 Aedes, Culex and Anopheles. Virus has been passaged successfully through Aedes salivary glands at 7 day intervals for 4 weeks. Preliminary observations suggest Culex and Anopheles are refractory; virus may be recovered from these species after one week but then it appears to disappear on passage. A longer incubation interval might overcome this situation.

Preliminary virus transmission attempts by feeding mosquitoes upon susceptible day-old mice appear to have been negative but studies are still in progress.

3. Studies in Singh's Aedes cell lines (S.M. Buckley)

Propagation in Singh's Aedes cell lines was attempted with the following rhabdoviruses: rabies (strain TR 5843) and two serologically related viruses from Africa, Lagos bat and Mokola (strain IbAn 27377).

Briefly, wet-frozen, infected mouse brain virus stocks were diluted 1:100 with a diluent consisting of growth medium (Mitsuhashi-Maramorosch medium supplemented with 20% of inactivated fetal bovine serum). In some experiments, the diluent was supplemented with diethylaminoethyl (DEAE) dextran in a concentration of 100 μg per ml. For primary infection of Aedes cells, cultures (three-ounce flint glass prescription bottles) of Aedes aegypti and Aedes albopictus cells were inoculated at a high multiplicity of infection. The viruses were allowed to adsorb for one hour at 36°C. The monolayers were then rinsed three times with Rinaldini's salt solution, refed with growth medium and incubated at 30°C for 20 days post-inoculation, thereafter at room temperature (25°C). Fluids were changed at 5, 12, 20 and 27 days after inoculation. At intervals, fluid and cell phases were examined for presence of virus by sub-inoculation into mice (intracerebral inoculation; animals 2-3 days old). Development of cytopathic effect (CPE) was not observed. Rabies and Lagos bat viruses failed to multiply in Singh's Aedes cells with or without DEAE dextran. Mokola virus, while not replicating in Singh's Aedes aegypti cell line, propagated in Singh's Aedes albopictus cell line as shown in figure 1. Over a period of 26 days, from 200 to 776 LD_{50} per 1.0 ml of fluid phase were produced. Subsequent determination of the percentage of infective centers revealed that only a small percentage of cells were infected, i.e. from 0.24% to 1.35%. Passages of Mokola virus in Aedes albopictus cells as well as transfers of persistently infected cells have been initiated. The virus has also been plaque purified in Vero cells with and without passage through Aedes albopictus cells. Specificity studies are currently underway.

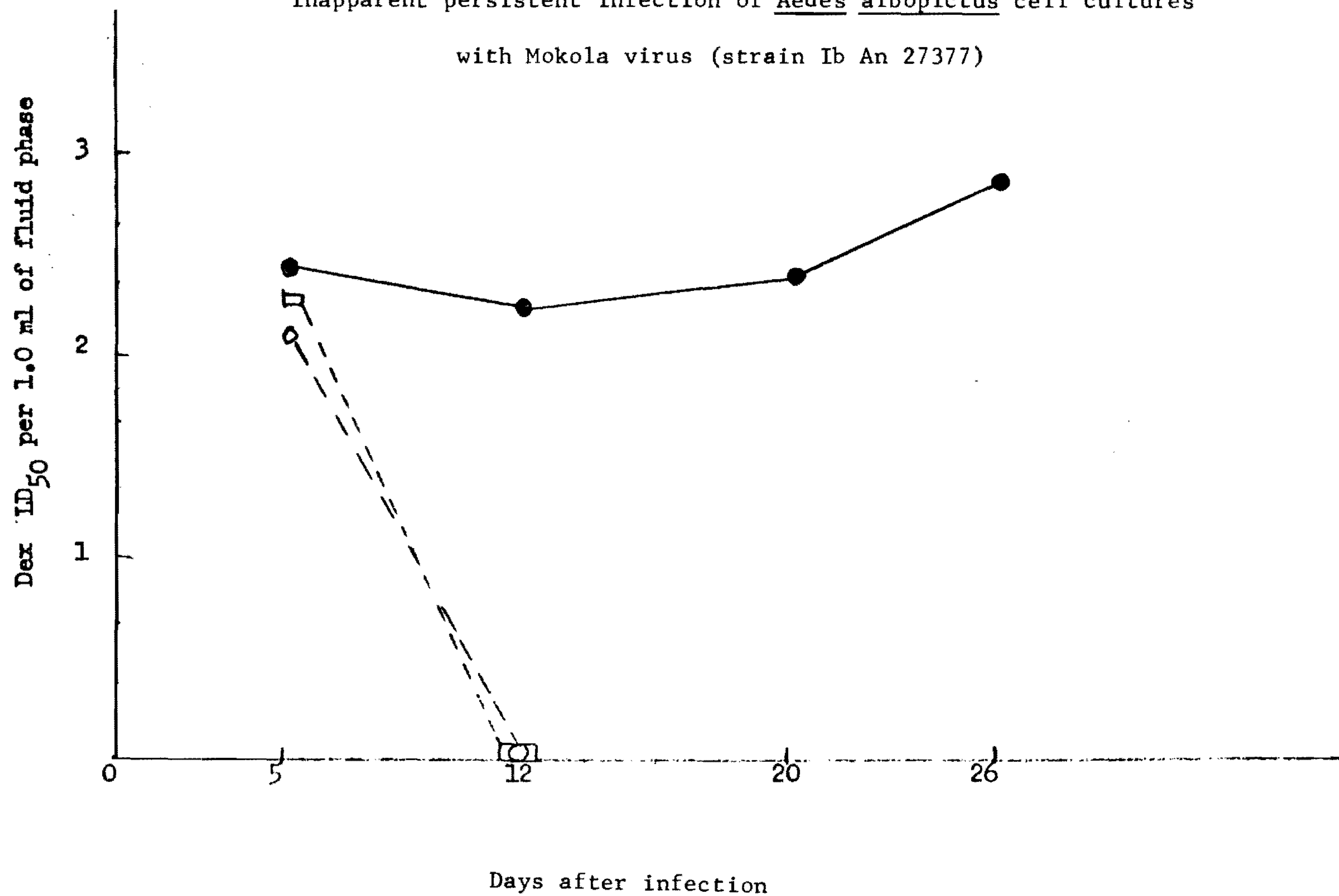


Figure 1. Growth curves of Mokola virus in *Aedes albopictus* (●———●), *Aedes aegypti* (o- - - -o) cell cultures and in growth medium without cells (□———□).

Results of earlier investigations carried out in this laboratory support an hypothesis that all mosquito-borne arboviruses, whether relatively resistant or sensitive to lipid-solvents, are able to multiply in one or both of Singh's Aedes cell lines; and that, of the arboviruses isolated from ticks and phlebotomine flies, those lipid-solvent resistant are able to multiply in one or both cell lines whereas those lipid-solvent-sensitive are unable to grow in either (J. Med. Ent. 9(2):168, 1972).

Neutralization and hemagglutination-inhibition tests with Congo-Crimean hemorrhagic fever (C-CHF) virus. (J. Casals and G.H. Tignor)

Serological studies with C-CHF virus have heretofore been done mainly by complement-fixation and agar gel diffusion tests. No hemagglutinating antigen is on record. The neutralization test by intracerebral inoculation of mice has been often difficult to interpret owing to the fact that there is a variable and frequently considerable non-specific neutralization of the virus by normal, or presumed normal, sera from different animal species including man. Tissue culture neutralization tests, either plaque reduction or CPE in fluid cultures, if available at all, have not been widely publicized or used.

This report describes attempts to develop a practical, reasonably dependable neutralization test in newborn mice and describes for the first time a specific hemagglutinin for this virus. Furthermore, additional information is supplied concerning the debated question as to whether C-CHF and Hazara viruses are antigenically related.

Neutralization test. Two strains of C-CHF virus had mainly been used in the past in NT tests in this laboratory: Congo 3010, the prototype strain; and the Drozdov strain, isolated from a case of CHF by Professor M.P. Chumakov. Neither strain had proved satisfactory in our hands; another strain, IbAr 10200, isolated from ticks in Nigeria proved, on the other hand, superior. The work reported here is, therefore, based on the use of strain IbAr 10200. However, very recent observations in our laboratory point to the fact that there may not be an inherent superiority of strain IbAr 10200 for the NT test, for it has also been possible to employ the ptototype strain satisfactorily, under certain conditions to be reported elsewhere, in neutralization test.

Seven tests were done with strain IbAr 10200 with the purpose to determine whether in an antibody survey with human sera a base line could be established above which inhibition of the virus could be reasonably assumed to be due to specific neutralizing antibodies, arising from contact with C-CHF virus or another virus serologically related to it, rather than to non-specific inhibitory substances.

To that end were tested: 1, sera from persons who, by their place of residence (USA), could hardly be expected to have been exposed to the virus; 2, sera from life-time residents of Nigeria, who may or may not have been exposed to the agent; 3, sera from temporary residents of Nigeria, mostly medical missionaries, who had no clinical history of hemorrhagic fever during their residence in that country; 4, three sera from humans, one a pool of convalescent sera from cases of CHF, kindly supplied by Prof. Chumakov, the other 2 from persons who had infection with Congo virus 10-11 years before, supplied by Dr. Kafuko. In addition and in the same tests, were tested sera from mice immunized against viruses antigenically unrelated to C-CHF virus.

The results of 2 of the 7 tests are shown in Table 1; none of the human sera neutralized the virus significantly, except two of the three convalescents. On the other hand, mouse immune sera against an assortment of viruses neutralized to a significant degree.

The combined results of the 7 neutralization tests (of which only 2 are shown here) were as follows: 52 sera from persons not known to have had Congo virus fever or Crimean hemorrhagic fever were tested. Fifteen gave neutralization indices between 0.9 and 0.5, the remaining 37 had indices of 0.4 or less. Two of the 3 persons, or sera, known to have had CHF or Congo virus disease protected against the virus.

While the non-specific effect of mouse sera, and a similar one from other lower animal species, remains to be elucidated, it appears that serum surveys by neutralization test with C-CHF virus, at least with strain IbAr 10200, are a practical possibility with human sera.

Hemagglutination and henagglutination-inhibition tests. In the past experience of this laboratory no definite evidence of a specific agglutinating antigen had been obtained with C-CHF virus by the routine sucrose-acetone method. Recently, traces of agglutination were noted on testing an antigen prepared with strain IbAr 10200; the observation led to the results reported below. It soon became apparent that specific agglutination of goose erythrocytes by strain IbAr 10200 could be achieved within, at the moment, narrow and precise conditions.

The following maneuvers gave a positive test; 1, the goose erythrocyte suspension used was adjusted to an optical density of 0.375, equivalent approximately to a 0.2 percent suspension of packed cells; this was in lieu of the routine optical density 0.750, or 0.4 percent suspension; 2, once the red cells suspension was added to the wells, either in the HA or HI test, the wells were tightly sealed using translucid adhesive tape, "magic tape," strips; the trays were vigorously shaken by hand in order to insure thorough mixing and placed in the warm room at 37C, to allow sedimentation; 3, the test was read as soon as the cell controls settled, usually 1-1 1/2 hours; the pattern of agglutination was perfectly clear at that time. However, when the trays were taken out of the warm room and left at room temperature,

there was a gradual slipping of the positive patterns, so that 2 to 4 hours later it was difficult to distinguish what had been complete agglutination from partial, or traces; 4, the pH range of activity of this antigen extended considerably to the alkaline side; the plateau of activity is between pH 7 and 7.7, and the optimal or selected pH for HI tests was 7.4.

The result of an antigen titration is shown in Table 2 and the result of an HI test with both C-CHF and Hazara viruses is shown in Table 3.

Only one attempt has been made to prepare an antigen with strain IbAr 10200 and as the titration shows its titer was low, 1:16. Additional attempts are in progress, first to see whether the results can be confirmed, and secondly, to see whether an improved antigen can be prepared by means of sonication, protamine sulphate precipitation, or other means. The specificity of the agglutination is beyond doubt, Table 3; furthermore, there was cross-reactivity reactivity observed between C-CHF and Hazara viruses, thus confirming by HI what had previously been found by neutralization and complement-fixation tests.

Table 1

Neutralization tests with C-CHF virus, strain IbAr 10200

Test #1			Test #2		
Serum	Titer	NI	Serum	Titer	NI
None, diluent	6.7		None, diluent	6.0	
Mouse			Man, Nigeria		
Normal	5.5	1.2	M.G.	5.9	.1
CETBE	4.5	2.2	L.S.	6.2	.2-
Bandia	4.0	2.7	L.B.	5.9	.1
Banzi	5.0	1.7	E.A.	5.6	.4
Punta Salinas	3.5	3.2	R.M.	6.1	.1-
CTF	5.5	2.8	D.C.	5.9	.1
Dengue 3	3.9	3.3	M.H.	6.3	.3-
Quaranfil	4.6	2.1	Ma.H.	5.9	.1
Kemerovo	4.7	2.0	E.E.	5.7	.3
Cam B 854	5.5	1.2			
Man, Nigeria			Convalescent,(MPC)	<2.5	3.5+
#115	6.4	.3	" , SE21089	3.9	2.1
#155	6.5+	.2	" , SG 3597	5.6	.4
# 81	6.4	.3			
#172	6.5+	.2			
#270	6.2	.5			
# 93	6.0	.7			
#131	6.4	.3			
#160	6.5+	.2			
#113	6.4	.3			
#185	6.5+	.2			

Intracerebral route of inoculation, mice 2-3 day old; serum-virus mixtures incubated 2 hours, 37C.

Titer: log, disregarding negative sign.

Table 2

Hemagglutination test, C-CHF virus, antigen from strain 16 Ar 10200

pH	Dilution of antigen, 1:						
	2	4	8	16	32	64	cells only
6.0	I	0	0	0	0	0	0
6.2	+	I	0	0	0	0	0
6.4	+	+	⊕	0	0	0	0
6.6	+	+	+	0	0	0	0
6.8	+	+	+	⊕	0	0	0
7.0	+	+	+	⊕	I	0	0
7.2	+	+	+	⊕	I	0	0
7.4	+	+	+	⊕	I	0	0
7.7	+	+	+	⊕	I	0	0

Microtest, one drop of antigen. + complete agglutination: ⊕ complete with faint ring; I little agglutination; 0 no agglutination.

Table 3

Hemagglutination-inhibition test

Serum	Antigen	
	C-CHF, 16 Ar 10200 4 units,pH 7.4	Hazara JC 280 4 units, pH 7.0
MOUSE		
C-CHF, JD 206, 2 injections	160	20
C-CHF, 16 Ar 10200, 1 inj.	20	0
HAZARA, JC280, 3 inj.	320	1280
DHORI 3 inj.	0	0
DENGUE 2 3 inj.	0	0
MAN		
Convalescent pool, MPC	10	0
CHF conv., Bash.	160	40
Congo Fever, Conval. L.	10	0
Congo Fever, " #3597	20	0
McD., normal	0	0
Sheep, #510, Dr.S.S., Iran	640	80

Reciprocal of titer; 0, no inhibition at dilution 1:10

REPORT FROM THE DIVISION OF LABORATORIES AND RESEARCH,
STATE OF NEW YORK DEPARTMENT OF HEALTH,
ALBANY, NEW YORK

SUMMARY: Three isolates of California encephalitis virus were obtained from pools of mosquitoes collected in St. Lawrence and Cattaraugus Counties in June and July, 1972 (Table 1).

Two cases of Powassan virus infection with onset dates in June, 1972, were identified by HI, neutralization and CF tests (Table 2).

(Rudolph Deibel and John Woodall)

Table 1

California Encephalitis Virus Isolations

Date Collected	Lab. No.	Species	Pool No.	County Site
6.7.72	72-30903	Aedes communis group	25	St. Lawrence
6.30.72	72-31105	Aedes stimulans	25	Cattaraugus Olean, New York
7.2.72	72-31122	Aedes stimulans	25	Cattaraugus Olean, New York

Table 2

Results of Serologic Investigations for Powassan Virus Infection

Patient	Date Onset	Lab. No.	1972 Date Coll.	CF Titer	HI Titer	Log_{10} Neut. Index
J.M. age 12, male	6.26	72-11992	6.27	<4	20	≧6.0
		72-12529	7.11	8	320	≧6.0
		72-13510	recd. 8.7	16	1280	NT
S.T.* age 1, male	6.20	72-12121	6.26	<4	20	≧4.6
		72-12457	7.12	4	1280	≧6.0

*The CSF yielded Echo Virus Type 31.

REPORT FROM THE U.S. ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES, FREDERICK, MARYLAND

Biological and chemical characteristics of plaque variants of Florida Venezuelan equine encephalomyelitis virus

A renewed interest in the disease potential of VEE virus was stimulated by the epizootic in the United States during 1971. The vaccine currently used to prevent infection with VEE virus is the attenuated TC-83 strain derived from the Trinidad strain of VEE isolated from the brain of a donkey (Tigertt, W.D., and W.G. Downs, 1962 . Amer. J. Trop. Med. Hyg. 2:822-834). However it has been suggested that a Florida strain of VEE (Fe 3-7c) might be used in place of TC-83 as it is apparently not pathogenic for horses (Henderson, B.W., W.A. Chappel, J.G. Johnston, Jr., and W.D. Sudia. 1971. Amer. J. Epidem. 93:194-205), and is already enzootic in southern Florida (Chamberlain, R.W., W.D. Sudia, P.H. Coleman and T.H. Work. 1964. Science 145:272-274). In this light we studied the Fe 3-7c strain of VEE and observed a distinct heterogeneity of plaque sizes. Upon subpassage in cell culture the ratio of small plaque (SP) to large plaque (LP) variants increased, a finding which has been reported for other viruses (Wigand, R. 1962. Arch. ges. Virusforsch. 11:718-731. Marshall, I.D., R.P. Serivani, and W.C. Reeves. 1962. Amer. J. Hyg. 76:216-224).

The isolated plaque variants had distinct biological and chemical characteristics. As seen in Table 1 the LP variant was uniformly lethal for weanling mice and was highly virulent for hamsters and guinea pigs. In contrast the SP variant was avirulent for hamsters and guinea pigs and less virulent for weanling mice than either the parental virus (PV) or the LP variant. Animals which survived the initial inoculation of Fe 3-7c viruses were generally protected from challenge with virulent Trinidad VEE virus.

Calcium phosphate chromatography reinforced the findings of biological differences between the variants (Figure 1). These differences may be a reflection of the charge on the membrane of the virion which would account for the individual elution profiles of the variants on calcium phosphate columns. In our system the LP variant was eluted at a lower ionic strength of buffer than the SP variant.

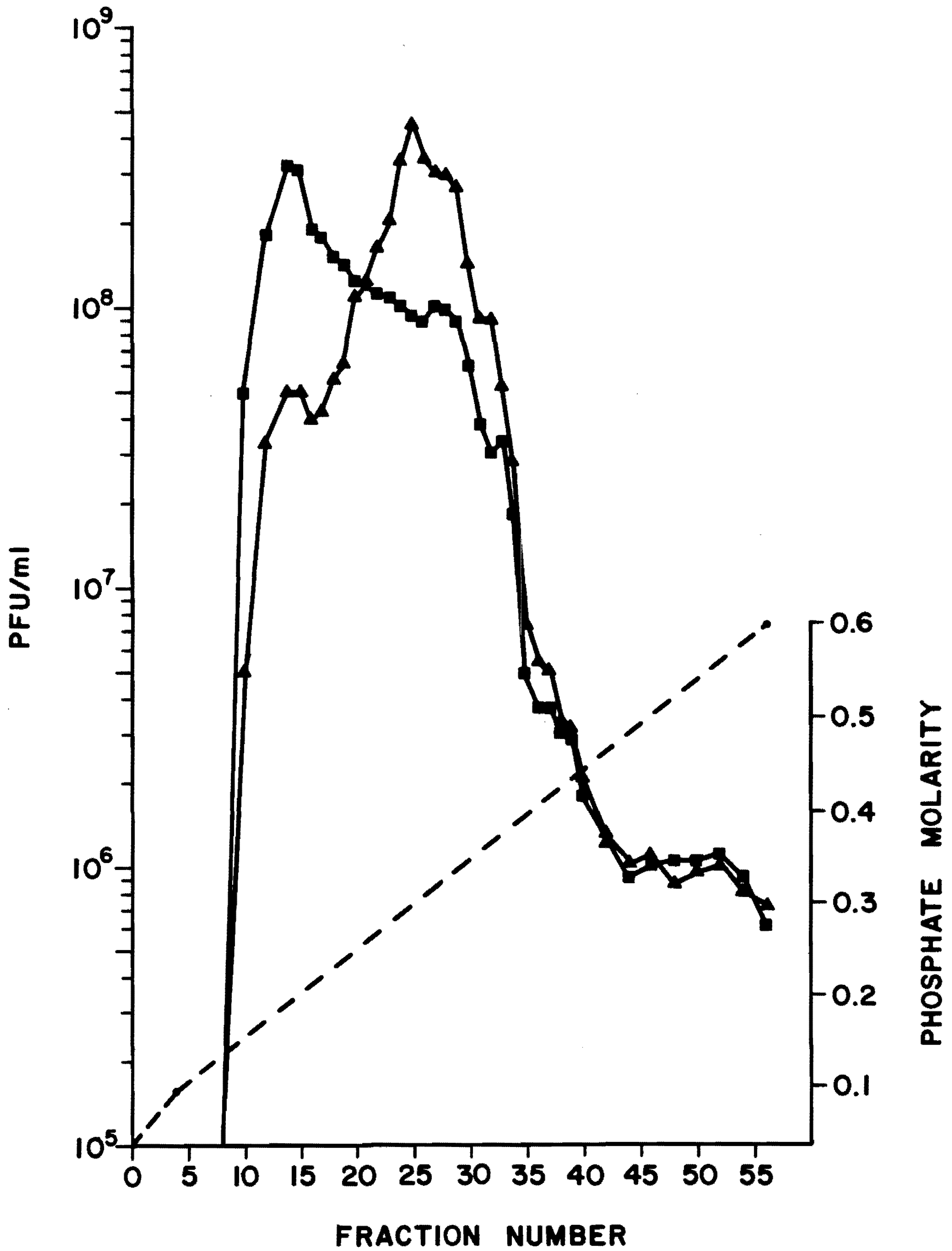
Although our studies indicate that the SP variant of Fe 3-7c would offer no advantage over the TC-83 vaccine strain currently in use, the SP variant is less virulent in hamsters than the LP variant of Fe 3-7c or the PV (Table 1). On this basis comparative studies in horses using the SP variant of Fe 3-7c and TC-83 vaccine strain might be warranted to determine their relative pathogenicity, antigenicity and duration of immunity.

(C.E. Pedersen, Jr.)

Figure 1

Elution pattern of the parental Fe 3-7c virus using calcium phosphate chromatography. Plaques were individually scored as large (>2 mm) or small (<2 mm) after 48 hr on DEC.

large plaques ■——■ small plaques ▲——▲


Fe 3-7c
PFU/ml
10^9
10^8
10^7
10^6
10^5
PHOSPHATE MOLARITY
0.6
0.5
0.4
0.3
0.2
0.1
0
5
10
15
20
25
30
35
40
45
50
55
FRACTION NUMBER

PROTECTION AFFORDED BY Fe 3-7c VEE AGAINST TRINIDAD VEE CHALLENGE (10^3 SMICLD$_{50}$ AT 21 DAYS)

VIRUS		WEANLING MICE (n=8,5)		HAMSTERS (n=5)		GUINEA PIGS (n=5)	
Fe 3-7c	CEC PFU* 2X	SURV. %	PROT. N/S	SURV. %	PROT. N/S	SURV. %	PROT. N/S
PV	10^5	0		20	1/1	100	5/5
	10^4	0		20	1/1	100	4/5
	10^3	0		20	1/1	100	4/5
	10^2	0		40	2/2	100	5/5
	0	100	0/8	100	0/5	100	0/5
LP	10^5	0		0		0	
	10^4	0		0		0	
	10^3	0		20	1/1	20	1/1
	10^2	0		40	2/2	80	2/4
	0	100	0/5	100	0/5	100	0/5
SP	10^5	20	1/1	100	4/5	100	4/5
	10^4	60	3/3	100	4/5	100	4/4
	10^3	60	3/3	100	4/5	100	4/5
	10^2	40	2/2	100	4/5	100	2/5
	0	100	0/5	100	0/5	100	0/5

* WEANLING MICE AND GUINEA PIGS: 0.5 ml IP
HAMSTERS: 0.5 ml SC

REPORT FROM THE DEPARTMENT OF VIRUS DISEASES
WALTER REED ARMY INSTITUTE OF RESEARCH
WASHINGTON, D.C.

Biophysical separation of major arbovirus serogroups

The close serological relationships within each of the 3 major serogroups of the arboviruses (A, B, and Bunyamwera supergroup) indicate that there are structural similarities within groups. In addition to the serological criteria currently employed, examination of biophysical characteristics of the virions could provide an additional basis for classification. Among the group A viruses that have been extensively studied, close similarities have been reported for sedimentation velocities and polypeptide composition. Among the group B arboviruses, however, significant variations have been reported, except when direct comparisons have been made in the same laboratory. Very little published data is available on the polypeptide composition or sedimentation velocities of viruses in the Bunyamwera supergroup. This report presents data that indicates that when arboviruses are analyzed in the same laboratory using basic biophysical techniques, virions in each of the 3 major serogroups have similar and readily identifiable characteristics.

The following agents were included in the comparative study: Sindbis (SIN), strain AR 229; Eastern equine encephalitis (EEE), Cambridge strain; Western equine encephalitis (WEE), McMillan strain; Venezuelan equine encephalitis (VEE), TC-83 strain; chikungunya (CHIK), Ross-168 strain; Japanese encephalitis (JE), M1/311; Langat (LAN), TP-21; dengue-2 (DEN-2), New Guinea C; Saint Louis encephalitis (SLE, Hubbard strain; yellow fever (YF), French neurotropic and Asibi strains; Bunyamwera (BUN), prototype; California encephalitis (CE), BFS 283; Tahyna (TAH); Oriboca (ORI), Be An 17; and Murutucu (MUR), Be An 974.

The group A and Bunyamwera supergroup viruses were propagated in primary chick embryo cells; the group B viruses were grown in continuous monkey kidney cells (LLC-MK_2). Viruses were intrinsically labelled with 14_C or ^3H amino acid mixture. The viruses were concentrated by either ammonium sulfate precipitation or pelleting in an ultracentrifuge. Final purification and analysis of sedimentation velocities were done by rate zonal centrifugation in 15-30% (w/v) sucrose gradients for 3 hours at 25,000 rpm in the Beckman 25.1 rotor. Polypeptide analysis of purified virions was done by polyacrylamide gel electrophoresis of samples that were boiled for 10 minutes in 1 percent sodium lauryl sulfate and 1 percent 2-mercaptoethanol.

Virions within each of the 3 major serogroups sediment similarly; comparisons between the 3 groups were done by cosedimenting a representative virus (SIN, DEN-2, and CF) from each group (Fig. 1). The Bunyamwera supergroup virions (about 350S) were always obtained over a broader region of the gradient than the group A virions (240S) and group B virions (200S). Polyacrylamide gel electrophoresis of degraded virions revealed similarities in the number and size of the structural polypeptides within each of the 3 groups. Patterns from four of the group A viruses that we examined are shown in Fig. 2; they each contain 2 polypeptides that appear to have molecular weights similar to those of SIN as originally determined by Strauss et al (PNAS 1968 and Virology 1969) to be 53,000 and 30,000 daltons, respectively. The polypeptide profile of VEE (not shown) was similar to CHIK. The larger polypeptide is associated with the viral envelope and the smaller with the viral nucleic acid as determined by dissociation with non-ionic detergents. Gel electrophoresis of degraded group B virions generally reveals 3 polypeptides (Fig. 3). The largest and smallest polypeptides are associated with the viral membrane and have previously been shown for JE and DEN-2 to have molecular weights of between 53,000 and 58,000 for the large polypeptide and from 7,700 to 8,700 for the small one (Shapiro et al and Stollar, Virology, 1971, 1969). The intermediate polypeptide is about 13,000 daltons and is associated with the nucleic acid core of the virion. A co-run of SIN marker proteins with JE is shown for comparison.

Polyacrylamide gel electrophoresis of Bunyamwera supergroup virions generally revealed 3 polypeptides (Fig. 4). Within this group, the polypeptides are remarkably similar and easily distinguishable from those of group A and B. Within the Bunyamwera supergroup, the separate groups, e.g., California encephalitis group and group C, cannot be differentiated by these methods. Coelectrophoresis of SIN virion polypeptides with CE and Oriboco (group C), is included for comparison of molecular weights. The estimated molecular weights for the 3 polypeptides of the Bunyamwera supergroup virions are 83,000, 30,000 and 20,000 daltons, respectively.

These data indicate that virions within a major arbovirus serogroup have a characteristic polypeptide composition and sedimentation rate which can provide a structural basis for classification. This is a confirmation and extension of previous impressions of structural homogeniety within serologic groups gained from physical, chemical and serologic techniques. Determining the polypeptide composition of an arbovirus virion may aid in the taxonomy of those viral isolates that are not readily classified by antigenic analysis.

(P.K. Russell)

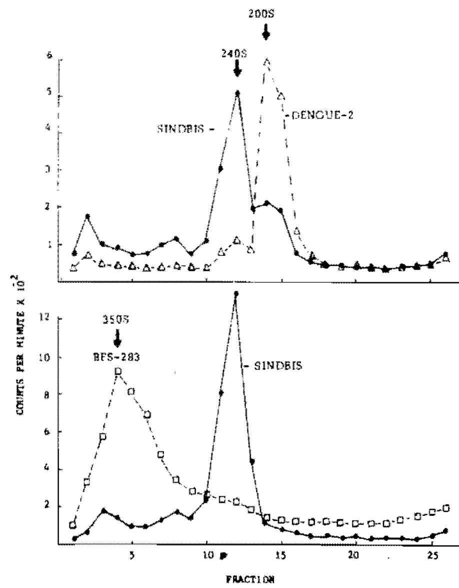


Figure 1. Rate-zonal centrifugation in 15-30 percent sucrose gradients of representatives of group A (SIN), group B (DEN-2), and the Bunyamwera supergroup (BFS-283).

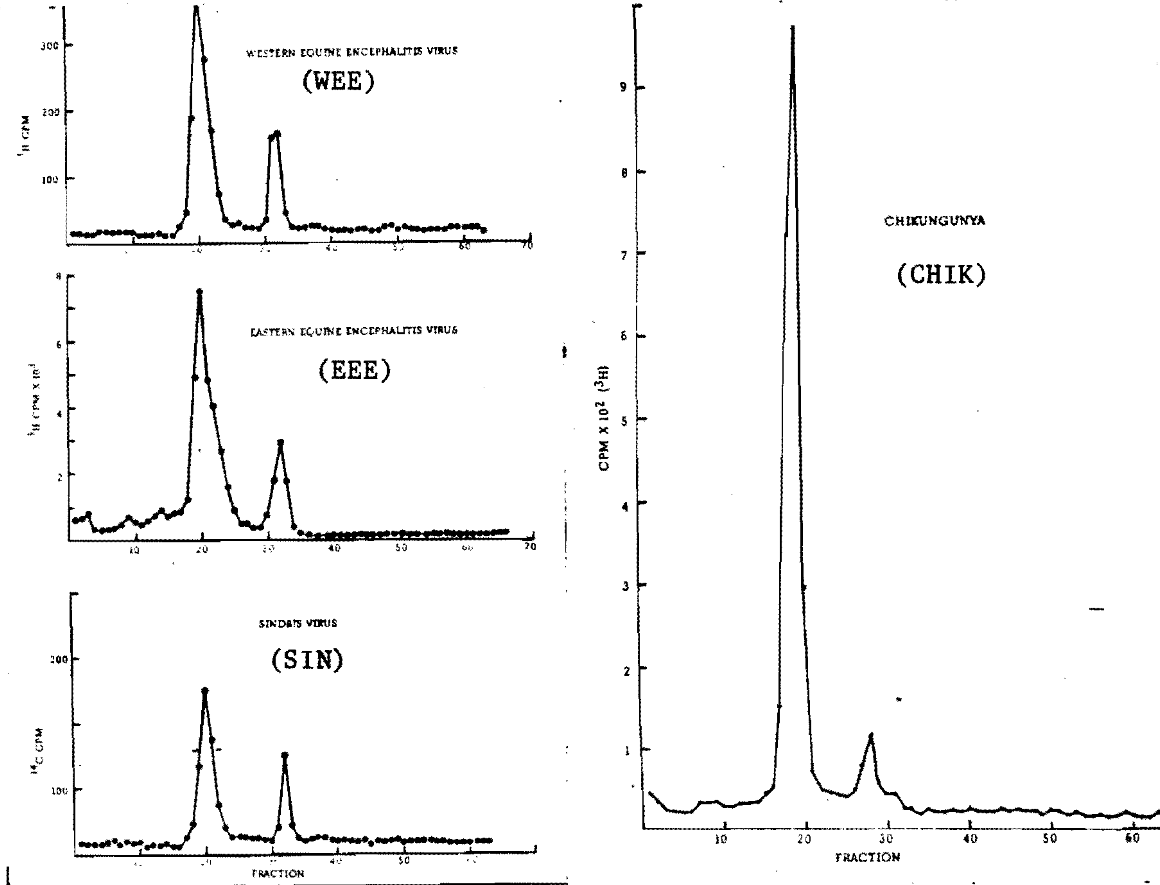


Figure 2. Polyacrylamide gel electrophoresis of degraded group A virions; migration is from left to right. The larger peak is the envelope protein and the smaller peak is the core protein, 53,000 and 30,000 daltons, respectively.

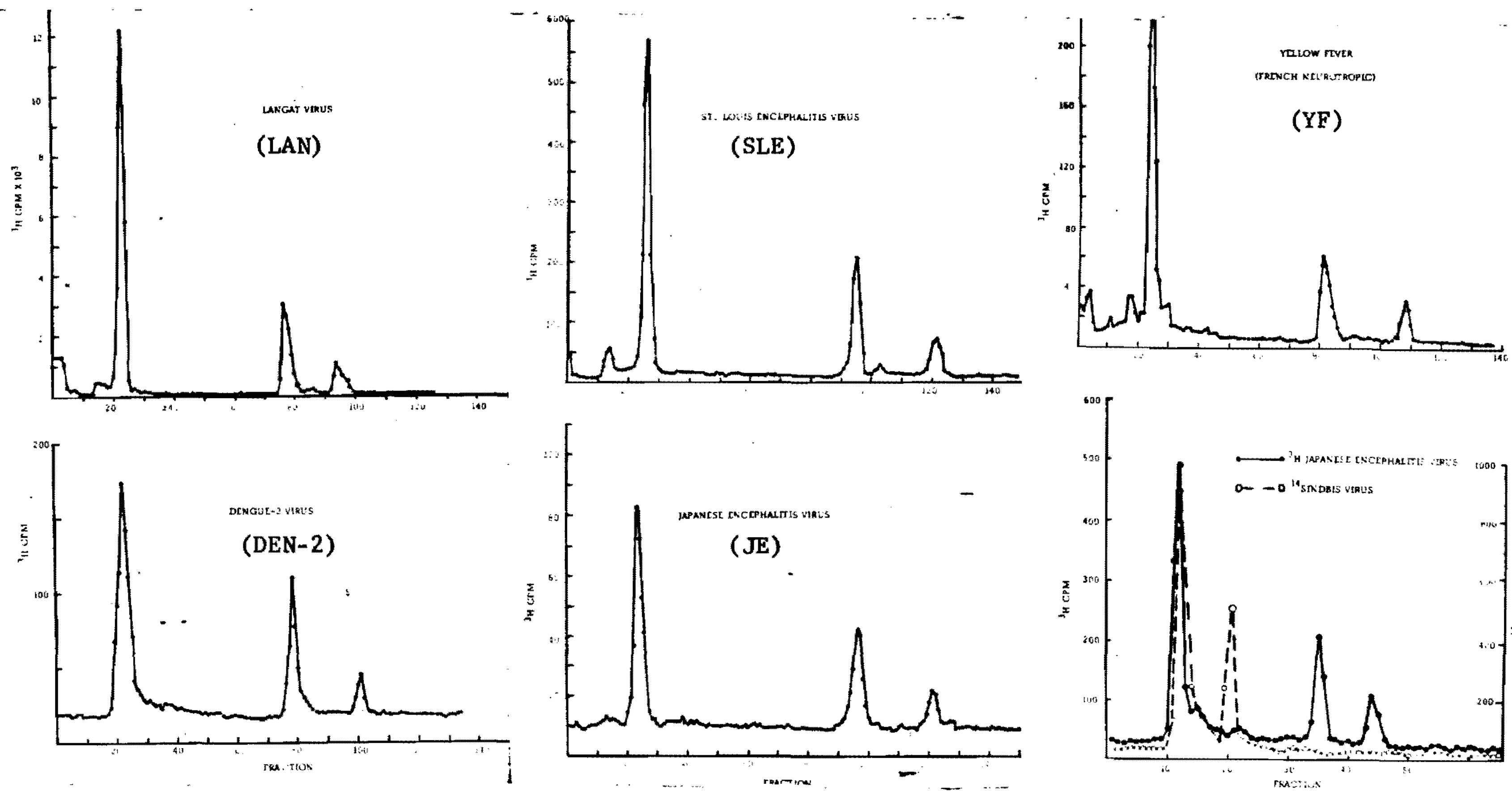


Figure 3. Polyacrylamide gel electrophoresis of degraded group B virions; migration is from left to right. The largest and smallest peaks are envelope (coat) proteins and the center peak is the internal (core) protein. Group A (SIN) marker proteins were coelectrophoresed with JE for comparison (bottom right panel).

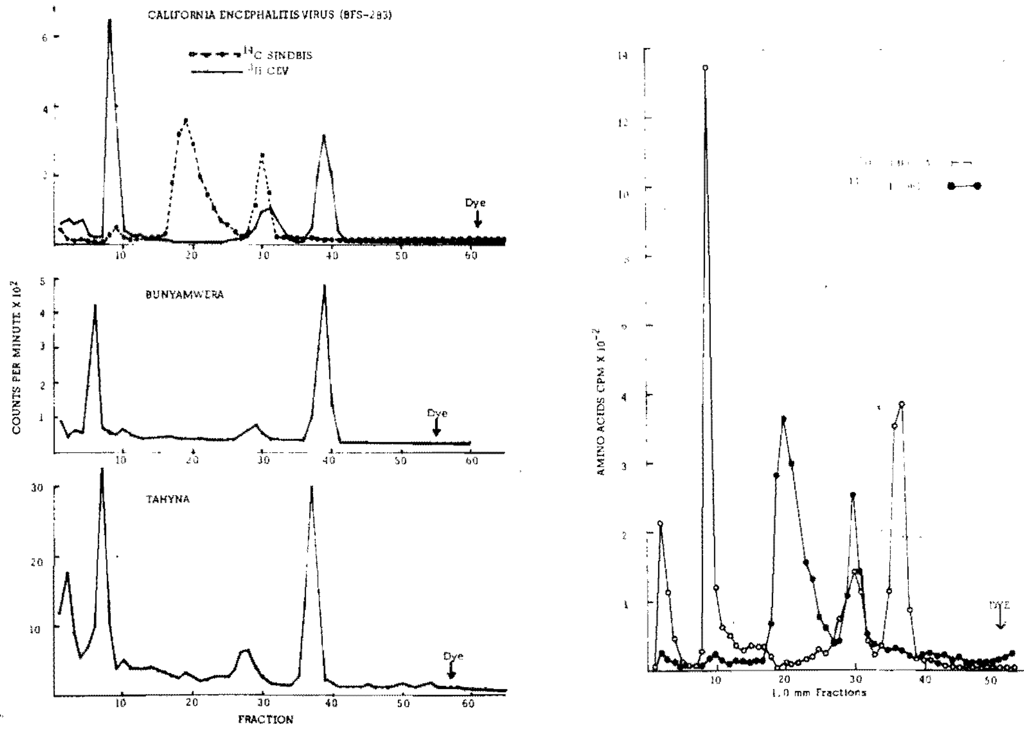


Figure 4. Polyacrylamide gel electrophoresis of degraded Bunyamwera supergroup virions. Migration is from left to right, the smaller proteins moving the fastest. SIN virion proteins were coelectrophoresed with CE (top panel) and Oriboca (right) for comparisons.

Recovery of dengue-2 viruses from patients and *Aedes aegypti* mosquitoes from Colombia

Serum specimens and mosquito pools collected in Colombia during February - May 1972 were received from Dr. Hernando Groot. Virus isolation was accomplished by the LLC-MK_2 plaque technique. Identification of plaque-forming agents was done by plaque reduction neutralization tests using hyperimmune mouse ascitic fluids made against reference dengue strains.

Fourteen strains of dengue-2 virus were recovered, 5 from serum, 9 from mosquito pools. All the strains appear identical. They form small plaques with considerable plaque-size variation in the LLC-MK_2 cells. Reisolation in suckling mice was accomplished with the HGL 1047 strain. One blind passage was necessary; the mice showed minimal signs of illness in first passage.

Table 1 - Acute serum specimens from which dengue-2 viruses were recovered

Specimen	Date of collection	Location
HGL-1047	2 Feb 72	Sahagún, Cordoba
306104	3 Feb 72	" "
306105	3 Feb 72	" "
306615	27 Apr 72	Pueblo Nuevo, Cordoba
306616	24 Apr 72	" "

Table 2 - Pools of *Aedes aegypti* from which dengue-2 virus was recovered

Pool No.	Date of collection	Location
3010-2	15 May 72	Monteria, Cordoba
3012-3	17 May 72	" "
3015	19 May 72	" "
3017-1	23 May 72	Buenavista, Cordoba
3017-2	23 May 72	" "
3017-5	" "	" "
3017-6	" "	" "
3019	25 May 72	" "
3020-2	26 May 72	" "

Table 3 - Plaque reduction neutralization test results on representative strains

Strain	Immune ascitic fluid			
	DEN-1 (HAW)	DEN-2 (N.G.C.)	DEN-3 (PR-6)	DEN-4 (1 & 241)
HGL 1047	15*	600	<10	<10
306104	<10	600	<10	<10
306615	<10	900	<10	<10
3010-2	20	900	10	<10
3015	15	700	10	<10
3017-1	15	850	20	<10
3020-2	15	1000	15	<10
(homologous)	1900	1200	650	100

* reciprocal of 50% plaque reduction titer

(P.K. Russell)

REPORT FROM THE ARBOVIRUS UNIT, DEPARTMENT OF EPIDEMIOLOGY & MICROBIOLOGY, GRADUATE SCHOOL OF PUBLIC HEALTH, UNIVERSITY OF PITTSBURGH, PITTSBURGH, PENNSYLVANIA

I. DENGUE VIRUSES

A. Search for and Evaluation of a Type Specific Dengue Complement Fixing Antigen

Because of the obvious need for a more type specific *in vitro* test for dengue and more specific antisera for *in vitro* identification tests of viral isolates, a search was made for the type specific CF antigen(s) of the dengue prototypes. An antigen closely approaching that sought has been found and a quick, inexpensive way to produce it. It appears to be an envelope component of the virion. An antiserum prepared against it will neutralize the virus. Adequate tests to evaluate it in patient serology have been limited due to delays and difficulties in obtaining suitably fresh, adequately defined human post infection sera. This should soon be a completed study.

B. Search for a Group Specific Dengue or B Group Antigen

Although well recognized as existing in all group B arboviruses and probably another existing for each subgroup, such has never been isolated. The uses for such an antigen(s) are many. An antigen common to the dengue group and other members of the same subgroup was extracted from the rapidly sedimenting hemagglutinin (RHA) described by others. Antibodies made to this antigen cross neutralize extensively and similarly inhibit hemagglutination. In addition to serving as a grouping antigen, it may have important vaccine potential. This is a completed study.

C. Search for Temperature Sensitive Mutants (TSM) of Dengue Virus for Attenuated Vaccine Strains

In conjunction with the Dengue Task Force of the CVI, AFEB, we are seeking for TSM's of types 2 and 3 dengue viruses. Passages at low temperatures are being made in three types of cell cultures, two of them diploid cell lines probably suitable for a most readily licensed vaccine product. Methods of cloning progeny and identifying markers are the immediate problems. Mutagens will probably be applied shortly.

D. Cross Protection between Group B Arboviruses: Resistance to Japanese B and St. Louis Encephalitis Viruses Induced by Dengue Virus Immunization

In mice, highly significant long term protection (20 weeks) against subcutaneously inoculated JBE and SLE were produced by immunization with any of the dengue virus serotypes, some giving better protection against one agent than another. Previously, only short term protection had been tested and only in hamsters. There is suggestive evidence that this protection may be cellular in nature. This study supports suggestive epidemiologic data that dengue infection prevents or modifies clinical manifestations of the more serious JBE and SLE in man. This is now a completed study.

E. Fluorescent Focus Unit (FFU) Method for Quantitating Dengue Neutralizing Antibody and Titrating Infectivity

This method has now been developed for use with dengue viruses so that it replaces the more difficult and time consuming PFU test. It has also proved more reproducible with certain strains than the latter more generally used test.

F. Studies Involving Peroxidase-labeled Antibodies

The tests developed first by Nakane and Pierce and modified by Graham and Karnovsky have been adapted to arbovirology for the first time and find usefulness in dengue virus studies. Staining occurs in the cytoplasmic and perinuclear areas. Results compare favorably with FA methods and so far appear to show no heterologous type crossing. The indirect method appears to have some advantage over the direct.

G. Comparative *In Vitro* Electron Microscopic Studies of the Four Dengue Virus Types

This is the first such comparative study of all four prototypes by any method. The sequential ultrastructural alterations of the infected cell, and the relative amount, size and morphology of the viruses developing in them were determined. Although the particle size of all viruses, the stages of development, and the mechanism of release of virus were the same, the ultrastructural cell changes varied significantly in respect to lattice-like crystals and annulate lamellae. This is a completed study.

II. CALIFORNIA ENCEPHALITIS GROUP VIRUSES

Studies on the possible role of large domestic mammals in the transmission cycle of Keystone and trivittatus viruses in Florida, plus the evaluation of a micro plaque reduction test for Keystone virus serological survey work have been completed.

III. JAPANESE B ENCEPHALITIS (JBE) VIRUS

A. *Trichinella spiralis* Infection and Susceptibility to JBE

Confirmation was obtained for a few scattered provisional observations suggesting that certain systemic helminth infections increased susceptibility to disease caused by JBE virus in certain animals. Two well-controlled experiments were carried out with mice infected with a dose of *Trichinella spiralis* larvae per os which alone failed to cause detectable disease. After challenge by peripheral inoculation of graded doses of JBE virus at 7, 14 or 28 days, mortality ratios were very significantly higher at 7 & 14 days and incubation periods shorter in the helminth infected than in the controls, while at 28 days no differences were noted. No *T. spiralis* could be found in the brains at 7 or 14 days to account for any break in a "blood-brain barrier."

B. Maternal Transfer of Japanese B encephalitis Virus Immunity

To investigate further the nature of maternally transferred immunity to arboviruses, female mice were immunized with live or inactivated JBE virus, mated and the progeny examined at various ages for susceptibility to peripheral inoculation of virus and for neutralizing antibody. The pattern found was entirely compatible with simple passive transfer. Results were identical whether live or inactive virus was used for immunization. No increased antibody response occurred following challenge of the passively immune progeny, apparently paralleling events in infants receiving live measles virus vaccine.

(W.McD. Hammon)

REPORT FROM THE ARBOVIROLOGY UNIT, VIROLOGY SECTION, CENTER FOR DISEASE CONTROL, ATLANTA, GEORGIA

A major outbreak of Venezuelan equine encephalomyelitis caused by type IB VEE virus occurred in the lowlands of Ecuadro and Peru in the winter and spring of 1969. In June-July, 1969, it had apparently jumped to Guatemala, then progressed into Honduras, El Salvador and Nicaragua. It continued into Mexico and Costa Rica in 1970, and by the spring of 1971, was active in the area of Tampico, northeast Mexico. In late June, 1971, it crossed over the U.S.-Mexican border into the lower Rio Grande Valley of Texas. Approximately 1500 horses died of VEE disease in southern Texas between July-October of 1971, and 110 human cases were eventually documented. The progression of this wave of infection -- Ecuador to the United States -- is illustrated in Figure 1.

The series of tables which follow summarize the findings of investigators from the Arbovirology Unit, CDC, who were actively engaged in survey activities, control evaluation and monitoring of human disease. The entomological data are those of Drs. W. Daniel Sudia, Verne F. Newhouse and Leslie D. Beadle; the wild bird and mammal data, of Drs. Sudia, Robert McLean (Epidemiology Program, CDC) and Newhouse; the human data, of Dr. G. Steven Bowen; and the laboratory data on equines, of Dr. Charles H. Calisher and Miss Gladys Sather.

Table 1 lists the mosquitoes collected from the affected lower Rio Grande Valley area of Texas during the epidemic period. Area I on this table includes the Boca Chica Flats, near the mouth of the Rio Grande River; Area II, the eastern half of the main Rio Grande Valley west of Boca Chica Flats; and Area III, the western half of the Valley proper. These subdivisions are illustrated in Figure 2. Table 2 summarizes the isolations of VEE virus made from the various mosquito species collected in these three areas. These virus isolations are also indicated in Figure 2. The findings indicate *Aedes sollicitans* to have been the species primarily involved in the brackish water area (Area I), while *Psorophora confinnis* and *P. discolor* were the mosquitoes mainly infected in the non-brackish areas. Other studies in Palo Blanco, Mexico (a non-brackish area), made in cooperation with Mexican authorities, also indicate the importance of *P. confinnis* and *P. discolor*; the majority of 679 VEE isolates obtained from the Palo Blanco area were from these two species.

Isolations of arboviruses other than VEE that were also isolated from Texas mosquitoes during the survey period are shown in Table 3. The large number of isolations of WEE virus from *Aedes sollicitans* in September are of some interest. *Culex tarsalis*, the generally accepted vector of WEE in western United States, was relatively scarce (refer to Table 1).

A post-epidemic vector surveillance (Table 4), conducted in September, October and November of 1971 and March of 1972, failed to yield additional isolations of VEE virus, indicating that the virus was no longer active.

Birds and small wild mammals were collected periodically in one of the most heavily affected areas (Boca Chica) to determine whether they had been exposed to VEE infection and might possibly serve as reservoirs to maintain the virus. VEE virus was isolated only once from the wild animals collected (from an opossum), and this animal was collected during the period of known epidemic activity in equines.

The serological results obtained from the wild-collected birds and mammals also failed to confirm a reservoir role or continued virus activity. The bird sera test results (Table 5) indicate some involvement of birds with VEE virus (up to around 10%) during the epidemic period, but with subsequent loss or absence of antibody in samples collected later. The mammal (and marsupial) results (Table 6) indicate the same trend, namely, a low level of involvement during the epidemic period with subsequent lack of further infection.

Incubation periods for human infections with VEE virus were estimated by interviewing of patients who were recent out-of-state visitors becoming ill shortly after arrival at Port Isabel, a high risk epidemic area where _Aedes sollicitans_ mosquitoes were abundant. For 10 persons of known recent arrival from non-epidemic areas, the periods of time between first possible exposure to infected mosquitoes and onset of symptoms compatible with VEE virus infection were (hours): 29, 36, 38, 48, 60, 72, 72, 72, 72, 84.

Clinical findings were as reported in previous epidemics of VEE IB virus infections except that there were no deaths or severe sequelae. The disease was more severe in children, with 5 of 23 under age 15 having seizures _versus_ no seizures in adults. Early paralysis was found in two children only. Sequelae occurred in 9 of 110 total cases and are as recorded in Table 7.

The results of CDC testing of human sera collected from suspect cases in the epidemic area are given in Table 8. Fifty of 67 of the cases were diagnosed by isolation of the virus from acute blood specimens. The other 17 were identified by detection of antibody rises by serological tests. Twenty-one other cases from the same general area were diagnosed by the Texas State Health Department, making a total of 88. Later, a retrospective survey in the Port Isabel area disclosed another 22 probable cases, for a total of at least 110.

It was of interest to determine the levels of viremia in VEE virus infected patients, since titers of $10^{3.5}$ SM i.c. LD_{50} or more per .02 ml of blood have been shown to be infective for susceptible vector mosquito species. The majority of virus isolations from humans (84%) were made from blood specimens drawn the day of onset of clinical symptoms or within the next two days. Peak titers occurred on day 1 after onset and averaged 4.2 logs/0.02 ml, whereas titers from day 0 and day 2 averaged 2.9 and 2.1 logs/.02 ml, respectively. Over half of the blood specimens from which virus was isolated contained 3.4 logs or greater of virus/0.02 ml and were therefore potentially capable of infecting vector mosquitoes.

Table 9 lists virus titers detected in naturally acquired human infections.

Very few subclinical infections occurred. Concurrent infections with other agents, such as Coxsackie B and Leptospira, mimicked the VEE infections, but over 90% of persons which subsequently developed VEE antibody had history of symptoms compatible with VEE infection: fever, headache, nausea, malaise, back pains, with or without diarrhea.

It was possible to conduct one-year follow-up serological tests on 36 of the VEE patients. It was found that the HI titers had persisted essentially unchanged ($\pm$ 1 dilution) in greater than 80%. CF titers were unchanged in 33%, and down 2 or more wells in 67%. Neutralization titers were essentially unchanged ($\pm$ 1 log) in 50%, and down $>$ 1 log in 36%. Two patients had no detectable neutralization titer at 1 year. These results are summarized in Table 10.

The VEE outbreak provided opportunity to make a number of observations on reactions of equines to natural infection with the epidemic IB strain and to vaccination with live TC-83. These observations are summarized in the various tables as detailed below.

Table 11: VEE (epidemic strain) viremias, listed by day after onset of symptoms. The viremias ranged from very low levels in some animals (below the infection threshold for vector mosquitoes) to very high levels in others (far above the levels required to insure mosquito infection).

Table 12: Isolations of VEE virus (epidemic and vaccine strains), listed by day after vaccination. The vaccine strain of virus was isolated most frequently on the third or fourth day post vaccination; the epidemic strain was detected most frequently on the sixth day, but was also noted at various other time periods.

Table 13: Isolations of epidemic or vaccine VEE virus from equine blood components. The data in this table suggest that the blood clot may be a better source for isolating virus than whole blood; and whole blood, better than serum. However, the whole blood and clot specimens were generally taken during acute stages of infection so that bias is in their favor.

Table 14: Isolations of epidemic VEE virus from equine tissues other than blood. Brain and spleen were both good sources of virus. It was less frequently found in liver, kidney, heart and lung.

Table 15: Comparison of HI and NT antibody titers in twenty randomly selected single equine sera from the epidemic area. This table presents representative examples of the types of results obtained. The general area of Texas from which these samples were taken experiences periodic outbreaks of WEE, and in addition some of the horses may have been vaccinated in the past with bivalent EEE-WEE killed vaccine.

Table 16: Neutralizing antibody responses in equines to VEE, EEE and WEE viruses $>$ 7 days after VEE vaccination. Of 69 sera which remained VEE antibody negative after administration of TC-83 vaccine, 44 (64%) were EEE and/or WEE positive. Of 328 sera which were VEE positive, 132 (40%) were EEE and/or WEE positive. These figures indicate some interference with VEE vaccination induced by pre-existing EEE and/or WEE antibody.

Table 17: VEE neutralizing antibody in equines, by day after vaccination, U.S. (excluding Texas), July-September, 1971. Although a small proportion of individuals (10/307) showed a significant level of antibody within six days, the great majority became detectably positive between the second and third week.

Table 18: Susceptibility of mice, guinea pigs and duck embryo cell culture to known strains of VEE virus and TC-83 (vaccine) variant. With the exception of type IE (Mena II), which goes poorly in guinea pigs, all of the epidemic (I) strains were found to infect all systems equally well. Type II (Everglades) has not yet been tested in 4-week-old mice i.p. or in guinea pigs, but was found to infect all other systems in the same manner as the epidemic strains. Pixuna was poorly infective for 4-week-old mice i.p. and for guinea pigs. The vaccine strain (TC-83, derived from a virulent IA type) had lost infectivity for 4-week-old mice i.p., newly weaned mice i.p. and for guinea pigs i.p.

(R.W. Chamberlain)

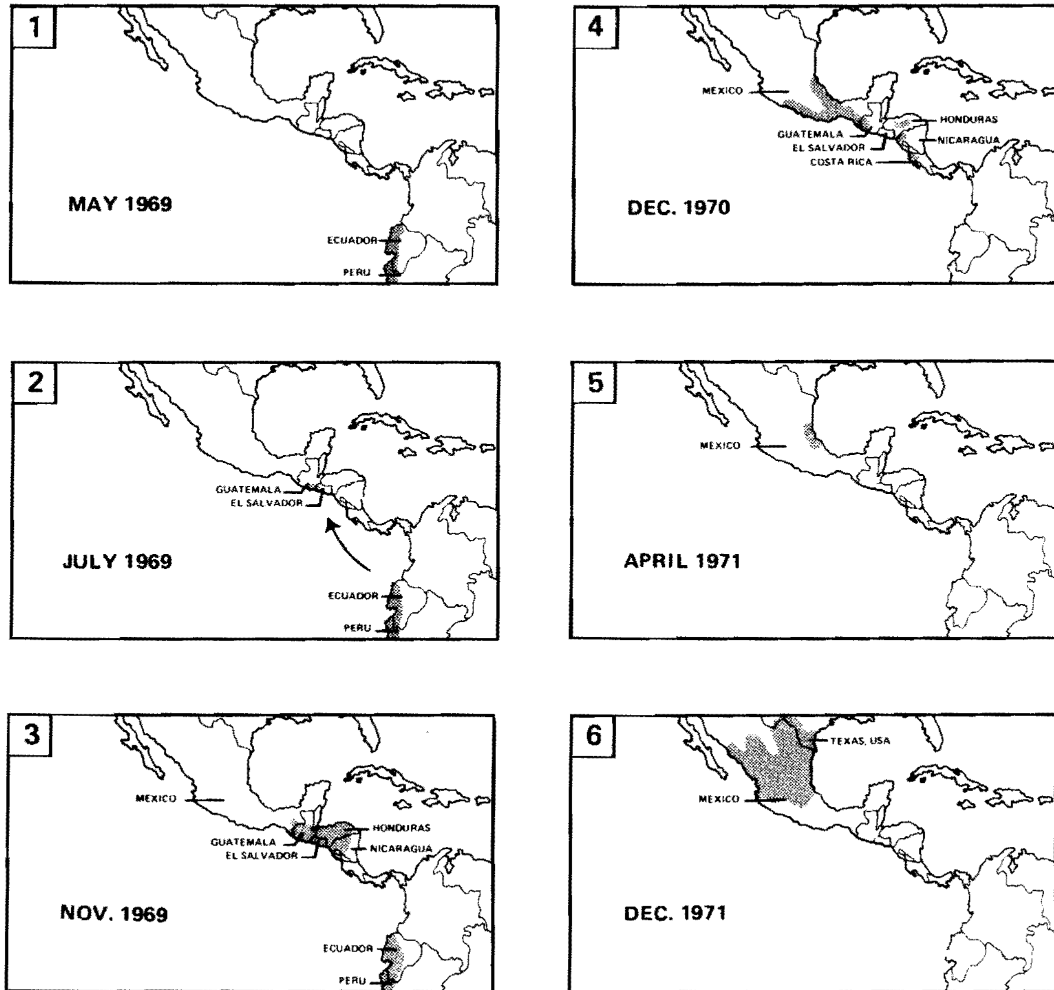


Figure 1. Progression of VEE, type IB, from Ecuador and Peru to the United States, 1969-1971.

TABLE 1

Summary of mosquitoes collected and tested for virus during the VEE epidemic in the lower Rio Grande Valley, Texas, 21 June-15 August, 1971.

	Area I	Area II	Area III	Misc. collections	Totals
	No. (Pools)	No. (Pools)	No. (Pools)	No. (Pools)	No. (Pools)
Aedes bimaculatus	1 (1)	-	-	-	1 (1)
scapularis	2 (2)	2 (2)	2 (2)	-	6 (6)
sollicitans	26,917 (1,309)	13,495 (459)	12,323 (419)	17 (3)	52,752 (2,190)
taeniorhynchus	799 (70)	17 (9)	7 (5)	1	824 (85)
thelcter	4,347 (165)	183 (20)	31 (11)	48 (5)	4,609 (201)
triseriatus	-	2 (2)	1 (1)	-	3 (3)
vexans	29 (10)	770 (50)	436 (35)	40 (6)	1,275 (101)
zoosophus	2 (1)	3 (2)	13 (4)	2 (2)	20 (9)
Anopheles crucians	4,403 (206)	163 (27)	15 (7)	-	4,581 (240)
p. pseudopunctipennis	81 (34)	201 (29)	30 (12)	288 (15)	600 (90)
quadrimaculatus	433 (49)	201 (26)	33 (12)	27 (4)	694 (91)
Culex coronator	315 (48)	1,084 (45)	39 (14)	220 (17)	1,658 (124)
p. quinquefasciatus	2 (2)	2 (2)	-	20 (7)	24 (11)
salinarius	12,191 (522)	207 (38)	56 (17)	-	12,454 (577)
tarsalis	393 (30)	1 (1)	2 (2)	61 (8)	457 (41)
(Mel.) sp.	2,738 (153)	365 (53)	67 (18)	159 (8)	3,329 (232)
Deinocerites mathesoni	29 (17)	1 (1)	-	-	30 (18)
pseudes	23,874 (805)	3 (1)	-	-	23,877 (806)
Mansonia indubitans	-		1 (1)	-	1 (1)
titillans	-	1 (1)	1 (1)	-	2 (2)
Psorophora ciliata	169 (28)	29 (11)	18 (7)	2 (2)	218 (48)
confinnis	5,185 (222)	9,787 (369)	2,868 (151)	25 (6)	17,865 (748)
cyanescens	1,557 (69)	1,094 (59)	81 (15)	5 (3)	2,737 (146)
discolor	5,103 (309)	88 (21)	6 (5)	3 (2)	5,200 (337)
signipennis	2 (1)	-	29 (2)	24 (5)	55 (8)
Totals	88,572 (4,053)	27,699 (1,228)	16,059 (741)	942 (94)	133,272 (6,116)

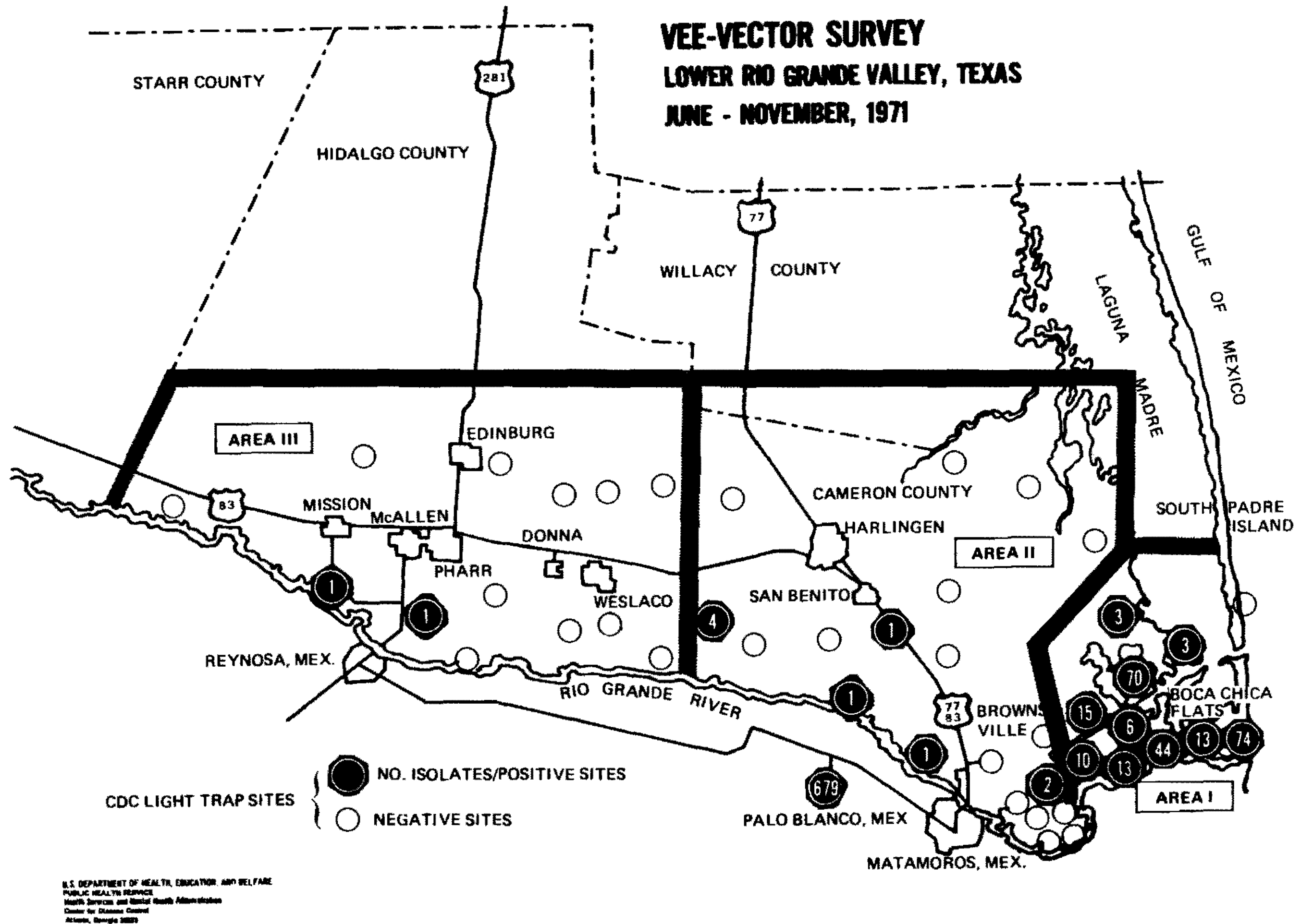


Figure 2

TABLE 2

Summary of VEE-IB viruses isolated from mosquitoes collected in the lower Rio Grande Valley, Texas, during the VEE epidemic of 1971.

Species	Areas				Total
	I	II	III	Misc.	
A. sollicitans	196	2	-	-	198
P. discolor	28	-	1	-	29
P. confinnis	4	7	1	-	12
A. thelcter	10	-	-	1	11
D. pseudes	9	-	-	-	9
A. taeniorhynchus	3	-	-	-	3
An. p. pseudopunct.	-	-	-	1	1
An. crucians	1	-	-	-	1
Total	251	9	2	2	264

TABLE 3

Arboviruses other than VEE isolated from mosquitoes collected in the lower Rio Grande Valley, Texas, 1971.

Species	June	July		Sept.	Oct.	Total
	SA*	SA	VEE-SLE*	WEE	WEE	Isol.
A. sollicitans	1	1	1**	34	3	40
A. taeniorhynchus					1	1
A. thelcter					1	1
P. confinnis					1	1
P. discolor					1	1
Totals	1	1	1	34	7	44

* SA, San Angelo virus; SLE, St. Louis encephalitis; VEE-Venezuelan equine encephalitis; WEE, Western equine encephalitis virus.
** Mixed virus isolation.

TABLE 4

Post-epidemic vector surveillance in Area 1, Boca Chica Flats, Cameron County, Texas, September-November, 1971, and March,1972.*

Species	Week of Collection 1971				1972	
	9/13-9/19	9/20-9/26	10/25-10/31	11/1-11/7	3/19-20/72	Total No. Mosquitoes
Aedes bimaculatus			4(4)			4(4)
fulvus-pallens	2(1)**					2(1)
sollicitans	1,772(37)	19,539(399)	7,301(163)	985(24)	6,490(134)	36,087(757)
taeniorhynchus	33(2)	143(9)	257(17)	26(3)	21(5)	480(36)
thelcter	1(1)	27(11)	740(27)	250(8)	38(6)	1,056(53)
vexans		1(1)	3(3)		6(3)	10(7)
Anopheles crucians	1,949(41)	4,704(101)	17,737(363)	6,057(122)	1,460(32)	31,907(659)
p. pseudopunctipennis	47(2)	34(8)	404(15)	24(3)	1(1)	510(29)
quadrimaculatus	62(2)		405(16)	29(3)	12(3)	508(24)
Culex coronator	4(2)	26(7)	958(27)	202(6)	8(3)	1,198(45)
salinarius	4,516(93)	7,907(167)	10,716(233)	819(19)	3,918(86)	27,876(598)
tarsalis	7(3)	68(9)	906(27)	112(4)	637(15)	1,730(58)
thriambus			17(6)	7(1)		24(7)
(Mel.) sp.	765(16)	3,926(84)	2,256(52)	1,042(24)	108(5)	8,097(181)
Deinocerites mathesoni	4(1)	5(4)	3(3)		1(1)	13(9)
pseudes	86(4)	60(4)		2(2)	35(1)	183(11)
Psorophora ciliata	1(1)	15(5)	42(13)	2(2)	1(1)	61(22)
confinnis	22(3)	751(23)	5,856(128)	53(3)	101(6)	6,783(163)
cyanescens	1(1)	58(9)	146(16)	14(3)	7(4)	226(33)
discolor	2(2)	22(8)	3,999(96)	23(3)	54(4)	4,100(113)
Uranotaenia lowii	98(2)	23(4)	5(2)			126(8)
Totals	9,372(214)	37,309(853)	51,755(1,211)	9,647(230)	12,898(310)	120,981(2818)
Trap Nights	23	43	13	4	20	103
Average/Trap Night	407	868	3,981	2,412	645	1,174

*No isolations of VEE virus were made from these collections.
**No. Mosquitoes (No. pools tested.)

TABLE 5

Venezuelan Equine Encephalitis N antibody rates in wild birds by residency and month of collection, South Texas, 1971-1972

Residency	1971										1972		Total: 1971-72	
	July		August		September		October		November		March			
Permanent	19/247*	8%**	15/172	9%	3/147	2%	2/132	2%	0/42	-***	0/199	-	39/939	4%
Summer	5/47	11%	1/18	6%	3/73	4%	0/5	-	0/2	-	0/3	-	9/148	6%
Winter	1/4	-	2/5	-	1/29	3%	0/8	-	0/15	-	0/60	-	4/121	3%
Transient	None		0/14	-	32/516	6%	1/59	2%	0/35	-	0/25	-	33/649	5%
Total	25/298	8%	18/209	9%	39/765	5%	3/204	1%	0/94	-	0/287	-	85/1857	5%

*No. positive/No. tested
**Percent VEE positive
***Zero percent, or insignificant numbers collected.

Table 6. Antibodies to VEE and WEE viruses in sera from mammals collected in the Boca Chica Area, Cameron County, Texas, 1971-72

Species	1971				1972		TOTALS			
	July-Sept.		Oct.-Nov.		March					
	VEE	WEE	VEE	WEE	VEE	WEE	VEE	%	WEE	%
Sylvilagus floridanus Cotton-tail rabbit	3/8	3/8	1/2	1/2	1/1	1/1	5/11	45	5/11	45
Myotis species Bat	3/13*	1/10					3/13	23	1/10	10
Liomys irroratus Pocket-mouse	5/42	1/36	0/24	0/24	0/12	0/12	5/78	6	1/72	1
Didelphus marsupialis Opossum	4/55	0/33	1/22	0/22	0/28	0/28	5/105	5	0/83	
Peromyscus leucopus White-footed mouse	18/330	4/309	0/179	0/180	0/187	3/188	18/696	3	7/677	1
Sigmodon hispidus Cotton rat	7/165	0/152	0/25	0/25	0/32	0/32	7/222	3	0/209	
Spermophilus mexicanus Mexican ground squirrel	1/37	0/34	0/9	0/9	0/11	0/11	1/57	2	0/54	
Neotoma micropus Woodrat	3/140	0/132	0/67	0/67	0/26	2/26	3/233	1	2/225	1
Canis latrans Coyote			1/1	1/1			1/1		1/1	
Rattus norvegicus Norway rat	1/2	0/1					1/2		0/1	
Procyon lotor Raccoon	0/5	0/2	0/1	0/1			0/6		0/3	
Eptesicus fuscus Big brown bat	0/5	0/5	0/1	0/2			0/6		0/7	
Nycticeius humeralis Evening bat					0/2	0/2	0/2		0/2	
Onychomys leucogaster Grasshopper mouse	0/1	0/1			0/19	0/19	0/20		0/20	
Lepus californicus Black-tailed jackrabbit		0/1			1/3	1/3	1/3		1/4	
Lynx rufus Bobcat			0/1	0/1			0/1		0/1	
Totals:	45/803	9/724	3/332	2/334	2/321	7/322	50/1456		18/1380	
Percent positive	5.6	1.3	0.9	0.6	0.6	2		3.4		1.3

*DEC, 90% Plaque reduction neutralization test; No. positive at 1:20 serum dilution/No. tested.

TABLE 7

Sequelae of VEE virus infection in nine patients from Cameron & Hidalgo Counties, Texas

Patient	Age (years)	Sex	Duration of sequelae	Sequelae noted
A.A.	6	F	> 1 mo.	Fearful child with a stiff, weak left leg and bilateral 6th cranial nerve palsy
			1 yr.	Recovered
J.Y.	5	F	> 1 mo.	Left side of mouth with partial paralysis. Recurrent neck, leg and abdominal pain
			1 yr.	Recovered
R.C.	48	M	1 mo.	Poor thinking, easily fatigued, decreased taste, smell, hearing
			1 yr.	Easily fatigued, decreased taste
A.G.	52	M	1 mo.	Easily fatigued, forgetful, recurrent headache
			1 yr.	Easily fatigued, recurrent headaches
E.S.	29	M	1 mo.	Drowsiness, easily fatigued
			1 yr.	Irritable, easily fatigued
S.K.	64	F	1 mo.	Weakness, easily fatigued
			1 yr.	Easily fatigued
B.S.	61	F	1 mo.	Easily fatigued
			1 yr.	Easily fatigued

TABLE 7 (CONT.)

Patient	Age (years)	Sex	Duration of sequelae	Sequelae noted
V.H.	44	F	1 mo.	Easily fatigued
			1 yr.	Easily fatigued
A.H.	15	F	4 days	Recurrent fever, chills, headache
			1 mo.	Recovered

TABLE 8

Human VEE virus infection diagnosed at CDC
by virus isolation and serological tests

No. Sera Tested		No. Patients	Virus Isolations Made	Positive Serology	Diagnosis First Made By:	
Acute	185	177	52		Isolation	50
Convalescent	117	110		36	Pos.Serology	17
Total	302	204			Total	67

TABLE 9

Titers of VEE virus in acute blood specimens of 43 naturally acquired human infections

No. of patients	Virus titers detected, log 10 *
18	< 1.5
2	1.6 - 3.3
12	3.4 - 4.4
8	4.5 - 5.4
1	5.5 - 6.4
2	6.5

* Per .02 ml.

TABLE 10

Serological tests of 36 blood specimens
drawn from VEE patients one year after infection

HI		CF		Neut	
Titer	No. Patients	Titer	No. Patients	Titer	No. Patients
≥ 1:20	35	≥ 1:16	10	≥ 3.0 log	11
1:10	1	1:8	14	1.7-3.0 log	23
Undetectable	0	Undetectable	12	Undetectable	2

TABLE 11

VEE viremias by day after onset in 40 naturally infected Texas
horses from which virus was isolated

Day After Onset	Number	Titer, log 10 ($SMicLD_{50}$/ml)	
		Range	Geometric Mean
0	7	2.2-8.2	5.6
1	5	5.2-8.0	6.5
2	4	2.2-8.3	5.3
3	1	0.5	0.5
Unspecified	23	2.2-6.8	4.2

TABLE 12

VEE virus isolations from horses, listed by day after vaccination, 1971

	Day after vaccination with TC-83													
Strain	0	1	2	3	4	5	6	7	9	10	11	14	25-61	Total
Vaccine (TC-83)	1	4	5	16	13	4	9	4	4	1	1	1	0	63
Epizootic*	1	2	1	2	1	1	6	0	2	0	0	0	7	23

* All from Texas

TABLE 13

Isolations of a VEE virus (either epidemic or vaccine type) from equine blood components, Texas, June 29-August 31, 1971

Blood Component	No. with virus/No. tested	% Positive
Whole blood*	6/37	16
Clot*	11/32	34
Serum	103/1585	6
Total	120/1654	7

* The whole blood and clot specimens were mostly from the early part of the epidemic and were acute specimens. Their results are therefore biased toward positive as compared with the serum specimens.

TABLE 14

Isolations of epidemic VEE virus from equine tissues other than blood, Texas, June 29-August 31, 1971

Tissue	No. with virus/No. tested	% Positive
Brain	10/44	23
Spleen	4/14	29
Liver	1/12	8
Kidney	1/10	10
Heart	1/9	11
Lung	1/6	17
Other *	1/3	33
Total	19/98	19

* Mesenteric tissue, lymph gland and pancreas. Virus was isolated from the mesenteric tissue specimen.

TABLE 15

Comparison of HI and NT antibody titers in twenty randomly selected single equine sera, Texas, 1971

	Antigen (HA)				Virus (NT)		
Specimen No.	(GJ9-1BJ) VEE	(FE3-7 C) VEE	(NJO) EEE	(Flemming) WEE	(TC-83) VEE	(NJO) EEE	(Flemming) WEE
1							
2	320	160	20	160	≥50		5
3	(b)		10	40		≥50	≥50
4 (a)				10		5	5
5				40		≥50	≥50
6	10		10	20		5	5
7	40	20	40	80		≥50	≥50
8 (a)							
9				40			≥50
10	80	40	20	20	5	5	5
11			10	10		5	5
12	10	10	80	80		≥50	≥50
13	40	40		10	5		
14	40	20	10	80	5		≥50
15	10			20	5		
16	≥1280	≥1280	80	160	≥50	5	5
17	160	80	40	80	5		5
18	80	160	20	20	≥50	5	5
19	10	10	10	10	5	5	5
20	320	160	40	40	≥50	5	5

(a) VEE virus isolated

(b) A blank indicates an HI titer of <1:10 or a NT titer of <1:5

TABLE 16

NT antibody responses in equines to VEE, EEE, and WEE viruses
>7 days after VEE vaccination, U.S., 1971[a)]

		WEE	
VEE	EEE	-	+
-	-	25	15
	+	10	19
+	-	196	43
	+	25	64

a) - = <1:5, duck embryo cells, 90% plaque reduction

+ = 1:5 or ≥1:50, duck embryo cells, 90% plaque reduction

Summary:

Of the VEE negative, 44/69 were EEE+ and/or WEE+ (64%)

Of the VEE positive, 132/328 were EEE+ and/or WEE+ (40%)

TABLE 17

VEE NT antibody in equines, by day after vaccination,
U.S. (excluding Texas) July-September, 1971

	0-6	7-14	15-21	>21	Total
No. horses with no antibody a)	94	28	5	5	132
No. horses with antibody b)	10	101	18	36	175
Total	104	129	23	41	307

a) <1:5, duck embryo cells, 90% plaque reduction

b) 1:5 or ≥1:50, duck embryo, 90% plaque reduction

TABLE 18

Susceptibilities of mice, guinea pigs and duck embryo cell culture to infection with known strains of natural VEE virus and the TC-83 (vaccine) variant [a)]

Type	Subtype	Strain	0.02 SMic	0.03 SMip	0.03 WMic	0.03 WMip	0.1 OMip [b)]	0.1 GPip [c)]	0.1 DECC
I	A	Trinidad donkey	>9.0	>9.0	>9.0	>9.0	>9.0	>9.0	>9.0
	B	ICA	>9.0	>9.0	>9.0	>9.0	8.0	7.6	>9.0
		PTF-39	>9.0	>9.0	>9.0	>9.0	>9.0	>9.0	>9.0
		Three Rivers	>9.0	>9.0	>9.0	>9.0	>9.0	8.5	>9.0
	C	P-676	>9.0	>9.0	>9.0	>9.0	>9.0	>9.0	>9.0
	D	3880	>9.0	>9.0	>9.0	>9.0	8.3	>9.0	>9.0
	E	Mena II	>9.0	>9.0	>9.0	>9.0	NT [d)]	≤1.0	>9.0
II	Everglades	FE3-7C	>9.0	>9.0	>9.0	>9.0	NT	NT	>9.0
III	Mucambo	BeAn8	>9.0	>9.0	>9.0	>9.0	NT	>8.0	>9.0
IV	Pixuna	BeAr356ys	>9.0	>9.0	>9.0	≤1.5	NT	≤1.0	>9.0
I	A	TC83(Vaccine)	>9.0	>9.0	>9.0	≤1.5	≤1.5	≤1.5	>9.0

a) Titers given are per inoculation dose, and indicate the titers of respective stock virus suspensions in the various host systems.

b) OM = Old mice (8-10 week)

c) 250 gram

d) Not tested

REPORT FROM FLORIDA DIVISION OF HEALTH, DEPARTMENT OF HEALTH AND REHABILITATIVE SERVICES BUREAU OF LABORATORIES, JACKSONVILLE, FLORIDA

Arbovirus Surveillance Report, January - August 10, 1972

The Bureau of Laboratories examined a total of 397 human, 315 chicken and 38 animal (dogs and raccoons) serological specimens for antibodies against EEE, SLE and VEE. There were no significant findings to report.

In addition, 76 horse bloods were examined for the above listed antibodies, plus WEE. We found two horses positive for EEE received in the laboratory from Duval County during the first week of August.

In summary, this has been a light year for arbovirus activities in Florida, judging from the diagnostic surveillance specimens tested in the Jacksonville laboratory.

(N.J. Schneider)

REPORT FROM FLORIDA DIVISION OF HEALTH, DEPARTMENT OF HEALTH AND REHABILITATIVE SERVICES EPIDEMIOLOGY RESEARCH CENTER, TAMPA, FLORIDA

Due to the outbreak of Venezuelan equine encephalitis (VEE) virus in Texas last year, there has been real concern expressed about the possible geographical extension of the 1B strain in the United States. During the first six months of 1972, the Epidemiology Research Center (ERC) has been engaged in VEE surveillance in the Gulf Coast areas of Mississippi, Alabama and North Florida. Fourteen mosquito pools have been tested with negative results. To date, 686 mammalian and 7 avian sera have been processed for isolation attempts and sero-serology. Four sera from 263 cotton rats trapped in Hancock County, Mississippi yielded Western equine encephalitis (WEE) virus isolates. The positive cotton rats were trapped on May 9th and 10th. Seven of the 263 had hemagglutination-inhibition (HI) titers ranging from 1:10 - 1:120 for St. Louis encephalitis (SLE) virus. Confirmation of these findings will be attempted in weanling mouse neutralization tests.

No evidence of VEE infection was found in the rodents tested. One of three raccoons, an adult female, trapped on July 22nd in the same area as the WEE positive rodents, had a serum neutralization plaque reduction (SNPR) titer of 1:20 against the TC-83 VEE vaccine strain in duck embryo cell culture (DECC). Since cells were not available immediately, the serum was tested against WEE and Eastern equine encephalitis (EEE) viruses in weanling mouse neutralization (N) tests. The neutralization index (NI) was 0.8 against EEE and 1.7 against WEE. In addition, serum from one of 22 raccoons trapped and bled at the Eglin Air Force Base on June 30th showed an 1:5 SNPR titer in a single-dilution-screening test. This serum from an adult male raccoon possessed an NI of 1.4 against EEE and 1.7 against WEE in the weanling mouse neutralization test. No conclusions can be drawn as to the significance of these findings until the SNPR tests in DECC against VEE strains 1B, TC-83 and Fe3-7C as well as EEE and WEE are completed.

Routine arbovirus surveillance in the Tampa Bay area yielded few positive findings this year. It appears that the limited rainfall in Florida, which has precluded large mosquito hatches thus far this year, has had its effect on arbovirus buildup. All human sera (291 paired specimens) submitted to ERC this year were negative when tested against EEE, SLE, VEE, CAL and dengue. Of the 524 sera from sentinel chickens and pauperized avian flocks tested by HI against EEE, WEE, VEE and SLE, seven were positive to EEE with titers ranging from 1:10 - 1:80 and one positive to WEE at a 1:10 titer. These have not been confirmed by N tests as yet. EEE virus was isolated from two of six equine central nervous system tissue specimens examined. Serological results on sera from three additional equines did not reveal evidence of recent arbovirus infection.

(F.M. Wellings)

REPORT FROM THE DEPARTMENT OF EPIDEMIOLOGY AND PUBLIC HEALTH
SCHOOL OF MEDICINE
UNIVERSITY OF MIAMI, FLORIDA

Endemic Venezuelan equine encephalitis (VEE) virus in the United States

Surveillance for VEE virus infection in South Florida has demonstrated continued VEE virus activity. In August 1971 a 75-year-old man from South Miami was hospitalized with Florida VEE disease (Table 1). Two VEE viral strains were isolated from sentinel hamsters and 2 seroconversions were found in 52 batches of serially collected sera from *Peromyscus gossypinus* and *Sigmodon hispidus*. In tests of single sera 9 of 13 dogs and 1 of two cats were found to have VEE antibodies (Table 2).

No VEE antibody was found in girls 11-20 years and from both sexes under 10 years of age, but was present in 16% of males over the age of 10 years (Table 3). Three serodiagnostic changes were observed in 112 paired human blood specimens collected 1968 to 1971.

(A.K. Ventura, E.E. Buff, N.J. Ehrenkranz and W.J. Bigler)

TABLE 1

TEST RESULTS OF PATIENT WITH VENEZUELAN EQUINE ENCEPHALITIS
DADE COUNTY, FLORIDA - AUGUST-SEPTEMBER 1971

Serologic Test	Date of Specimens Aug. 31	Sept. 10	Sept. 24
Complement-Fixation			
1. VEE (TC-83, Trin)[1]	<1:8	1:32	1:64
2. VEE (Fe3-7C, Fla)[2]	<1:8	1:32	1:32
3. VEE (GJ9-1BJ, Guat)[2]	<1:8	1:32	1:32
4. Herpex simplex[1]	1:32	1:32	1:32
Hemagglutination-Inhibition			
1. VEE (Fe3-7C, Fla)[3]	1:10	1:320	1:160
Plaque Reduction (Neutralization)			
1. VEE (Fe3-7C, Fla)[2]	1:80	≥1:640	1:640

[1]Bureau of Laboratories, Florida Division of Health

[2]Arbovirus Reference Laboratory, CDC

[3]University of Miami School of Medicine

TABLE 2

HEMAGGLUTINATION-INHIBITION RESULTS ON DOMESTIC ANIMALS FROM PINECREST TESTED WITH FLORIDA VEE STRAIN Fe3-7C

Animals	Number Pos.	Number Tested
DOGS	9	13
CATS	1	2
RABBITS	0	8
CHICKENS	0	11

Range: 1:80 to 1:640

TABLE 3

SEROLOGIC SURVEY IN PINECREST*

Age Years	Male #Pos/#Tested	Female #Pos/#Tested	Totals #Pos/#Tested
1-10	0/10	0/9	0/19
11-20	6/20 (30%)	0/9	6/29 (20.7%)
21-30	1/17 (5.8%)	0/14	1/31 (3.2%)
31-40	2/15 (13.3%)	1/3 (33.3%)	3/18 (16.7%)
41-50	1/11 (9.09%)	0/8	1/19 (5.26%)
≥ 51	4/13 (30.8%)	1/6 (16.7%)	5/19 (26.3%)
TOTALS	14/86 (16%)	2/49 (4.1%)	16/135 (12%)**

* Tested by HI method against 4-8 units of Florida VEE (Fe3-7C) antigen.

** This figure does not reflect the 112 re-bleedings collected from these individuals.

REPORT FROM THE TRINIDAD REGIONAL VIRUS LABORATORY,
PORT OF SPAIN, TRINIDAD

Current arbovirus studies are being centered in the forests at Aripo-Waller Field in northeastern Trinidad, near Turure Forest. Emphasis is being given to a study of Eastern equine encephalitis (EEE) virus.

EEE - In 1971 some 62,000 mosquitoes were tested for the presence of virus in 703 pools. Three species (Culex portesi, C. taeniopus and C. declarator) accounted for 72 per cent of the mosquitoes caught. Fourteen strains of EEE virus were isolated; 5 came from portesi; 5 from taeniopus; one each from C. declarator and A. serratus and 2 from C. amazonensis. Twenty-two other strains were isolated from sentinel mice, while another was recovered from a hamster which was used as a bait animal in a mosquito trap.

More EEE isolates were made in 1971 than in any previous year. Three of the 37 isolations came in January which can be considered as part of the 1970 transmission season. The first appearance of EEE in the 1971 transmission season, however, was in June with two isolations, but peak months were in October and November with 8 and 12 isolations respectively.

In association with the EEE programme, birds were netted-bled-banded and released. By the end of 1971, 753 blood specimens were taken from 43 species of birds. No virus was isolated from bird material, but 23 sera (of 745 tested) possessed HI antibodies to EEE virus.

Other Virus Isolations - Apart from EEE, 123 strains of other viruses were isolated in 1971. Of these 85 were of VEE (probably Mucambo). Sentinel mice and sentinel hamsters yielded 72 strains, while 9 came from C. portesi; 3 from C. taeniopus and one from C. sp. #17.

Twenty strains of Guama group viruses and 18 of Group C were also isolated. Since members of these two groups are outside of our present scope of interest we do not make specific identification of these agents.

(E.S. Tikasingh)

REPORT FROM THE VIRUS RESEARCH LABORATORY
UNIVERSITY OF IBADAN, NIGERIA

Isolation of bovine ephemeral fever virus in Nigeria

Three-day-fever has been recognized for many years as a clinical entity causing fever, soreness and shifting lameness primarily in 1 to 2 year old cattle on the University of Ibadan farm. This illness has been associated with fresh green grass and its reoccurrence with the beginning of the rainy season.

On 31st July 1971, blood samples were taken from three animals between 1-2 years of age having typical signs suggestive of bovine ephemeral fever (B.E.F.) infection. Virus was isolated from two of the samples - Ib AN 59688 and Ib AN 59689. The isolates were tested in complement fixation tests with twenty immune ascitic fluids. The isolates reacted in complement fixation tests with 2 strains of bovine ephemeral fever M.A.Fs - only South African and Australian strains obtained from Dr. Erasmus, Veterinary Research Center, Onderstepoort. Ib AN 59689 was later used as prototype in subsequent tests.

MAF

Antigen	BEF (SA)	BEF (AU)	59689
AN 59689	64/8*	64/8	128/64

*Serum dilution/antigen dilution.

The acute phase serum (AN 59689) from which virus was isolated was compared in neutralization test (NT) with the convalescent serum. The convalescent serum neutralized B.E.F. virus by 3.6 log greater than the acute phase serum.

Recent studies on virus UG MP 359

Eight isolations of strains of virus Ug MP 359 (first isolated from a pool of *Aedes funestus* in Uganda), have so far been made in Nigeria. Of the strains one was isolated from a pool of *Aedes dentatus* mosquitoes collected at Du near Jos. The others were isolated from humans in Ibadan and Umuahia.

From February through June epidemics of a febrile illness with nausea, muscular pains, headaches have been reported in and around Jos. Two deaths were recorded. Blood samples were collected and screened in complement fixation test against a battery consisting of twenty-four viral antigens. Of the 45 samples screened, 38 showed presence of antibodies to Ug MP 359 of which 31 showed positive results to Ug MP 359 agent only. Further investigation is on. The virus has not yet been reported to cause fatal disease in man.

Rift Valley Fever: Typing of the Nigerian strain

Two strains of Rift Valley Fever, Neurotropic and Lunyo, were reported from East Africa. An attempt was made to type the Nigerian strain of Rift Valley Fever isolated from *Culicoides* spp.

The Nigerian strain Ib AR 55171 reacted in CF and NT tests with the two strains of Rift Valley Fever supplied by the East African Virus Research Institute (EAVRI).

C.F.T. RESULTS

M.A.F.

Antigen	Ib AR55171	R.V.F. Lunyo	R.V.F. Neurotropic
Ib AR 55171	256/128*	32/8	-
Lunyo strain	32/8	8/128	-
Neurotropic strain	4/8	8/8	128/128

* = Serum dilution/antigen dilution

\- = less than 1/4

Preliminary results show Ibadan strain to be the Lunyo strain. However, neutralization tests show the three to be undistinguishable . Ref: A variant of Rift Valley Fever virus, S.A. Medical Journal, 1957, September 21. V. 31. No. 38 pp. 951-957.

Recent isolates from arthropods

In the course of research in the Nupeko forest area for an endemic zone of yellow fever infections, two isolates of Semliki Forest virus were made from a mixed pool of mosquitoes - Aedes spp. and Mansonia spp. Hitherto isolates of Semliki Forest virus have been from Atelerix albiventris and Queles erythrops both from Kware, and sentinel mice in Ibadan. These isolates from Nupeko Forest were the first in mosquitoes in Nigeria. 11 strains of Bwamba virus were also isolated from other pools of mosquitoes collected at the same time. Previous isolates of Bwamba virus were from human beings in Ibadan, one isolate was also made from mosquitoes in Jos.

Serological studies

A. Chandipura. The prevalence of Chandipura virus immunity was investigated in six species of domestic animals: cattle, sheep, goats, pigs, horses and camels. Results showed that 5/66 cattle, 1/50 sheep, 2/50 goats, 4/49 pigs, 7/26 horses and 16/62 camels had neutralizing antibodies.

B. Tataguine. Sera from humans and animals in three ecological zones in Nigeria lowland, rainforest, derived guinea savannah and plateau, were tested for neutralizing antibodies. Neutralization tests were performed in mice. The highest prevalence (61%) of antibodies was found in derived savannah zone followed by lowland rainforest 42%. The percentage of positive sera on the Jos plateau was significantly lower than in other zones. In persons tested from all areas, adults had a higher prevalence of antibodies than did children. This finding indicates the endemic presence of the virus.

All animal and bird sera tested were negative.

Experimental infection of white Fulani calves with AR 23380

Three white Fulani calves were inoculated with IB-AR 23380 (Kotonkan virus), a member of the rabies-related virus group. One of the calves developed a high temperature of 104.4°F and anorexia on the 11th day post viral inoculation. This was preceded by a slight serous milky nasal discharge, which later became profuse and stringy. A leucocytosis with absolute neutrophilia was noted a day prior to onset of fever and during the febrile period.

A second calf exhibited a mild response by developing a serous nasal discharge on the 5th day post viral inoculation.

However, no virus was isolated from blood samples or nasal discharges, but all the three calves developed increased antibody titres.

(Akinyele Fabiyi)

REPORT FROM THE ARBOVIRUS LABORATORY, INSTITUT PASTEUR AND ORSTOM, DAKAR, SENEGAL

During the period January 1, 1971 through June 30, 1972 studies initiated in Bandia and Saboya field stations in 1965 were continued. In addition a new field station has been opened at Kedougou, a suspected yellow fever enzootic area in the south eastern part of Senegal, in a Guinea savanna vegetation zone.

1. VIROLOGICAL STUDIES

1.1. Human blood samples

953 human blood specimens have been collected from febrile patients (mostly children under 14 years of age) attending the dispensary open at Bandia village by the Institute. No virus has been yet isolated from these materials.

A strain isolated from the blood of a 6 year old child in October 1970 (Dak HD 10674) reported as group B virus in the last report (AVIE 1972, n° 22) has been shown to be a Dengue 2 virus strain. This is the second area in Africa where Dengue virus has been isolated, numerous strains of Dengue 1 and 2 having been obtained in 1968 in Nigeria by the workers of the Arbovirus Laboratory, Ibadan (AVIE, 1962, n° 19, 52-53).

1.2. Wild vertebrate samples

1300 specimens have been processed for virus isolation. Most of them were collected at the Bandia, Saboya and Kedougou field stations. Investigations were also conducted in Richard Toll, a selected rest place for migrating birds in the delta of the Senegal River.

1.2.1. Bandia

One strain of Koutango virus has been isolated from a *Mastomys*. The prototype strain of Koutango (Dak An D 5443) was isolated in 1968 from *Tatera kempi*. It seems to be a new group B virus.

1.2.2. Saboya, Kedougou, Richard Toll

No strain has been yet recovered from specimens collected in these field stations.

1.3. Arthropods

No virus was isolated from 526 mosquito pools collected in the field.

926 pools of ticks collected at the Dakar abattoirs yielded three strains of Jos Virus (Ib Ar 18735), all isolated from *Amblyomma variegatum*. One strain of Congo Virus was isolated from *Hyalomma truncatum* ticks removed from febrile oxen at Bambylor, a cattle grazing station 20 miles from Dakar.

2. SEROLOGICAL STUDIES

2.1. Human sera

2.1.1. Bandia

320 sera collected from febrile children were tested for HI antibodies to the following viruses: CHIK, ONN, YF, UGS, DAK, WN, ZIK, BUN. This study has brought out that Zika virus is widespread in this village. Effectively 3-, 2- and one-year-old children were found with HI antibodies for Zika virus and 27% of the HI positive sera showed CF antibodies for this virus.

2.1.2. Kedougou

This new area has been selected for intensive field studies. Serological results on human sera collected in 1965 showed that yellow fever virus was active in this area. In 1970 and 1971, serological surveys were carried out on school children, mostly under 6 years of age. A high proportion of these sera tested for HI antibodies reacted with yellow fever, West-Nile and Zika antigens. CF and neutralization tests were done on HI positive sera. 32 of the 351 sera collected (8.1%) reacted with yellow fever antigen and 11/351 (3.1%), with Zika antigen. 15 per cent of children under 2 years of age showed a specific response to yellow fever virus.

2.2. Wild vertebrate sera

2.2.1. 1394 sera taken from wild vertebrates caught in Bandia, Saboya and Richard Toll have been tested for HI antibodies. Saboya virus (a new group B member) seems to be very active in Senegal, especially in Bandia area.

2.2.2. Monkey sera. In order to understand the dynamic of the maintenance of yellow fever in enzootic areas of West Africa, a serological survey is carried out on sera collected from West African monkeys, using HI, CF and NT tests.

Animals are taken by shooting by the ORSTOM entomological team. The survey will cover a wide area spreading from the lowland forest in Ivory Coast to the sahelian area in Upper Volta. The geographical prospecting unit is the square degree. About ten monkeys are shot and bled in each square degree. 26 square degrees have been studied. Though incomplete, the results point out two main features:

a. There is a high incidence of recent infection in the area where yellow fever broke out in human beings during the 1969 epidemic (North of 11° N).

b. South of 10° N, yellow fever is enzootic.

(G. le Gonidec and Y. Robin, Institut Pasteur, Dakar and R. Taufflieb, M. Cornet and J.M. Klein, ORSTOM, Dakar)

REPORT FROM THE ARBOVIRUS LABORATORY,
DEPARTMENT OF BACTERIOLOGY AND VIROLOGY,
AND THE
DEPARTMENT OF PARASITOLOGY AND ENTOMOLOGY,
NATIONAL SCHOOL OF PUBLIC HEALTH AND TROPICAL MEDICINE,
LISBON, PORTUGAL

After March 1971 our laboratory had been mainly concerned with the virological and serological studies related to the Yellow Fever epidemic in Luanda, Angola, which started in January 1971 and to the Chikungunya outbreak which started several months before the Yellow Fever epidemic. A summary of the studies done in Luanda and in Lisbon with the material collected in Angola is the following:

1 - The Yellow Fever epidemic

During January 1971 a few cases of a severe hepatitis were admitted to the Infectious Diseases Hospital of Luanda with haemorrhagic symptoms, black vomitus, jaundice and rapid fatal progress. In February more cases were admitted with the same symptomatology. The diagnosis of Yellow Fever was soon confirmed by the histopathology of five fatal cases. The Yellow Fever epidemic had its peak in March, and it was under control in the beginning of April. The number of hospitalized cases was 65 patients with 42 deaths. (Wkly Epidem. Rec., 1972, 47, 229-236).

Yellow Fever was not known in Luanda since the end of the last century. An entomologic survey made in Luanda in 1970 did not show the presence of *Aedes aegypti* in the town and suburbs. However, as soon as Yellow Fever was recognized, an entomologic survey showed that the *A. aegypti* had been introduced in Luanda and the captures were especially abundant in an area where rubber tires were kept in the open air near the town. (Pinto, M. & Filipe, A.R., Bull. Soc. Patho. exot. 1971, 64, 708-710). (Ribeiro, H., Rev. Med. Angola, 1971, 13 (50) 67-91).

The isolation of the virus was attempted in baby mice by inoculation i.c. and i.p. of sera from 28 cases and from a liver fragment taken during necropsy. Nine strains of a virus with marked neurotropism to the mice were isolated, eight being isolated from the sera and one from the liver fragment. Two of these strains were isolated from mild infections not recognized as Yellow Fever.

Immune mice sera were prepared against all the isolated strains and tested by HI test with a battery of antigens from group A, B and Bunyamwera and the antigens extracted by the sucrose-acetone method from baby mice brains inoculated with the isolated viruses. Haemagglutinins were easily extracted from baby mice brain according to the technique cited. Immune reference sera against Yellow Fever, Asibi strain, was also included in all the performed

tests. All the isolated strains were studied by the neutralization test with immune reference sera and these studies showed that the isolated strains were Y.F. virus.

The Y.F. virus could not be isolated from the captured mosquitoes. At the same time that the virological studies started in Luanda, a serological survey was done with 589 sera from several groups of people living in the town and suburbs. Some of the sera were from family contacts of people with the diagnosis of Yellow Fever. Other groups of sera were from people with diagnosis of Y.F. and from suspected cases of illness with Y.F. All these sera were locally studied by the HI test with several antigens from group A, (Sindbis, Chikungunya) group B (Y.F., West Nile, Wesselsbron, Ntaya, Banzi, Zika, Dengue 1, Dengue 2) and Bunyamwera. The results of this survey have shown that the population of Luanda and suburbs had been in contact with two arboviruses: Y.F. and Chikungunya.

2 - The Chikungunya epidemic

Some months before the start of the Yellow Fever epidemic, the physicians of Luanda and suburbs observed the presence of a Dengue-like disease locally called Katolu-Tolu (dialect quimbundo). This disease was characterized by a sudden onset, headache, photofobia, severe pains in the joints and muscles and in some cases a mild rash. We had the opportunity to follow three typical cases and the serology showed an antibody rise against the Chikungunya antigen, the acute sera being negative and the convalescent sera positive in titers ranging from 1:40 to 1:160. A virus was isolated from the blood of one of these typical cases and another strain was isolated from a pool of *Aedes aegypti* mosquitoes. Both of the viruses have been studied using the same methodology followed in the studies done with the Y.F. and both proved to be strains of the Chikungunya virus. The serological survey for antibodies to arboviruses showed that the immunological response from the studied group was against the Y.F. and the Chikungunya virus. But while antibodies against Y.F. were to be found mostly in people living inside the town, antibodies against Chikungunya were found also in people living in the suburbs and small villages to the north of Luanda.

It seems that it should be emphasized that in the same area and during a certain time two arboviruses from groups not related antigenically were actively circulating among the population and transmitted by the same mosquito vector, and that the Chikungunya epidemic preceded the Yellow Fever epidemic. To our knowledge it was the second situation reported in the recent years (Chik. and Y.F.), the first being reported in the Republic of Zaire in 1958/60 (Osterrieth, P.M. & Blanes-Ridaura, G. Ann. Soc. Belge Med. Trop. 1960, 40, 199-203).

(A.R. Filipe)

3 - Entomological studies and vector control during the Luanda Yellow Fever epidemic of 1971

Fourteen mosquito taxa were recorded at the time of the epidemic, *Culex pipiens quinquefasciatus* representing 84 per cent of all catches and the recently introduced *Aedes aegypti* accounting for 15.3 per cent. The suburban belt was, by far, the main ecological area of the local population of *aegypti*, there the indoor pretreatment *aegypti* density (females/man/hour) attaining 5.2. Vector control was started the 17th of March with ULV technical malathion at the dosage of 500 ml/ha sprayed by a "Piper Pawnee" aircraft equipped with four "Micronair AV 3000" rotary atomizers. Three treatment cycles were carried out at about a week interval. Bioassays show 100 per cent killing both for outdoors exposed adults and larvae as well as for indoors exposed adults. Indoor *aegypti* density was drastically reduced immediately after each treatment (84,93.6 and 96 per cent, respectively), reduction range being 77-98.4 per cent during the 24 day observation period following last treatment. Dissections of *aegypti* females show that all of them were nulliparous, non-transmitting females, after the second treatment cycle, at the end of March (Ribeiro, H., Rev. Med. Angola, 1971, 13(50) 67-91, Ribeiro, H. to be published).

(H. Ribeiro)

REPORT FROM THE LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE, LONDON, ENGLAND

A long term study of the effects of irrigation on arbovirus and other diseases: MRC Project, Kisumi

The overall objective of the Project is to study the effects of the irrigation of a predominantly dry area on arbovirus infections in man and on their arthropod and animal hosts. Wherever irrigation has been carried out on a large scale it has been followed by profound changes in mosquito populations and often in the incidence of mosquito-borne diseases of man and his domestic animals. The Kenya National Irrigation Board's proposed irrigation scheme close to the shore of Lake Victoria on the Kano Plain near Kisumu, Nyanza Province offers a unique opportunity to study these changes in depth and will allow information to be collected before, during, and for several years after the irrigation scheme is put into operation. At the same time the nearby existing pilot Ahero Rice Scheme allows the Project to study some of the changes which are already taking place as a result of irrigation.

The Project, set up jointly by the Kenya Ministry of Health and the British Medical Research Council and intended to last at least 10 years began operations early in 1971 but the staff complement was not completed until June 1971. The Project Leader is Dr. D.I.H. Simpson. The Ministry of Health kindly provided 2 buildings, which have been converted into laboratory space in the grounds of the old Nyanza General Hospital. A temporary building has also been erected at the Project's expense. The study is jointly formed by the MRC and the Overseas Development Administration.

The work during the year has been concerned with the area of the proposed Kano II irrigation scheme, with the established rice scheme at Ahero and with an "undisturbed" area north-west of Kisumu centered around Ngege village. The need for this "undisturbed" control area is under consideration and Ngege may be eliminated from detailed studies in the future programme.

The programme is designed to measure the prevalence and incidence of arbovirus infections and diseases in the human population on the Kano Plain and to detect any changes in prevalence or incidence following the introduction of irrigation and rice cultivation; and also to detect and identify the introduction or emergence of new diseases. These measurements require closely correlated concurrent studies of a defined human population and of infections in arthropods, birds and animals. The studies will assess the clinical significance of arbovirus infections on the Kano Plain as causes of febrile illness and their relationship to malaria. These base-line studies will also make it possible to study the relationship of arbovirus infections to other conditions such as malnutrition.

The medical demography and population census programme directed by Dr. D.H. Smith began serious work in September and has made excellent progress. By the end of 1972 there should be a defined population of some 50,000 people in and around the area to be irrigated. Detailed maps covering 2 areas close to the proposed Kano II irrigation scheme, Nyamware and Kapiyo, have been prepared and mapping of the whole of the Ahero Rice Scheme and the immediate off-scheme area is almost complete. It has been decided to use the "clan" unit or gweng which is a well defined unit both in terms of population and geographical locations. Each gweng consists of approximately 200 homesteads and the population ranges from 1,500 to 3,000 people. The population census has been completed in Nyamware and Kapiyo and a considerable amount of other information dealing with social habits, the number of domestic animals in each homestead and anti-malarial measures has been collected. A large number of field clinics have been held in all the study areas and have proved to be very popular. A large amount of information on existing disease prevalence has been acquired but these clinics are inefficient as a means of collecting meaningful data and only give a rather patchy cover. It is proposed in future to hold static clinics in each area dealing with specific aspects of health.

The entomological team organised by Messrs M.N. Hill, J.A. Chandler and R.B. Highton have been carrying out detailed studies at Ahero, Kano II and in Naege village using a variety of techniques to collect juvenile and adult mosquitoes. Particular emphasis has been directed towards those mosquitoes biting man and his domestic animals. Human bait collections were used in the early work on adult mosquito populations but were found to be wasteful in terms of manpower. The use of CDC light-traps gave comparable results and these and Monks Wood light traps have been used extensively inside and outside houses.

Ninety seven per cent of Anopheles gambiae taken in all the study areas were collected at the Ahero Rice Scheme. The results for An. funestus and An. ziemanni were 79% and 65% respectively. These figures clearly illustrate the changes which irrigation has already brought about. The populations of Anopheles species in the Ahero scheme are strongly influenced by the rice cultivation cycle. When the rice fields were flooded in November there was a marked peak of An. gambiae, whilst after the December harvest the population of An. funestus increased dramatically. Mansonia species were found to be abundant everywhere but the largest numbers were collected at Nyamware. Culex antennatus and Aedes species were found in the largest numbers at Kapiyo. Mosquitoes have been pooled by species, refrigerated at -70°C and despatched to the Microbiological Research Establishment (MRE), Porton, Salisbury, England for virus isolation studies.

Larval studies in all the study areas have been designed to complement the adult collection data. Methods of standardising collection techniques are still being devised. Larvae have been collected from a wide range of sites including papyrus and reed swamps, ditches, hippo wallows, water-holes, rice fields and temporary pools.

Collections of sera from a wide variety of vertebrate species have been made under the direction of Mr. W.E. Grainger. Sera have been collected from rodents, bats and other small mammals as well as from snakes, birds and domestic animals. In addition a start has been made on a method of estimating small mammal populations in different habitats on the Kano Plain. It is hoped to expand these investigations to include estimates of the bird populations found on the Kano Plain and on the Ahero rice scheme.

Virus isolation work and antibody studies on human and animal sera have been carried out in the United Kingdom in the Arbovirus Epidemiology Unit, MRE, under the direction of Mr. E.T.W. Bowen. This laboratory will move to the London School of Hygiene and Tropical Medicine during 1973.

Antibody studies on sera collected from school children on the Kano Plain in 1969 show that there has been considerable recent activity of the chikungunya/o'nyong nyong complex of viruses. Sixty per cent of the children tested had antibodies and the probability of infection has been calculated as almost 10 per cent per annum, suggesting that infection with o'nyong nyong virus is

endemic on the Kano Plain. Group B virus activity appears to be low but Bwamba virus appears to be endemic. Nyando virus seems to have caused an epidemic about 10-15 years ago but there is little evidence of recent infection. Preliminary studies on bovine, sheep and goat sera show that Germiston virus is active in goats and cattle. Very few arbovirus antibodies were found in small mammal sera but the white toothed shrew (*Crocidura* sp.) and *Arvicanthis niloticus* at Ahero had evidence of group B virus activity.

(C.E. Gordon Smith)

REPORT FROM THE DEPARTMENT OF VIROLOGY, UNIVERSITY OF VIENNA, AUSTRIA

Serologic evidence suggests the presence of the tick-borne viruses Tribec and Uukuniemi in Austria. 4% of sera of cattle from Carinthia were found to possess neutralizing antibodies against Tribec virus (473 sera were investigated). In HI tests conducted with sera of nonmigrating birds which were netted in the East of Austria during winter 1971/72, of 69 Blue Tits 5 had antibodies against Uukuniemi virus.

Tick-borne encephalitis (TBE) virus continues to present a major public health problem in Austria. This is clearly indicated by the figures presented in Table 1.

Upon reading the Table it must be considered that the figures are not complete. In particular in Styria, where most of the diagnostic studies are done by another laboratory, our sampling was inadequate. Thus each year the actual number of persons in Austria who contract overt TBE ranges between 400 and 500.

Presently studies are being done by us in order to find out whether or not foci of TBE Virus in nature can be eradicated by either reduction of small mammals or application of the insecticides Malathion^{R} and Gardona^{R} against ticks. Thus far results are encouraging .

A candidate arbovirus was isolated in spring of 1972 from the blood of a migrating bird (robin) just after arrival in Austria.

(Ch. Kunz)

T A B L E 1

Cases of the TBE diagnosed between 1964 and 1971 in the Department of Virology, University of Vienna

Year	Vienna	Lower Austria	Upper Austria	Burgenland	Styria	Carinthia	Total
1964	30	56	9	6	0	0	101
1965	43	67	8	11	0	0	129
1966	34	63	14	15	4	6	136
1967	8	38	7	4	1	2	60
1968	22	30	23	3	0	0	78
1969	26	36	12	10	0	51	135
1970	45	56	27	7	73	112	320
1971	38	87	29	4	26	101	285

REPORT FROM THE VIROLOGICAL DEPARTMENT, RESEARCH INSTITUTE OF EPIDEMIOLOGY AND MICROBIOLOGY, BRATISLAVA, CZECHOSLOVAKIA

Embryonic human skin and muscle in organ cultures were found to be a suitable system for the propagation of viruses of the California group.

Human skin-muscle tissues from 8 - 12 week embryos were cut into fragments measuring approximately 2 cmm and placed onto scratched areas of Petri dishes and plastic plates (Linbro FB-16-24-TC), respectively. Fragments were maintained in Leibowitz (L-15) medium with 10% fetal calf serum. Forty-eight hours after explantation followed inoculation of virus. After 2 hours of adsorption period fragments were washed 5 times with BSS and release of the virus into the medium was observed in one-day intervals.

Infection of single fragments with La Crosse virus (10 times passaged in young or suckling mice) at the multiplicity $5x10^1$ - 10^5 LD_{50} per fragment resulted in appearance of new virus in the culture medium. The titres obtained at 1st - 7th day were dependent on the amount of virus inoculated. The growth curve of the virus at the multiplicity 10^5 LD_{50} per fragment showed that virus multiplied continuously and reached the highest titres ($5x10^6$ LD_{50} per fragment) at 48 hours after inoculation. At the 7th day the titer more as 10^3 LD_{50} was detected in tissue culture fluid.

The organ cultures from human embryo skin-muscle supported also the multiplication of "extraneural" variant of Tahyna virus (10 times passaged in hamster blood). The titres of infectious virus at different days after inoculation were remarkably lower in comparison with those obtained by La Crosse virus.

These preliminary results show that the use of human embryonic organ cultures provide a possible additional method in the study of host-arbovirus relationships.

(I. Schwanzerová)

REPORT FROM THE VIRUS RESEARCH CENTRE, INDIAN COUNCIL FOR MEDICAL RESEARCH, POONA, INDIA

Dengue-like epidemic in Jaipur

An extensive outbreak of febrile illness resembling dengue occurred in Jaipur city, Rajasthan, during the latter half of 1971. Investigations were conducted during October-November 1971 by the Virus Research Centre (VRC). As far as could be ascertained haemorrhagic manifestations were generally absent.

Nineteen virus strains were isolated from 109 acute-phase sera in mice and/or Singh's *A. albopictus* cell line. Of these 4 have been identified as dengue type 2 and 5 as dengue type 1. The other strains have not yet been identified.

Three virus strains were isolated from 217 *Aedes aegypti* mosquitoes, collected at the same time, which were processed in eight pools; two strains have been identified as dengue type 1.

Thirty nine paired serum samples were tested in haemagglutination inhibition and complement fixation tests with JE, WN, DEN-1, DEN-2, DEN-3, KFD, CHIK and SIN antigens. Conversion or significant rises in titre of CF antibodies to group B viruses were detected in 21 cases, of which 11 represented conversions or rise in titre to the dengue viruses.

One hundred and ninety seven 'single' convalescent - phase sera were also tested for HI antibodies to the above-mentioned antigens. A high proportion (approximately 60 per cent) of the sera had titres above 1:640 to JE, DEN-1 and DEN-2 antigens.

The aetiological and serological studies described above have indicated that dengue types 1 and 2 were involved in the present outbreak. JE virus was probably not a factor in the aetiology as studies carried out in other parts of Rajasthan State have shown the presence of the activity of this virus in the area.

(V.S. Padbidri, C.N. Dandawate, M.K. Goverdhan, U.K.M. Bhat, F.M. Rodrigues, L.V. D'Lima and N.P. Gupta)

Isolation of Phlebotomus Fever Virus from Aurangabad District, Maharashtra State, India

During 1970-71, 26,734 sandflies divided into 264 pools were processed for virus isolation. These sandflies were collected indoors, either in human dwellings or cattle sheds. In 1970, 17 strains were isolated from a total of 11,376 sandflies divided into 109 pools. In contrast only three strains of virus were isolated in 1971 from 15,358 sandflies divided into 155 pools.

The details of these virus isolations are given below:

VRC Specimen Nos.	Month & Year of collection	Locality	No. of Sandflies in the pool.
701737-1	June 1970	Gevrai	149 Females
701737-2	"	"	152 "
701742	"	Padegaon	180 "
701738	"	Bidkin	223 "
701736	"	Farola & Chitegaon	227 "
701735	"	Gevrai & Bidkin	150 "
701773-3	"	Nakshatravadi	85 "
701774-1	"	Gevrai	87 "
701775-2	"	Padegaon	106 "
701775-3	"	Padegaon	71 "
701771-2	"	Bidkin	69 "
702292-2	August 1970	Dhorkin	236 "
702279-4	"	Pir Pimpalgaon	66 "
702290-1	"	Bidkin	82 "
702290-2	"	Bidkin	106 "
702289-1	"	Chitegaon	43 "
703252-3	October 1970	Gevrai	173 "
714913-1	August 1971	Aurangabad	166 "
715371-1	October 1971	Padegaon	200 "
715371-2	October 1971	Padegaon	29 "

(M.K. Goverdhan, V. Dhanda and G.B. Modi)

Successful colonization of Trombiculid mites in the laboratory

A simplified technique for the successful colonization of Trombiculid mites has been established. The technique is a modification of the one used by earlier workers (Shirasaka, A. and Sasa, M. 1967. Jap. J. Exp. Med., 37: 129-140; and Nadchatram, M. 1968. J. Med. Ent., 5:465-469). Laboratory reared colonies of three species of mites, namely, Leptotrombidium (Leptotrombidium) deliense (Walch, 1922), Leptotrombidium (L.) akamushi (Brumpt, 1910) and Herpatacarus (Cricarus) longisetosa (Hiregaudar, 1958) are being maintained at ambient room temperature. Each has completed 3 to 4 generations in the laboratory and a large number of larvae are available for experimental purposes.

(S.M. Kulkarni, T. Ramachandra Rao and P.V.M. Mahadev)

REPORT FROM THE ARBOVIRUS RESEARCH UNIT, HOOPER FOUNDATION AND UNIVERSITY OF CALIFORNIA ICMRT, SAN FRANCISCO AND THE UNIVERSITY OF MALAYA, KUALA LUMPUR

Evidence continues to accumulate to support the hypothesis that enzootic dengue cycles exist in Malaysian forests. In addition to the isolation of dengue type 2 virus from the blood of a sentinel monkey, several type-specific dengue serological conversions have been seen in wild and sentinel monkeys. Infection of an experimental monkey with the monkey dengue strain produced a significant viremia. 68% of 585 wild monkeys, captured in forest areas, were serologically positive for group B and 8% had dengue antibody only.

Experimental infection of monkeys with "wild" strains (not passed in laboratory systems) of the four types of dengue resulted in clear-cut serological conversions. On the other hand, the slow loris, a lower primate, failed to elicit any response to inoculation with the four dengue types.

Infection of monkeys with "wild" strains of Zika virus and P8-1542 virus (isolated from the blood of a sentinel monkey in Malaysia) resulted in viremia and development of antibody also. In contrast, "wild" strains of Tembusu, Japanese encephalitis, Lanjan, Keterah (a new ungrouped arbovirus), and P9-314 (a new group B virus) failed to stimulate antibody production and attempts to isolate these viruses from the blood failed. These results are consistent with our survey results to date, which indicate that wild monkeys are commonly infected with dengue and Zika viruses, but not with Tembusu and Japanese encephalitis, the other principal group B viruses in Malaysia.

On the basis of the evidence available so far in these studies, we believe that we can draw some reasonable conclusions:

1) Enzootic dengue infection exists in Malaysia among the several species of monkeys examined and is widespread in the three major types of forest environments (mangrove swamp, fresh-water peat swamp, and primary dipterocarp).

2) Those species of monkeys with wider territories, such as *M. fascicularis*, have a greater chance of acquiring dengue infections, but those species with more limited territories, such as the leaf monkeys, also are significantly involved.

3) No vertebrate species tested so far, other than primates, appear to be significantly involved in a dengue cycle.

4) *Aedes albopictus*, a known vector of dengue, is the only *Stegomyia* species found in significant numbers in all of the forest environments studied at canopy and ground levels and is the most likely suspect forest vector.

Studies of a case of 2 episodes of dengue fever within 5 months in an individual in Malaysia have been completed. This case is of special interest, since it is the shortest interval yet recorded for 2 such episodes in the same individual.

The first episode showed the development of specific neutralizing antibody for dengue type 4 and no crossing with the other 3 dengue types.

In the second episode, dengue type 2 virus was isolated, the preexisting dengue type 4 antibody was boosted to a very high level, and it would have been impossible to determine the infecting virus by serology alone.

P9-621 strain isolated from the acute-phase serum taken in the second episode showed a 50% plaque-reduction titer of 180 with dengue 2 immune monkey serum, while there was no plaque reduction with dengue 1, 3, and 4 immune monkey sera.

Both episodes were reported as typical mild dengue fever.

New viruses presently being studied include:

1) P5-350 from *Mansonia uniformis* -- ungrouped and closely related to Sud Ar 1169 from the same species in the Sudan.

2) P8-1542 from the blood of a sentinel monkey -- ungrouped.

3) Eight strains representing three probably new group B viruses -

a) P9-314 from Aedes (Cancraedes) sp., P70-1861 from Uranotaenia sp., and P70-762 from the blood of a Cynopterus brachyotis bat.

b) P70-1215 from the salivary glands of C. brachyotis, P70-1391 and P70-1486 from the salivary glands of Macroglossus lagochilus bats.

c) P70-1459 from the salivary glands of C. brachyotis and P71-1030 from the salivary glands of Eonycteris spelaea.

Additional strains of Bakau virus have been confirmed: P8-1519 from the blood of a wild Macaca fascicularis in Carey Island Mangrove, P9-365 from Culex sp. no. 1 (an undescribed species) in fresh-water swamp in Pahang, and P70-1577 from Culex sp. no. 3 (another undescribed species) from Carey Island Mangrove. A previous isolate, E-210, was made from Culex sp. no. 1 collected in fresh-water swamp forest in Selangor. With two isolations of Bakau from the same species taken from widely-separated localities and several years apart, there is an indication that this undescribed Culex is an important vector of the virus. A limited serological survey of wild monkeys has revealed the presence of Bakau neutralizing antibody.

P-378 and V-323 viruses, previously reported from Suncus murinus shrews, produce clear, distinct plaques (2 mm in diameter) with irregular edges in Vero cells under agar. Complete cell destruction is observed within the plaques. These agents are not similar to Thottapalayam virus reported from the same animal in India.

Two agents (SP71-14 and SP71-26) have been isolated from cattle ticks collected in the Philippines. Their behavior in suckling mice is not dissimilar to that of Wad Medani virus, with which they will be compared.

From a collection of ticks received from Ethiopia, 4 agents have been isolated in suckling mice. Three of these (SP8-324, SP8-328, and SP71-75), from Ornithodorus capensis ticks taken in white-necked cormorant nests, will be compared with Soldado virus. Similar agents, isolated by Dr. Harald Johnson of the Rockefeller Foundation from ticks taken in the same collections, have proven to be strains of Soldado virus. The fourth agent (SP71-69), from Argas ticks collected in the nest of Abdim's stork, may prove to be a strain of Chlamydia.

Strains of a virus very closely related to Umbre have been isolated from the blood of a sentinel chicken and from at least three pools of _Culex_ mosquitoes. A comparison being made with Turlock and the original strain of Umbre from India show considerable crossing in neutralization tests, but the Malaysian strains appear to be closer to Umbre than to Turlock. In Malaysia, Umbre and Tembusu viruses appear to have similar cycles involving birds and the same _Culex_ vector species.

(A. Rudnick and R.W. Dewey)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY
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The viruses of dengue type 1 (DEN-1) and chikungunya (CHIK) have been regarded as important causes of the hemorrhagic fever prevailing in Southeast Asia during the past few decades. The main purpose of our recent works is to elucidate these two viruses from various angles, and in addition, to compare them as representatives of the groups B and A arboviruses. Routinely in this laboratory the viruses for test are grown in monolayer cultures of BHK-21 cells. The infectivity is determined by counting plaques formed under the methyl cellulose overlay; HA and HI titers are measured by the microtiter techniques, and NT titers either by mouse-intracerebral method or by plaque reduction method.

Purification of virus

A comparatively short and straightforward method of purifying the viruses was designed. The procedures consisted of: precipitation of virus from infectious tissue culture fluid by zinc acetate (0.05 M), filtration through a Sephadex G200 (lower 10 cm)-Sepharose 6B (upper 40 cm) column (2.5 cm diameter) by upward elution, concentration of the filtrate in a collodion bag _in vacuo_, and sucrose density gradient (10-40% w/v) centrifugation (Suc-DGC). Usually the final preparation was 100 to 500 times purer than the original material. Electron microscopic observations revealed particles of 50-55 mμ (DEN-1) and 50-60 mμ (CHIK) diameter mostly.

In the case of CHIK virus, the infectivity and HA activity of the final purified preparation coincided with each other, forming one peak which corresponded to a single visible band. Distinct from it, the DEN-1 virus showed two peaks of HA in Suc-DGC, the rapid-sedimented one of which was infectious.

In comparison, DEN-1 virus derived from mouse brains and purified by protamine sulfate precipitation and Suc-DGC was examined by electron microscopy. Spherical particles, of essentially the same magnitude with that described above, were revealed. Some more details of what was considered as dengue virion were also observed: an envelope (5 mμ in thickness) with fine projections (5-7 mμ in length), fine "doughnut"-like structures on the surface of the virions, etc. No such structures were seen in control samples from normal mice or mice injected intracerebrally with non-infected diluent.

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Virus-specific RNA formed in infected cells

Total RNA was extracted from the infected culture cells (actinomycin-treated) with sodium dodecyl sulfate (SDS)-phenol at 60 C. Extraction of RNA from the purified virions was made by the same method. Assays of the infectious RNA were done by using DEAE-dextran 300-1,000 μg/ml. In the case of CHIK virus, the following results were obtained:

In analyzing the extracted RNA by Suc-DGC and Sepharose column chromatography, three kinds of RNA were obtained, i.e., 42S, 26S, and 20S. The 42S RNA was infectious and regarded as the same as that from purified virions; the 26S was non-infectious and RNA-sensitive; and the 20S was infectious and contained RNase-resistant portions. No such RNA forms were obtained from control uninfected cells either in the presence or absence of actinomycin. Base compositions of these RNA's were almost the same with each other, and with that of purified virions; and each was relatively high in adenine and low in uridine. In continuous labeling or pulse labeling experiments, it was shown that the 20S RNA was labeled first, the 26S secondly, and the 42S last.

For further fractionation of these RNA forms, agarose and benzoylated diethylaminoethyl cellulose (BD cellulose) chromatography techniques were applied. By the agarose chromatography, double-stranded 20S RNA was separated from single-stranded 42S RNA and 26S RNA which were also fractionated from each other on the same column. The 20S RNA from the agarose fractions was separated into two components by the BD cellulose chromatography eluted with a linear NaCl gradient plus urea. These two components appeared to have the nature of "replicative form" ("RF") RNA and "replicative intermediate" ("RI") form RNA, respectively.

As to selected members of the group B arboviruses, on the other hand, it has been reported that two kinds of RNA (42-45S and 20S) were detected but 26S RNA was not detectable or of very small quantity (as for DEN-2, reported by Stollar, V., Schlesinger, R.W. and Stevens, T.M., in Virology, 33, 650, 1967; and for JE, reported by Kumano-Fukui, K., of this laboratory, in Abstracts, 19th Annual Meeting, Society of Japanese Virologists, October 1971). Problems of whether certain quantitative or qualitative difference(s) may exist between the groups A and B arboviruses in the RNA replication processes are needed to be further investigated.

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Effect of low salt concentrations of culture media upon production of virus

By lowering NaCl concentrations of culture media, release of DEN-1 and CHIK viruses from BHK-21 cells into fluid phase was inhibited. This inhibitory effect was reversible, and the inhibition and its recovery were repeated practically indefinitely by changing the media from normal to low NaCl concentrations, and vice versa. The ionic strength rather than osmolarity of media was shown to be involved in the phenomena. Electron microscopic observations indicated that the process of "viral budding" was influenced by the low ionic strength, and this was particularly evident in the case of CHIK virus.

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Immunogenicity of purified virus

The virus samples used for test were highly purified by the methods described in the previous sections; and amounts of contaminating components from the host cells were thought to be practically minimum.

The purified, active DEN-1 virus (Mochizuki strain), injected sub- or intracutaneously into Japanese monkeys (Macaca fuscata), induced production of specific anti-DEN HI and NT antibodies which were of essentially the same magnitude with those produced by the crude virus. No abnormal signs were noted in the inoculated animals. The immunogenic effect decreased markedly when the virus of the same dose was inactivated, for instance, by treatment with ether.

The purified CHIK virus (African strain) was completely inactivated by UV irradiation within several minutes under the conditions studied. The inactivating effect was apparently of the first order reaction. Exposure of the virus to 0.05% formalin at 4 C for 7 weeks brought about complete loss of its plaque-infectivity, although the same virus treated with 0.025% formalin for 17 weeks was shown to be still infective.

The UV or formalin-inactivated CHIK virus, injected subcutaneously into Japanese monkeys, induced production of anti-CHIK NT antibodies of significantly high titers. So far as the antibody titers were concerned (at least under the conditions studied), the "UV vaccine" appeared to be superior to the "formalinized vaccine."

In relation to the immunogenicity, two strains of CHIK virus were compared: one, African strain isolated in Africa, and the other, BaH 306 strain isolated in Southeast Asia (kindly supplied by Drs. A. Igarashi and K. Fukai, of Osaka University, and their Japanese and Thai colleagues). Using homologous and heterologous antisera from mice inoculated intramuscularly with each of the viruses, kinetic neutralization curves (expressed by the rates of plaque reduction) were examined. Little difference was noted between the two strains, at least under the conditions studied. The results suggested that "anti-CHIK vaccine" made of one strain (African strain, in the case of our monkey immunization study as stated above) may be sufficiently effective against the virus of the other strain (BaH306, of Asian origin, in this case).

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(S. Hotta)

REPORT FROM THE DEPARTMENT OF PREVENTIVE MEDICINE,
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1. Growth of Japanese encephalitis virus in established lines of mosquito cells.

Growth of Japanese encephalitis virus was studied in three kinds of mosquito cell lines, i.e. Singh's *Aedes albopictus* (SA), Singh's *Aedes aegypti* (SE) and Peleg's *Aedes aegypti* (PE) cells. These cells were grown as monolayers at 28°C with 1:1 mixture of Mitsuhashi-Maramorsch's medium (MM) and Eagle's minimal essential medium (MEM) supplemented with 10% calf serum. After virus inoculation and adsorption for 2 hr, unadsorbed virus was removed by two washings with PBS. Virus phase medium was 1:1 mixture of MM and MEM supplemented with 2% calf serum. Virus growth at 28°C was checked every day until 5th day after inoculation, by taking out a portion of virus phase medium and assaying infectivity by plaque formation on BHK21 cells. Results using several different passage histories are summarized in Table 1. Virus growth at 37°C in BHK21 cells was used as a control.

The virus yield was highest in SA, followed by in PE, then in SE cells. Viruses with passage in MK or BHK21 cells seem to grow to a higher titer in SA cells than those with suckling mouse brain passage only, although the latter can grow well in BHK21 cells at 37°C.

JaOH-0566 strain with passage in MK and BHK21 cells was studied in SA cells thereafter. Growth curve of the virus at 28°C with input moi of 10 showed that virus titer increased from 6 hr until 36 hr after infection, remaining at a plateau for several days. Active growth of the virus was observed between 22 and 37°C, although the growth rate at 22°C was much slower. Temperatures below 15°C or above 40°C were not suitable to detect positive growth of the virus.

2. Purification of Japanese encephalitis virus grown in Singh's *Aedes albopictus* cells by polyethylene-glycol precipitation.

In order to test the basic characteristics of the virus grown in Singh's *Aedes albopictus* (SA) cells, partial purification of the virus was carried out as shown in Fig. 1. Seed virus was JaOH-0566 strain with passages in MK and BHK21 cells. Virus grown in BHK21 cells was used as a control. Average Of 4 experiments with SA cells and that of 5 experiments with BHK21 cells are summarized in Table 2.

By polyethylene glycol (PEG) precipitation, recovery of hemagglutinating activity (HA) was complete and that of infectivity (PFU) was 40-80% with residual protein of 3-4%, so that the purification factor was 30-40 for HA and 10-30 for PFU. When PEG precipitated specimen was centrifuged onto cushion of 50% and 20% sucrose, visible band was observed at the interphase of two sucrose layers. In this band almost 100% of HA and 5% of PFU was recovered with residual protein of 0.1%, so that purification factor was more than 800 for HA and 30-80 for PFU. Both the viruses grown in SA and BHK21 cells showed the same buoyant density of 1.224 g/cc in potassium tartrate density gradient centrifugation. For both viruses, optimal pH of HA was 6.2-6.4. No significant difference was observed between these two viruses by HI, NT using plaque reduction or heat inactivation.

(A. Igarashi)

Table 1. Growth of Japanese encephalitis virus in mosquito cells.

Seed virus inoculated			Maximum titer*(days after inoculation)			
Strain	Passage history	Titer*	SA	SE	PE	BHK21
Nakayama	$MB^{26}SMB^{6}$	7.6	5.8(2)	4.5(3)	5.0(3)	7.6(1)
	$MB^{26}SMB^{6}BHK^{8}$	6.3	7.6(2)	5.6(4)	5.6(4)	7.0(2)
JaGAr-01	SMB^{9}	7.3	6.7(4)	4.6(5)	6.7(2)	7.4(1)
	$SMB^{9}BHK^{11}$	7.7	7.4(3)	5.5(2)	6.4(3)	7.2(1)
JaOH-0566	SMB^{2}	9.0	6.9(4)	4.7(4)	5.4(1)	7.5(1)
	$SMB^{2}MK^{62}$	5.3	8.3(3)	3.5(5)	7.4(3)	7.9(1)
	$SMB^{2}MK^{62}BHK^{14}$	7.1	9.1(4)	5.8(4)	7.7(3)	8.7(2)
	$SMB^{2}BHK^{8}$	6.4	7.0(3)	4.0(2)	5.6(2)	7.0(2)

* log PFU/ml

Passage in BHK21 cells includes three times of plaque isolations.

Growth in BHK 21 cells was at 37°C, and that in SA,SE and PE cells was at 28°C

MB: adult mouse brain, SMB: suckling mouse brain.

Figure 1. Purification of Japanese encephalitis virus

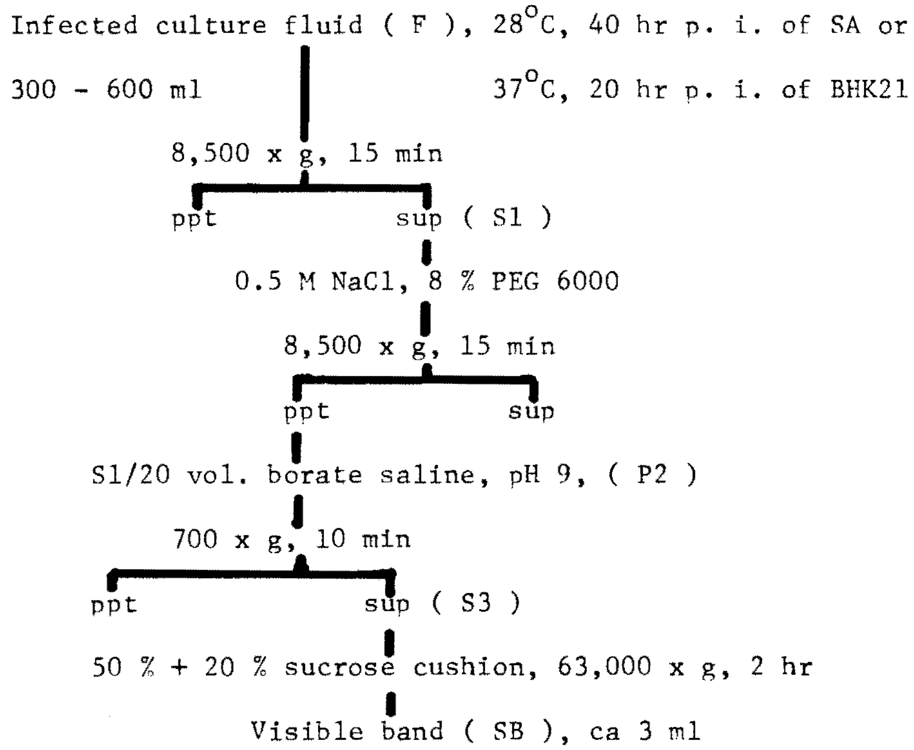

Table 2. Purification of Japanese encephalitis virus.

Host cell	Sample	Protein mg/ml	Protein recovery	HA log	HA recovery	HA PF*	PFU log	PFU recovery	PFU PF*
SA	F	3.43	100	1.58	100	1	8.76	100	1
	S1	3.32	96	1.51	88	0.9	8.58	70	0.8
	P2	2.03	4	2.58	91	19	9.59	40	11
	S3	2.02	4	2.58	133	35	9.64	41	12
	SB	0.49	0.1	3.77	152	1116	9.69	5	80
BHK	F	2.10	100	0.96	100	1	8.35	100	1
	S1	1.76	84	0.90	90	1	8.25	86	1
	P2	1.70	4	1.56	83	22	9.51	78	24
	S3	1.28	3	2.19	127	44	9.50	75	27
	SB	0.44	0.1	2.86	75	817	8.72	6	30

* Purification factor, i. e. Ratio of HA/protein or PFU/protein of a given sample to that of the starting material.

REPORT FROM THE NATIONAL INSTITUTE OF HEALTH,
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Three sublines of Japanese encephalitis virus derived from a Nakayama strain, based on a Tw 80^{+} marker

The Nakayama strain of JE virus (the prototype strain) was isolated by Kasahara et al in 1935 from spinal fluid of a patient showing the typical encephalitis symptoms. After World War II the strain was obtained again from the U.S.A. and maintained at two different laboratories in Japan, the National Institute of Health (NIH) and the National Veterinary Assay Laboratory (Yakken) by intracerebral passage in 4- and 3-week-old mice, respectively. The fact that the formalin-killed vaccine prepared with Nakayama-NIH was rejected for animal use by the national assay potency test in which immunized mice were challenged with the Nakayama-Yakken, despite similar origin of the NIH and Yakken strains, focused our attention on the variability of JE virus. The Nakayama-RFVL strain, which had been kept at the Rockefeller Foundation Laboratory, was then obtained through the courtesy of Dr. Theiler, and comparative studies of the antigenic characteristics and immunizing potential of the three strains performed.

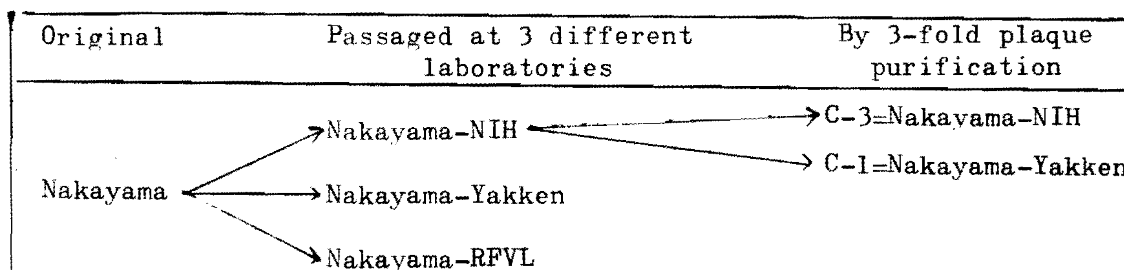
As for variation of virion density among the 3 sublines, C_sCl density gradient equilibrium centrifugation revealed that each has its own buoyant density (Nishimura et al, 1968); and immunodiffusion studies showed, besides a common line, different patterns of precipitation between the anti-Nakayama-NIH serum and each top fraction (ρ = less than 1.26) and high density fraction (ρ = higher than 1.30). Different patterns were also noted between anti-JaGAr-01 (a JE virus strain) serum and those antigens (Kitaoka and Shimizu, 1972).

The EID_{50} value obtained by challenge with Nakayama-NIH and -Yakken viruses of mice immunized with vaccine prepared with each of the 3 sublines was not high against the heterologous one, except for Nakayama-RFVL.

The optimal pH of HA activity of the 3 sublines (NIH, Yakken and RFVL) is known to be 6.4, 6.8 and 6.8, respectively. However, the latest studies by authors (1972) concerning the effect of 1.0% Tween 80 on those 3 sublines shows that the HA activity of Nakayama-NIH at pH 6.4 was reduced markedly by Tween 80 treatment but was actually enhanced at pH 6.8. Similar Tween 80 treatment had essentially no effect on the pH optimum of Nakayama-RFVL or -Yakken.

The question arose as to how the 3 sublines originating from a single Nakayama strain could have developed such different biological and immunogenic characteristics. It is likely, however, that variations or mutations of the Nakayama strain can occur spontaneously under different circumstances during long-term passages, as has been seen in other viruses. In attempt to determine whether or not all progenies of Nakayama-NIH have the same immunogenic characteristics, plaque purification was carried out in chick embryo cell monolayer culture in Petri dishes inoculated with the Nakayama-NIH (suckling mouse brain passage). Two strains, C-1 and C-3, were isolated by 3-fold plaque purification. The C-1 strain was passed 3 times in adult mouse brains and then in suckling mice. Irrespective of whether the C-1 antigen was prepared from infected suckling mouse brain or from fluid of infected chick embryo or baby hamster kidney-21 cell cultures, the optimal pH 6.4 of its HA activity was not shifted by Tween 80, and in this respect was as seen in Nakayama-Yakken. On the contrary, the C-3 antigen from suckling mice or cell culture fluid gave a shift of the optimal pH due to Tween 80 similar to that shown by its parent strain, Nakayama-NIH. It seems highly possible that the three sublines (Nakayama-NIH, -Yakken and -RFVL) may be genetically interchangeable and that progeny such as C-1 strain would be missed by the ordinary passage of Nakayama-NIH; plaque purification permitted it to be detected (Table 1).

Table 1



It is possible that each Nakayama subline has the potential to change into another Nakayama type under certain conditions, but that the new Nakayama type could be masked due to the parent strain growing predominantly. Therefore plaque purification of the strain should be undertaken prior to undertaking characterization studies of variants or mutants. The shift of HA activity induced by Tween 80 treatment (Shimizu and Kitaoka, 1972) is apparently a stable reaction which might be used as a marker (Tw 80^{+}) for grouping.

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(M. Kitaoka and A. Shimizu)

Pattern of immune response in mice following double-infections with Japanese encephalitis virus and Getah or Apoi virus

There are two known mosquito-borne viruses in Japan, Japanese encephalitis (JE) and Getah. The former is well-known as a disease producer and is distributed widely throughout Japan except for the northern part of Hokkaido; the latter has been isolated incidentally from mosquitoes in the limited areas, accompanied by only subclinical infections in a low percentage of animals and man. Apoi virus, on the other hand, is presumably transmitted by ticks. It was isolated first from rodents trapped at the Apoi area and next in the Teshio Nakagawa area in Hokkaido. HI antibody to Apoi has been found in domestic animals, especially horses, and very seldom in man living in Hokkaido only. An attempt was made to determine the pattern of immune response in mice experimentally infected with two strains, JE virus and Getah or Apoi virus, one after the other.

I. HI-antibody pattern in mice sequentially infected with JE and Getah viruses.

Strains: JE virus, the Nakayama-NIH strain; Getah, the JaGAr-185-65 strain isolated from mosquitoes in Itakura, Gumma Prefecture.
Mice: Four groups of 5-week-old mice, DD strain, 10 mice per group.

Each of the first and second groups of mice were inoculated intraperitoneally with 0.5 ml of $10^6 LD_{50}$ of Nakayama-NIH strain. Conversion to HI-antibody positive was confirmed on serums collected by partial bleeding of individual mice 2 weeks after the initial infection. Sixteen days after initial infection the first group was reinoculated intraperitoneally with 0.5 ml of $10^6 LD_{50}$ of JaGAr-185-65 strain and the second one with Nakayama-NIH strain. On this same day the third and fourth groups were infected with JaGAr-185-65 and Nakayama-NIH strains, respectively. Eleven days later all the mice of each group were sacrificed by bleeding for HI-tests.

No cross-reaction, interference or enhancement was noted in HI antibody patterns obtained in the doubly infected mice, probably because JE and Getah are quite unrelated, the former belonging to group B and the latter to group A.

II. Enhanced HI-antibody response in mice sequentially infected with JE virus and Apoi virus

Strains: JE virus, the JaTH-160 strain isolated from autopsied brain of a JE patient; Apoi virus, the prototype strain isolated from rodents trapped at the Apoi area, Hokkaido.
Mice: Six groups of 5-week-old mice, DD strain, 10 mice per group.

Six groups of mice (1, 2, 3, 4, 5 and 6) were each inoculated intraperitoneally with a dose as low as $10^3 LD_{50}$ of Apoi, JaTH-160, JaTH-160, JaTH-160, Apoi and Apoi, respectively. All the mice of each group were partially bled for the confirmation of HI-antibody being still negative on day 10 after the first inoculation. Four groups (3, 4, 5 and 6) were each reinoculated on day 14 with a similar small dose of Apoi, JaTH-160, JaTH-160 and Apoi, respectively. Individual serum of mice for HI-test was collected by heart puncture 10 days later.

No HI-antibody was found in 2 groups (1 and 2) 24 days after single inoculation of a small dose of Apoi and JaTH-160. Mice of 2 groups (4 and 6), inoculated twice with the same strain (JaTH-160 or Apoi) gave the HI-antibody positive for homologous antigen but a little bit or none for the heterologous one. However, the other 2 groups (3 and 5) which were inoculated first with JaTH-160 or Apoi and then with Apoi or JaTH-160 were HI-antibody positive for both antigens. In other words, HI-antibody responses for both antigens followed sequential infections with the JaTH-160 and Apoi strains.

Both JE and Apoi belong to group B arboviruses. Accordingly a cross-reaction between both strains in the HI test is to be expected.

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(M. Kitaoka and A. Shimizu)

HI antibody survey for Japanese encephalitis virus in serums of rodents trapped at selected areas in Japan

Japanese encephalitis (JE) is a mosquito-borne disease; however the virus can be experimentally transmitted by the oral route in mice and monkeys (unpublished). In spite of the fact that herons, horses, swine, etc. are known to serve as amplifiers of JE virus for mosquitoes, and that swine, bats, birds and cold-blooded animals have been reported to carry JE virus during the non-epidemic season, no generally agreed conclusion about the virus reservoir for mosquitoes has yet been reported. Another hypothesis which should be investigated is that the virus might be maintained by a vertebrate host-ectoparasite or endo-parasite cycle. At the moment it can still be said that the whereabouts of the virus during the inter-epidemic season is still under veil.

Since the virus was found to be transmitted by mosquitoes (Mitamura et al, 1936) our attention was focused on the ecology of JE virus in nature, and any animals and birds, even cold-blooded animals which could be bitten by vector mosquitoes were thought to take a certain part of the ecology of JE virus in nature.

In 1936 the study of susceptibility of rodents to JE virus revealed that after the intracerebral inoculation with JE virus (Kalinina strain), Apodemus speciosus speciosus remained apparently well while Microtus montebelli died, showing the typical encephalitis symptoms; and the virus could be passed serially from brain to brain. Subsequently, isolation of virus from house rats (Rattus norvegicus) during the epidemic season of 1937 indicated that these animals could be incidentally infected. In order to know further how much rodents take part of the ecology of JE virus in nature, HI-antibody for JE virus was surveyed on serums of various rodents trapped at selected areas in Japan. Results so far obtained are briefly summarized as follows.

Hokkaido: Seven rodents, Clethrionomys rufocanus bedfordiae, were trapped at the Apoi area in June, 1966. Out of them 2 were HI-antibody positive for Apoi antigen but not for JaGAr-01 antigen. At the same area 45 rodents of the same species were trapped in 1968. Three of them were found HI-antibody positive for both JaGAr-01 and Apoi antigens, probably due to a cross-reaction between these two group B arboviruses. One other rodent was positive for Apoi antigen but not for JaGAr-01 antigen.

Niigata: Twenty-four rodents, Microtus montebelli, were trapped at the Gosen area in 1968. Two of them were HI-antibody positive for JaGAr-01 antigen.

Oita: Twenty-three rodents, Apodemus speciosus speciosus, were trapped at the Kunisaki area in 1968. Two of them were HI-antibody positive for JaGAr-01 antigen.

These findings indicate that approximately 10% of the rodents trapped at the Apoi area, an area presumed to be endemic for Apoi virus, had probably been infected with Apoi virus but not with JE virus; and that approximately 10% of the rodents trapped at Niigata and Oita, areas endemic for JE virus, had presumably been infected with JE virus. The Niigata and Oita serums were not tested against the Apoi antigen.

Spleen-liver suspensions from more than 7000 rodents trapped by the study group on scrub typhus all over Japan since 1954 were found negative for JE virus upon inoculation into mice by the peritoneal route. Only Apoi virus was isolated, restricted to rodents from the Teshio Nakagawa and the Apoi area, Hokkaido. It appears, therefore, that the positive JE HI antibody response detected in rodents were the result of incidental bites of infected mosquitoes, with the rodents playing no important role in perpetuation of JE

virus. The positive isolation of Apoi virus from rodents, however, suggests that rodents may be a reservoir for Apoi. Further investigations are in progress.

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(M. Kitaoka and T. Ogata)

REPORT FROM THE DEPARTMENT OF MEDICAL MICROBIOLOGY, INSTITUTE OF PUBLIC HEALTH, UNIVERSITY OF THE PHILIPPINES, MANILA

Five cases of human dengue virus infections with shock and hemorrhagic manifestations were studied for their 7S and 19S Ig responses. The Ig classes were separated by sucrose density gradient centrifugation, and identified by either sensitivity or resistance of their HI antibody activity to 2-ME. The kinetics of their biosyntheses showed a simultaneous appearance of 7S and 19S Ig on the 5th day of illness, both attained peak titers between the 10th and 14th day, and tended to decline after the 2nd week. The 19S Ig disappeared after a month, while 7S Ig persisted at a fairly high level. The 19S Ig was produced in primary and secondary infections.

The diagnostic value of 19S Ig determination in cases of human dengue virus infections was underscored.

(V.F. Chan)

REPORT FROM THE PACIFIC RESEARCH SECTION, NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES, HONOLULU, HAWAII

Because of the relative insensitivity of both mice and cell cultures to dengue viruses, an attempt was made to use mosquitoes of a susceptible species (Aedes albopictus) as amplifying hosts, Employing prototype strains of each of the four dengue serotypes which had never been passed in mice or cell cultures, it was found that individual mosquitoes inoculated intrathoracically consistently contained between 10^5 to 10^7 infectious doses of virus (as measured in mosquitoes) after a suitable incubation period. Furthermore, essentially equal titers were found in male and female insects. The latter observation has made it possible to use the technique on a large scale without the inherent danger of having large numbers of infected female mosquitoes in the laboratory. Mosquitoes were tested for the presence or absence of virus by assay in a LLC-MK_2 cell plaque system.

Comparative titrations of human sera containing dengue virus and infected mosquitoes have shown that the intrathoracic inoculation technique increased dengue isolation sensitivity at least 100-fold as compared with direct plaque assay in LLC-MK_2 cells. Differences as high as 100,000-fold or greater were observed in studying certain dengue strains which grow poorly in LLC-MK_2 cells. Sera collected from persons with dengue during the first three days of their illness consistently yielded titers of 10^5 to 10^7 infectious doses of virus per ml. when assayed by the mosquito inoculation technique.

In an effort to further improve the usefulness of the technique, an attempt was made to demonstrate hemagglutinin or complement fixing (CF) antigen in infected insects. Hemagglutinin was not found, but it was possible to demonstrate CF antigen. CF antigen could not be consistently demonstrated in single infected insects, but pools of 5 mosquitoes were satisfactory. Large numbers of dengue virus strains from recent epidemics in the South Pacific were isolated and typed without recourse to either mice or cell cultures by using CF antigens prepared in mosquitoes. The technique might thus prove useful to laboratories without cell culture facilities and, obviously, might prove of value in the study of other mosquito-borne viruses.

(Leon Rosen)

ANNOUNCEMENT FROM THE EDITOR

Contributions for the next issue (Number 24) of the Arthropod-borne Virus Information Exchange will be due March 1, 1973.

Please be reminded that a successful Info Exchange depends upon your interest, and the best way to express your interest is to contribute brief resumes of your current progress. A serious attempt is being made by your editor to place the Info Exchange back on a regular twice-a-year schedule.

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