ARBOVIRAL DISEASE SECTION
ECOLOGICAL INVESTIGATIONS PROGRAM
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ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

Number Eighteen December 1968

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Reports from (continued):

This Arthropod-borne Virus Information Exchange is issued by a Sub-Committee on the Information Exchange of the American Committee on Arthropod-borne Viruses.

REPORT FROM THE CHAIRMAN OF THE AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

Arbovirologists attending the open meeting of the American Committee on Arthropod-borne Viruses at the Sheraton-Biltmore Hotel, Atlanta, Georgia, at 9:00 a.m. on Wednesday 31 October 1968, heard the following reports:

Subcommittee on Information Exchange

Dr. T. O. Berge, who was appointed to this Subcommittee November 1, 1967, began direction of the Catalogue and abstract distribution service on July 1, 1968 following receipt of a new contract from the National Institutes of Health. He reported that Catalogue operations are now running smoothly.

Subcommittee on Birds and Other Vertebrates in Relation to Arboviruses

This Subcommittee is now dissolved following the conduct of a training program on the methodology and philosophy of studying vertebrates in public health research on arboviruses. This two-day course, which was held at Laurel, Maryland on 18-19 March, 1968, was organized jointly by Dr. R. W. Dickerman, Chairman of this Subcommittee, in association with the Bird Population Station and the Patuxent Wildlife Center of the U.S. Fish and Wildlife Service.

Subcommittee on Immunological Relationships among Catalogued Arboviruses (S.I.R.A.C.A.)

Dr. J. Casals, Subcommittee Chairman, reported discussions regarding nomenclature of arbovirus groups and common essential attributes of arboviruses, at the International Congress of Virology at Helsinki, Finland, July, 1968, the International Congresses of Tropical Medicine at Tehran, Iran, September, 1968, and Sir Christopher Andrews in London, England. The A.C.A.V. concept, that Arbovirus is a functional term embracing all agents which are transmitted in nature by arthropods in which the agents undergo biological cycles, will be embodied in any system of virus nomenclature adopted by the International Committee on Virus Nomenclature. However, a definition of the Arbovirus group based solely on the presence of ribonucleic acid in enveloped

virions with cubic symmetry and particle diameters 20 to 60 nm, would include viruses which obviously are not transmitted by arthropods such as rubella and LDH, but arthropod-transmitted agents such as vesicular stomatitis with bullet-like morphology would be excluded.

Current guidelines for the nomenclature of arbovirus groups should show two syllables preceding virus, and each serological group should be named by the typical member of the group which is studied most extensively. For example:

Sindbivirus = group A (Sindbis)
Flavivirus = group B (flavi = yellow fever)
Marivirus = group C (Marituba)
Bunyvirus = Bunyamwera group

Difficulties have arisen regarding the terminology of arboviruses named for syndromes (e.g. western equine encephalomyelitis) for which several abbreviations are used in publications. Both authors and journal editors desired some guidance regarding uniform nomenclature and recognized abbreviations. It was resolved that the A.C.A.V. Executive, in consultation with journal editors, would devise guidelines and formulate specific suggestions regarding certain arboviruses, for distribution to the membership and possible publication in journals.

Dr. W. McD. Hammon proposed votes of thanks to Drs. Berge, Casals, Nathanson and Taylor, for meritorious services on their respective Subcommittees.

Nominating Committee

Dr. W. F. Scherer, Chairman, nominated Dr. Philip K. Russell, Chief of Virology at the Walter Reed Army Institute of Research, Washington, D.C. as a member of the A.C.A.V. Executive in succession to Dr. E. L. Buescher who rotated off. Dr. Russell was elected unanimously to serve a term of six years.

Abbott Laboratories Richard Moreland Taylor Award

An engraved medal was presented to the initial recipient, Dr. R. M. Taylor, by the A.C.A.V. Chairman. This medal recently became available, almost two years after the citation and presentation of a plaster replica was made to Dr. Taylor in Puerto Rico during 1966.

Dr. W. G. Downs delivered the citation and presented an engraved medal to Dr. J. Casals who was named as the 1968 recipient of the Richard Moreland Taylor Award for achievement in arbovirology.

There followed a Symposium on Current Developments in Arbovirology, presented by scientific staff members of the National Communicable Disease Center, Atlanta, Georgia.

Electron Microscopy of Arboviruses

Dr. F. Murphy showed a series of electron micrographs of thin sections of tissues infected with arboviruses, and of preparations stained negatively with phosphotungstic acid. Arboviruses in groups A and B showed enveloped virions with diameters 50 and 40 nm, which separated from host tissues by budding. Bunyamwera virions showed diameters of 98 nm. Colorado tick fever virions were 75 nm diameter which grew by maturation. Virions of the Tacaribe group showed helical symmetry reminiscent of myxoviruses in phosphotungstic acid preparations. Vesicular stomatitis and the green monkey virus (Marburg 1967) revealed bullet-like morphology (Rhabdoviruses). Nodamura virus showed crystals reminiscent of coxsackieviruses in infected mouse tissues.

Collection and Processing of Mosquitoes

Dr. V. F. Newhouse gave a pictorial demonstration of the use of the CDC light trap in the field, followed by procedures for identification of mosquitoes and isolation of arboviruses by injection of suckling mice with ground-up suspensions.

Collection and Processing of Vertebrates

Dr. Rex Lord demonstrated pictorially the use of (i) Sherman traps baited simultaneously with both apples (for herbivorous rodents) and peanut butter (for seminivorous or carnivorous rodents); (ii) National traps for collection of larger mammals such as groundhogs and rabbits; (iii) mist nets for collection of birds. He also discussed field bleeding procedures.

Crimean Hemorrhagic Fever Virus

Dr. J. Casals reported the isolation of several strains of arboviruses

from the blood of patients with Crimean hemorrhagic fever and pools of Hyalomma marginatum ticks collected during 1967 in the Ukraine and Crimea by inoculation of suckling mice. On complement fixation tests, these agents showed cross reactions only with Congo virus. Paired sera collected from Russian patients with Crimean hemorrhagic fever virus during 1962 and 1963 showed rising titers of complement fixing antibodies both to the Crimean agent and Congo virus, but not to other arboviruses.

Human Case of Venezuelan Equine Encephalitis in Florida

Dr. J. O. Bond described the clinical history and conclusive serologic findings of a case of VE encephalitis in a human resident of the southern area of Florida during summer 1968. This appears to be the first virologically documented human case of VEE infection in the Continental United States.

Next Open Meeting

The next open meeting of the American Committee on Arthropod-borne Viruses will be held in conjunction with the 18th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Shoreham Hotel, Washington, D. C., 3-7 November 1969.

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*S.I.R.A.C.A.: Subcommittee on Immunological
Relationships among Catalogued Arboviruses

REPORT FROM THE FLORIDA STATE BOARD OF HEALTH, JACKSONVILLE, FLORIDA

Venezuelan equine encephalitis was diagnosed in a 53-year-old Negro female from Homestead, Dade County, Florida in October, 1968. On September 2, 1968, the patient became ill with headache and respiratory distress while fishing in a nearby canal. Over the next two weeks, malaise increased and was associated with chest pains, shortness of breath and headache. On September 15, she was seen by a local physician who suspected "flu" and prescribed analgesics. Because of increasing severity of symptoms, she was admitted to Jackson Memorial Hospital, Miami, Florida, on September 19, 1968.

Aseptic meningitis was suspected. A chest film was considered normal, blood pressure was 170/100, temperature 102° F and she complained of severe headache and chest pain. A spinal tap revealed 900 WBC with 65% mononuclear cells. A peripheral WBC was 8,300 with essentially normal differential. Blood sera collected on September 19, 23, and 27 were submitted by N. Joel Ehrenkranz, M.D., Professor of Medicine, University of Miami to the Florida State Board of Health, Virus Laboratory, Jacksonville, Florida. HI, CF, and SN tests provided serologic confirmation of acute VEE infection. No antibody was demonstrated to WEE, SLE, EEE, mumps, Herpes simplex, Influenza A & B, Parainfluenza 1 & 3, Adenovirus Mycoplasma pneumoniae, and Leptospirosis. No viral isolation attempts were performed. This is the first naturally acquired human case of VEE infection reported in the United States. The patient is making a satisfactory but gradual recovery.

Currently, coordinated investigations into the nature and extent of VEE virus activity in the human, animal and mosquito populations of the Dade County area are underway. Staffs of the Dade County Department of Public Health, Florida State Board of Health, National Communicable Disease Center, and University of Miami School of Medicine are cooperating in these efforts.

(Nathan J. Schneider and Elsie E. Buff)

REPORT FROM THE ANIMAL DISEASE AND PARASITE RESEARCH DIVISION, PLUM ISLAND ANIMAL DISEASE LABORATORY, GREENPORT, LONG ISLAND, NEW YORK

Vesicular Diseases

A vesicular disease sample was received on Plum Island on August 11, 1967. The specimen consisted of swine serum samples and vesicular tissue from pigs located in Catahoula County, Louisiana. Upon clinical examination, the USDA Animal Health Division diagnostician suspected a vesicular disease. Serum and tissue samples were sent here and examined by complement-fixation tests. The serum sample was determined to have antibodies against vesicular stomatitis virus (VSV) type New Jersey, and the tissue sample was determined to contain vesicular stomatitis (VS) viral antigen, type New Jersey. We also inoculated the vesicular tissue into swine, tissue cultures, embryonating chicken eggs, pigs, cattle, and a pony. All attempts at demonstrating infective virus failed. Presumably the fixation which we obtained with the tissue specimen was due to inactivated virus in the tissue sample. Serum from the experimental animals did not contain complement-fixing antibodies.

Additional serum samples were obtained from the farm; (there was no further evidence of a vesicular disease on the farm) and complement-fixing antibodies against New Jersey type VSV were also demonstrated in these samples.

This was the only recorded incidence of VS in the U.S. in 1967.

(J.J. Callis)

REPORT FROM THE NORTH CAROLINA STATE UNIVERSITY, RALEIGH, NORTH CAROLINA

A study of birds in residential areas of Raleigh has been initiated using local funds. The objectives are to determine the late winter and early spring movements within a section of suburban housing consisting of three parts; 1) rather dense human population, 2) medium human population, 3) sparse population. The objectives will be to determine the interaction between the migrating birds that arrive late in the winter and the winter residents. The procedures will be essentially the capture of birds in

mist nets, banding and recapture. At a later time the collection of bloods will be started.

(David E. Davis)

REPORT FROM THE ARBOVIRUS INFECTIONS UNIT, NATIONAL COMMUNICABLE DISEASE CENTER, ATLANTA, GEORGIA

I. St. Louis Encephalitis (SLE) Surveillance Studies

A. Memphis, Tennessee

Memphis experienced a small SLE outbreak in 1964. Since then, the National Communicable Disease Center (NCDC) has been cooperating with the Memphis-Shelby County Health Department by testing mosquitoes, primarily Culex quinquefasciatus-pipiens, to detect rapidly the presence of SLE and provide information for control purposes. One SLE virus isolation was made in September, 1967. In 1968, 4,063 mosquitoes were tested from July through September, Table 1. Twelve Flanders virus isolations were made from the 3,319 Culex quinquefasciatus-pipiens tested. One additional isolation of Flanders virus was made from Culex restuans, Table 1. No SLE virus was isolated.

B. Corpus Christi, Texas

Surveillance for SLE was continued in Corpus Christi from May through September, 1968. Peri-domestic mosquitoes and birds were collected by Corpus Christi Health Department personnel and sent to NCDC for testing. The results of mosquito testing are presented in Table 2. Five Flanders viruses were isolated from the 2,337 Culex quinquefasciatus tested. No SLE virus was found.

Between May and September, 298 immature house sparrows were bled and their sera tested for HI antibody to SLE, Western encephalitis (WE), Eastern encephalitis (EE), and Venezuelan encephalitis (VE). Prior to the end of July, from 2 to 9% of the sera tested were positive for SLE with HI titers of 1:20 or greater, Table 3. Since that time no SLE antibody has been detected. No human cases were detected in Nueces County this year. Either early SLE activity in the immature house sparrows, which subsequently diminished, or maternal antibodies might account for the SLE antibodies detected. In the future, late July and early August samples may provide the clue to early detection of

Table 1

Summary of Identifications of Mosquitoes Collected during 1968 from Memphis, Tennessee, during Arbovirus Surveillance Studies

Mosquito Species	7/15-18/68	8/19-21/68	9/9-20/68	Total
Aedes vexans	8*(2)**		25 (4)	33 (6)
A. species	1 (1)			1 (1)
Anopheles crucians	1 (1)			1 (1)
An. punctipennis	3 (3)	1 (1)	2 (1)	6 (5)
An. quadrimaculatus	53 (5)	92 (4)	127 (4)	272 (13)
Culex quin.-pipiens	1614 (34)	326 (9)	1379 (29)	3319 (72)
C. restuans	26 (5)		26 (4)	52 (9)
C. salinarius	8 (3)		1 (1)	9 (4)
C. tarsalis			6 (3)	6 (3)
C. territans	10 (4)		27 (4)	37 (8)
C. (Melanoconion) sp.	45 (5)	12 (3)	251 (7)	308 (15)
C. species	12 (2)			12 (2)
Psorophora confinnis	1 (1)	4 (2)	1 (1)	6 (4)
Uranotaenia sapphirina	1 (1)			1 (1)
Total	1783 (67)	435 (19)	1845 (58)	4063 (144)

* Number of mosquitoes collected.
** Number of pools tested.

Isolations of Flanders Virus from Memphis, Tennessee during 1968

Mosquito Species	7/15-18/68	8/19-21/68	9/9-20/68	Total
Culex quin.-pipiens	12	0	Not Completed	12
Culex restuans	1	0		1

Table 2

Summary of Identifications of Mosquitoes Collected in 1968
from Corpus Christi, Texas, during Arbovirus Surveillance Studies

Mosquito Species	5/29/68	6/12-17/68	7/1-5/68	7/27-29/68	8/19-21/68	9/16/68	Total
Aedes species	1*(1)**	1 (1)	1 (1)				3 (3)
A. taeniorhynchus			2 (1)				2 (1)
Anopheles crucians		5 (1)	32 (1)	1 (1)	1 (1)		39 (4)
An. pseudopunctipennis	1 (1)				1 (1)		2 (2)
An. quadrimaculatus	1 (1)	4 (1)	19 (1)	7 (1)	2 (2)	1 (1)	34 (7)
Culex (Melanoconion) sp.	6 (1)	179 (4)	201 (4)	51 (1)	46 (2)	45 (1)	528 (13)
C. quinquefasciatus	809 (17)	541 (11)	547 (11)	181 (4)	132 (3)	127 (3)	2337 (49)
C. salinarius		2 (1)	1 (1)				3 (2)
C. tarsalis						27 (1)	27 (1)
Psorophora confinnis			3 (1)				3 (1)
Total	818 (21)	732 (19)	806 (21)	240 (7)	182 (9)	200 (6)	2978 (83)

*Number of mosquitoes collected. **Number of pools tested.

Summary of Isolations of Flanders Virus from Corpus Christi, Texas

Mosquito Species	5/29/68	6/12-17/68	7/1-5/68	7/27-29/68	8/19-21/68	9/16/68	Total
Culex quinquefasciatus	2	1	1	1	Negative	Negative	5

Table 3

Results of HI Tests of Immature House Sparrows
Collected in Corpus Christi, Texas, May through September, 1968

Date Collected	HI Antigens			
	SLE	WE	EE	VE
5/20-24/68	3/51* (6%)	1/51 (2%)	0/51	0/51
6/11-17/68	1/49 (2%)	3/49 (6%)	0/49	NT
7/3/68	3/35 (9%)	0/35	0/35	NT
7/23-26/68	0/66	1/66 (2%)	0/66	0/66
8/16-20/68	0/47	0/47	0/47	0/47
8/29/68-9/16/68	0/50	1/50 (2%)	0/50	0/50

NT = Not Tested

* Number positive/number tested

SLE activity, since increasing rates at that time might reveal a buildup of virus activity in the peri-domestic bird population and the potential for virus spill-over into the human population.

II. Eastern Encephalitis Epizootic in Maryland

Members of NCDC investigated an EE outbreak in pheasants near Willards, Maryland, in 1965. This summer, at the request of the Maryland State Departments of Health and Agriculture, an NCDC team, assisted by members of the Maryland Livestock Sanitary Service, investigated an outbreak of EE in horses in the same area.

Equine cases were found to be localized in the Willards-Salisbury, Maryland area where seven horse cases occurred; one of these had been confirmed as EE. One of the 11 horse cases in southern Delaware also had been confirmed as EE. No human cases were reported in these areas.

Mosquito-virus isolation studies were conducted at four sites beginning at Willards, the site of the epizootic; Campbell, an intermediate site; Shingle Landing, in the vicinity of salt water; and Newport Neck, an area in the salt marsh. These sites were selected to determine the relative populations of Culiseta melanura and Aedes sollicitans, both potential vectors of EE. Biting collections on horses were also made from 8:00 p.m. to 12:00 p.m. on five occasions.

Mosquito identification summaries are presented in Table 4. The largest number of C. melanura were found at Willards, and their numbers dwindled to a low in the salt marsh site. A brood of A. sollicitans was reported to have emerged two weeks prior to this study, and as evidenced by these collections, very few were left. Only 194 sollicitans were collected at the salt marsh site.

Aedes vexans (257), Culex salinarius (161), and Psorophora confinnis (142) were the species of mosquitoes found to be biting horses. No C. melanura were taken in the biting collections; however, at least three were taken from horses in an adjoining state in a later study.

Both suckling mice (SM) and duck embryo tissue culture (DETC) were used for virus isolation attempts. Virus isolation results are found in Table 5. A total of 59 isolations were made: 52 EE from C. melanura in both SM and DETC, four Flanders from C. salinarius in SM only, and three WE in DETC only (A. triseriatus - two and Aedes vexans - one).

The location from which EE virus was isolated corresponded to the distri-

Table 4

Total Collections of Mosquitoes from the Willards, Maryland Area
July 31 through August 5, 1968

Mosquito Species	Willards	Campbell	Shingle Landing	Newport Neck	Biting Collection	Totals
Aedes atl.-tor.	24(3)* **	20 (2)	13(2)	5(3)		62(10)
A. canadensis	57(5)	12(2)		6(1)		75(8)
A. cantator	1(1)	1(0)	1(1)	10(3)		13(5)
A. infirmatus				6(3)		6(3)
A. sollicitans		2(1)	3(1)	194(5)	2(1)	201(8)
A. triseriatus	32(6)	6(2)	28(2)			66(10)
A. taeniorhynchus	3(3)		3(1)	621(13)		627(17)
A. vexans	200(7)	497(11)	171(4)	66(3)	257(6)	1191(31)
A. species	2(1)					2(1)
Anopheles crucians	7(4)		149(3)	382(9)		538(16)
An. punctipennis	111(7)	47(2)	4(2)	1(1)	37(2)	200(14)
An. quadrimaculatus	2(2)	1(1)		5(2)		8(5)
Culex quin.-pip.		1(1)	2(1)	1(1)		4(3)
C. restuans	15(5)	2(1)	4(2)		1(1)	22(9)
C. salinarius	72(6)	447(10)	1213(25)	1168(25)	161(4)	3061(70)
C. territans	3(3)					3(3)
C. species	23(3)	77(2)	59(2)	1(1)		160(8)
Culiseta melanura	3458(72)	632(14)	66(2)	3(2)		4159(90)
Mansonia perturbans	7(3)			1(1)	31(1)	39(5)
Orthopodomyia signifera		1(1)	1(1)			2(2)
Psorophora ciliata	2(1)	21(2)	1(1)	1(1)	6(2)	31(7)
P. confinnis	17(3)	173(4)	31(2)	52(3)	142(4)	415(16)
P. cyanescens					2(1)	2(1)
P. ferox	74(6)	40(2)	14(2)	61(3)		189(13)
P. howardii				1(1)		1(1)
Uranotaenia sapphirina	1(0)	1(0)				2(0)
Totals	4111(141)	1981(58)	1763(54)	2585(81)	639(22)	11,079(356)

* Number of mosquitoes collected.
** Number of pools tested.

Table 5

Comparative Isolations of Viruses in Suckling Mice (SM) and Duck Embryo Tissue Culture (DETC) from Mosquitoes Collected in Willards, Maryland, August 1968

Mosquito Species	Isolations						Total	
	EE		WE		Flanders			
	SM	DETC	SM	DETC	SM	DETC	SM	DETC
Aedes triseriatus			0	2			0	2
A. vexans			0	1			0	1
Culex salinarius					4	0	4	0
Culiseta melanura	52	52					52	52
Total	52	52	0	3	4	0	56	55

Total number of viruses isolated: 59

bution and abundance of C. melanura, Table 6. This table also gives the EE infection rates for C. melanura which ranged from 1:44 to 1:105. In view of their numbers and extremely high field infection rates, it seems possible that only an occasional feeding by them upon a horse might account for the equine cases that occurred. On the other hand, the presence of a brood of A. sollicitans earlier also could have accounted for the transmission of EE to horses.

III. California Virus Studies in Winona, Minnesota

Human cases of CE have been reported from the Winona area since 1959. Several cases were reported again during August, 1968. A cooperative study was undertaken with the Minnesota State Department of Health to determine the extent of CE virus activity in the area. Winona, situated on the Mississippi River, and Gilmore Valley, one of numerous, shallow valleys surrounded by forested hills which extend several miles from the river, were selected for study.

Blood samples were taken from 155 human volunteers during an initial survey. Of these, 2/116 (1%) persons living in Winona had HI antibody to CE virus. On the other hand, 7/20 (35%) persons living in Gilmore Valley had CE-HI antibody. None of 19 persons inhabiting rural areas other than Gilmore Valley had CE antibody.

Subsequently, over 95% of the human population of the valley was sampled and pertinent information regarding age, length of residence, and date and character of recent illness was gathered. Pickwick Valley, nearby and ecologically similar to Gilmore Valley, was selected as a control site and simultaneously studied. Also, over 500 Winona school children were bled to determine exposure to California virus in the age group most susceptible to clinical infection. A large group (706) of townspeople of all ages was sampled, including 28 recently ill with a viral syndrome characterized by fever and headache. Because of the unusually high prevalence of mental retardation in Winona, mentally retarded children and their families were also studied.

Results of HI tests on portions of the human population sampled, Table 7, indicate highest exposure to California virus in the two valleys near Winona. Fifteen percent of Gilmore Valley residents and 16% of Pickwick Valley residents had HI titers of 1:10 or greater to the LaCrosse strain of California virus. Activity of this agent was significantly lower in urban areas (4% HI positive) and in rural areas other than the two valleys (4%). Moreover, of 19 persons living in Gilmore Valley recently ill with fever and headache, six, or 32% had HI antibody, whereas none of 17 townspeople with recent

Table 6

Isolations of EE Virus from C. melanura with Field Infection Rates
Willards, Maryland Area
July 31 through August 6, 1968

Site	Number	Pools	Isolates	Infection Rate
Willards				
Hudson's Farm	266	6	6	1:44
Bunting's Farm	903	19	8	1:113
Burnt Mill Branch	44	1	1	1:44
Truitt Farm	214	5	3	1:71
Davis Pheasant Farm	1197	24	17	1:70
Littleton Road	834	17	10	1:83
Intermediate				
Campbell	632	14	6	1:105
Salt Water Vicinity				
Shingle Landing	66	2	1	1:66
Salt Marsh				
Newport Neck	3	2	0	0
Total	4159	90	52	1:79

Table 7

Results of HI Tests for California Virus, Human Population
Winona, Minnesota, and Environs

	Location	Number Tested	Positive to Calif. HI $\geq$ 1:10 Number	(Percent)	Negative to Calif.
Rural	Gilmore Valley	224	35	(15%)	189
"	Pickwick Valley	131	21	(16%)	110
"	Other Areas	94	4	(4%)	90
Urban	Winona	706	30	(4%)	676
Total		1155	90	(8%)	1065

febrile headache had antibody. This seems to indicate California virus as the causative agent of some of the febrile headache syndromes in Gilmore Valley, and to suggest another etiology for viral illness in Winona proper.

The results of HI tests performed on the sera of school children have not yet been evaluated. Mentally retarded children do not have an unusually high rate of exposure to California virus based on results of the HI test (5% positive). Serum neutralization tests are being run to determine the possibility of remote (fetal) infection. Ten percent of a small sample of the mothers of retarded children was positive by HI test.

Mosquitoes were collected from Gilmore Valley by dry ice supplemented CDC light traps, by sweep net, and from horses. Cold weather hampered the efforts, but a total of 5, 288 mosquitoes were obtained between September 9 and October 1. Mr. Ron Zwonitzer of the College of St. Teresa's Biology Department assisted in these studies. The mosquito collection consisted of 16 species of which Aedes vexans was by far the most prevalent. Very small numbers of all other species were collected, Table 8.

Eighty percent of the mosquitoes collected have been tested for virus in SM. Two isolations have been obtained, one from a pool of seven Aedes triseriatus, and one from a pool of 50 Aedes trivittatus. The triseriatus isolate has been identified as California encephalitis virus by CF test, but it has not yet been typed. The other isolate is as yet unidentified.

Small mammals were collected throughout the valley by live trap, bled from the retro-orbital sinus by capillary tube, marked, and released. A total of 114 wild mammals representing 11 species and two horses were bled between September 5 and 13, Table 9. No virus was isolated from any of the mammal sera and serological testing is incomplete.

Table 8

Total Mosquitoes Collected for Virological Study
from Gilmore Valley, Minnesota, September 6 to October 1, 1968,
and Viruses Isolated to Date

Species	Number	Pools	Virus Isolations	Identification
Aedes intrudens ?	2	2		
Aedes triseriatus	24	5	1	California
Aedes trivittatus	189	5	1	Unidentified
Aedes vexans	4807	194		
Aedes species	3	2		
Culex erraticus	3	2		
Culex quin.-pips.	5	2		
Culex restuans	24	4		
Culex salinarius	26	2		
Culex tarsalis	7	1		
Culex territans	7	4		
Culex species	1	1		
Culiseta inornata	44	4		
Culiseta minnesotae	2	1		
Culiseta morsitaus	40	5		
Culiseta species	4	1		
Anopheles punctipennis	53	6		
Anopheles walkeri	12	4		
Uranotaenia sapphirina	35	3		
Total	5288	248	2	

Table 9

Mammals Trapped and Bled in
Gilmore Valley, Winona, Minnesota
September 5-13, 1968

Species		Total
Wood Mouse	Peromyscus spp.	61
Field Mouse	Microtus spp.	18
Cottontail Rabbit	Sylvilagus floridanus	9
Opossum	Didelphis marsupialis	4
Jumping Mouse	Zapus hudsonicus	3
House Mouse	Mus musculus	2
Chipmunk	Tamias striatus	2
Striped Skunk	Mephitis mephitis	1
Short-tailed Shrew	Blarina brevicauda	1
Big Brown Bat*	Eptesicus fuscus	10
Little Brown Bat*	Myotis lucifugus	3
Horse		2
Total		116

*Hand collected.

REPORT FROM THE ECOLOGICAL INVESTIGATIONS PROGRAM, N.C.D.C., U.S.P.H.S., FORT COLLINS, COLORADO

Colorado

Fifteen encephalitis cases among human and two among equines have been reported during 1968. Serologic tests (HI) on specimens from 14 of the cases indicate that one may have been due to an arbovirus (WE), and the convalescent serum specimen from the remaining case has not yet been obtained. The equine sera were negative for WE antibody. Through September 8, 1968, neither a WE nor a SLE virus isolation has been obtained from Culex tarsalis in Weld and Larimer Counties and no antibody conversion for either WE or SLE has been detected in the two sentinel chicken flocks through August 13, 1968. Four isolations of Turlock virus have been made from mosquitoes during the season, three from C. tarsalis and one from C. pipiens. The turlock infection rates 15.3/1000 among C. tarsalis and 2.0/1000 among C. pipiens.

Texas

This year no confirmed human arbovirus illness has been detected among 30 suspect cases in Hale County through September 4, 1968. Populations of C. tarsalis in the Hale County study area were markedly lower than in previous years. Ultra low volume spraying was carried out over several of the study sites; however, C. tarsalis populations were low in untreated areas as well as the treated areas. One possible explanation being investigated is the influence of large-scale parathion spraying to control a serious infestation of aphids (Schizaphis graninum) occurring on grain sorghum. Although agricultural spraying has been carried out in the past, principally against the grain sorghum midge (Contarinia sorghicola), the wide-spread spraying began much earlier this season. Despite the low population levels of C. tarsalis, WE virus isolations from pools of C. tarsalis remained high. The first isolation of WE virus occurred the week of 6/2. From the week beginning 7/7 through 8/3, 38 percent or more of the pools of 25 C. tarsalis tested yielded WE virus. The peak week of WE isolations was the week beginning 7/21 when 55 percent of the pools were positive. Isolations of WE virus from C. tarsalis has continued through the week of 9/8 with about 20% of the pools positive each week except for a two week period from 8/4 through 8/17.

Turlock virus was isolated from C. tarsalis early in the year and isolations have continued throughout the season. Two SLE virus isolations have been made from C. tarsalis, one each from the weeks of 9/1 and 9/8.

Isolations from nestling house sparrows (Passer domesticus) have been

lower than in the previous three years of study. The week of the highest proportion of WE positive blood samples was 7/21 when 16 percent of 61 nestlings tested yielded WE virus. This week coincides with the week of highest WE isolations from C. tarsalis pools.

A breeding colony of house sparrows (Passer domesticus) was successfully established at the Plainview Field Station during the year. A study was initiated on WE virus response among groups of nestling birds from WE-immune mothers and from WE-susceptible mothers. Preliminary results of the viremia responses following WE virus inoculation indicate high levels of virus among both groups of young nestlings.

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

In Collaboration With

THE DISEASE ECOLOGY SECTION, NATIONAL COMMUNICABLE DISEASE CENTER, USPHS AND THE CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH

This report reviews field and laboratory studies on arboviruses during the period May 1, 1967 through April 30, 1968.

Unusual water availability and developing populations of Culex tarsalis in the spring of 1967 alerted mosquito abatement districts in California to the possibility that an epidemic of WEE and/or SLE might develop. There was also an unusually cool April and June and a hot July and August. Mosquito control programs in Kern County were begun in the spring several weeks ahead of schedule and on an intensified basis. Intensive control of mosquito breeding was extended through the summer to cover all major mosquito sources. The populations of C. tarsalis were lower in 1967 than at any time during the past 10 years. WEE viral activity was detected at only 1 of 15 evaluation sites and there was a relatively high vector population index at this location. There was no evidence that SLE virus was active in Kern County. Turlock virus continued to be active although the C. tarsalis population was reduced and this may indicate a different mechanism or vector for maintenance and transmission of this virus.

General epidemiologic observations in Kern County revealed no evidence of WEE or SLE in humans for the tenth consecutive year. There also were no confirmed cases of equine encephalomyelitis.

Hemagglutination-inhibition tests on 63 sera from normal cattle, sheep, and pigs from Kern County gave little evidence that these animals had been infected with WEE, SLE, Powassan, Modoc, California encephalitis, Buttonwillow, Turlock, Lokern, or Main Drain virus. Neutralizing antibody to Turlock virus was found in 40 percent of 136 horses bled in the summer of 1966.

Relationships between the levels of encephalitis virus activity and vector populations and the intensity of mosquito abatement programs were reviewed and extended to include current data from Kern County and other areas in the Central Valley of California. Conclusive evidence is now available on the level of C. tarsalis control that must be achieved to eliminate effective transmission and maintenance of WEE and SLE viruses from an environment. A reduction of female C. tarsalis population indices to an average of 10 or less per light trap or bait trap night over a sufficiently wide area and time span will greatly reduce the risk of WEE in man and horse and will most likely result in the disappearance of SLE virus from the environment. Female C. tarsalis population indices of 1 or less females per light trap might be required to eliminate WEE virus.

An intensive study on the ecologic relationships between wild mammalian and ectoparasitic populations and the levels of arboviral activity was continued for the fifth year. Mammals were trapped, banded, and released on a 40 acre grid located in a saltbush desert and slough habitat adjoining an irrigation canal and agricultural area. Mammalian populations fluctuated markedly over the five year period, both in abundance and species composition. Populations of Dipodomys heermani and Perognathus californicus declined yearly and Perognathus inornatus disappeared from the grid area. The mean numbers of Dipodomys nitratoides captured decreased from 217 to 114, but their relative abundance in the total mammalian population increased from 55 to 81 percent. Populations of Ammospermophilus nelsoni and Peromyscus maniculatus fluctuated yearly with no consistent upward or downward trends. Similar trends in mammalian populations were observed in similar habitats that were under less intensive trapping pressure.

Supplemental trapping of wild mammals was continued for the third year at another saltbush desert habitat near Cecil Tracy's Ranch and at a foothill-streamside habitat along Poso Creek. At Cecil Tracy's Ranch, D. nitratoides, A. nelsoni, and P. maniculatus were captured most frequently, whereas at Poso Creek, P. maniculatus, D. heermani, Reithrodontomys megalotis, and P. californicus predominated. Large concentrations of Lepus californicus were found in alfalfa fields and adjacent desert areas near Mojave in the southeastern part of Kern County. The rodent species in the Mojave area were different from those found on the valley floor.

HAI antibodies to WEE, SLE, Powassan, Modoc, and California encephalitis viruses were rarely found in rodents collected at Lerdo Grid, Cecil Tracy's Ranch, Poso Creek, or Mojave. The 1 exception was at Poso Creek where 13 percent of P. maniculatus were positive for Modoc virus. Both species of leporids, L. californicus and Sylvilagus audubonii, had high prevalances of antibodies to California encephalitis, Buttonwillow, Main Drain, and Lokern viruses but had low prevalances of antibodies to WEE and group B viruses.

No virus was isolated from 420 blood samples collected from 276 rodents at Lerdo Grid nor from 46 and 56 bloods from L. californicus and S. audubonii respectively that were collected on the valley floor. Bloods from 120 L. californicus collected near Mojave yielded two strains each of Buttonwillow and Main Drain viruses and three strains of Lokern virus.

Ticks were the only ectoparasites removed from mammals and tested for virus. Rodent species were infested only with Dermacentor parumapertus. Otobius lagophilus and D. parumapertus were found on most L. californicus whereas Haemphysalis leporis-palustris and D. parumapertus most frequently infested S. audubonii. An unidentified virus was isolated from a pool of 2 O. lagophilus. The remaining 8,159 ticks that were collected during 1965-1967 were tested as 462 pools and no virus was isolated.

Studies on bird populations were continued for the third year at Cecil Tracy's Ranch and Poso Creek. A total of 765 resident birds, 648 winter visitant birds, and 102 summer visitant birds were netted or trapped, banded, bled, and released. From 84 to 97 percent fewer Song Sparrows, Yellowthroats, and Red-winged Blackbirds were captured in the 1967-1968 season than in the preceding two years, whereas the overwintering population of Gambel's White-crowned Sparrows was 31 percent higher than in the two previous years. The number of House Finches captured in 1967-1968 was the lowest of any of the three years. Recapture rates of birds banded during 1967-1968 ranged from 10 percent for the Hermit Thrush to 29 percent for Bullock's Oriole.

Biologic data and blood samples were obtained on 492 nestlings of eight common avian species in the period April through August 1967. Mean clutch sizes ranged from 2.0 for Mourning Doves to 4.7 for Brewer's Blackbirds, and mean brood sizes ranged from 2.0 for Mourning Doves and Yellow-headed Blackbirds to 4.2 for Brewer's Blackbirds. Smaller clutch and brood sizes for Brewer's Blackbirds were observed at F.C. Tracy's Ranch than at C. Tracy's Ranch. This disparity may reflect the higher densities of House Sparrows at F.C. Tracy's Ranch.

Turlock virus was isolated from the blood of one nestling House Finch collected at Cecil Tracy's Ranch on July 27, 1967. HAI antibody to WEE, SLE, or Modoc virus was found in 1-3 percent of nestling sera. The validity of these serologic results is questionable, especially with group B viruses, since Modoc virus rarely infects experimentally inoculated birds and SLE virus has been absent from Kern County since 1963. Serologic evidence for infection of immature and adult birds with WEE, California encephalitis, and Turlock viruses was found more frequently at Cecil Tracy's Ranch than at Poso Creek. As in previous years, House Finches and House Sparrows were the avian species most commonly infected with these viruses. Infection of birds with Turlock virus occurred in July and early August, as samples collected in June were negative for antibody and 29 and 10 percent of immature House Finches and House Sparrows respectively that were captured in August had HAI antibodies to Turlock virus. No HAI antibody to WEE virus was found in sera from immature House Finches and House Sparrows collected from April through October 1967.

A nonspecific inhibitor of WEE viral hemagglutinins that did not neutralize WEE virus was found in a high proportion of sera collected in April 1968 from female but not male House Sparrows. This inhibitor was removed by treatment of sera with protamine sulfate. This phenomenon was demonstrated previously for sera from laying chickens.

Sera from 119 cold-blooded vertebrates, mostly western toads and western bullfrogs, were tested for HAI antibodies to nine arboviruses. One bullfrog (Rana catesbiana) and two snakes (one Petuophis catenifer and one Lampropeltis getulis) had antibodies to SLE and WEE viruses respectively.

Studies of Culicoides were begun in Kern County in 1963. At least 13 species have been collected and Culicoides variipennis predominates. Tests of over 100,000 female C. variipennis yielded 77 viral isolations that represent three new viruses: Buttonwillow virus in the Simbu group and Main Drain and Lokern viruses in the Bunyamwera group. No viral isolations have been made in tests of 5,000 females of other Culicoides species, Leptoconops or Simuliidae.

The principal flight periods of C. variipennis are near sunset and sunrise, and this may reflect an inherited circadian rhythm. However, data still support earlier findings that environmental variables such as light intensity, temperature, and humidity have at least secondary influences on flight patterns. Preliminary data on five other species of Culicoides indicate a wide variation in flight patterns, as some species are equally active all night or only become active after sundown. Small numbers of all life stages of C. variipennis were collected throughout the winter in

Kern County, which indicates some breeding occurs throughout the year. Efforts to colonize this species have met with limited success.

The blood-feeding habits of mosquitoes were studied in a wide variety of localities. Over 5,000 engorged mosquitoes from the Hawaiian Islands were tested in 1967. Culex quinquefasciatus had fed predominantly on birds (54 percent) including the pelagic red-footed booby. Dogs were the most frequent mammalian host. Aedes albopictus and Aedes aegypti that were collected in and around houses both had over 56 percent of feedings on man. Aedes albopictus from rural collections had a wide host range, but fed predominantly on mammals (94 percent). Aedes vexans fed exclusively on mammals. This study is now terminated.

Identifications of mosquito blood meals from collections in Illinois and Missouri were terminated after a four year period. Culex pipiens from both states fed predominantly on birds, predominantly passeriforms, and with little variation in the several years and areas. Culex restuans and Culex erraticus fed almost exclusively on birds and Culex territans fed mostly on frogs. Anopheles quadrimaculatus and Anopheles punctipennis fed on mammals.

An opportunity arose to study the feeding habits of mosquitoes from Panama. Deinocerites pseudes, Deinocerites epitedeus, and Deinocerites cancer included all major groups of vertebrates in their blood meals. Over 50 percent of the first two species had fed on cold-blooded hosts, but they included birds and mammals in their host range. Deinocerites melanophyllum fed almost exclusively on lizards.

A fifth year of studies in Hale County, Texas, confirmed that a high proportion (76 percent) of C. tarsalis fed on birds.

Seven species of mosquitoes were collected in Minnesota. Culex tarsalis, C. restuans, C. pipiens, Culex salinarius, and Culex morsitans fed predominantly (90 percent of more) or birds. Culiseta inornata and Anopheles earlei fed almost exclusviely on mammals.

Culiseta melanura and C. pipiens from Maryland and New York fed almost exclusively on birds.

Studies were continued on arboviruses as etiologic agents of undiagnosed central nervous system disease and febrile illness of man in California. In 1967, three of 120 human cases were diagnosed as WEE by HAI or CF tests. None of these human cases had diagnostic rises in HAI antibodies to SLE, Powassan, Modoc, California encephalitis, Buttonwillow, Lokern,

or Main Drain virus. Stationary levels of HAI antibodies were found to California encephalitis virus in six cases, and to Lokern virus in one case. Neutralizing antibody to Turlock virus was found in six of 458 human cases that occurred from 1965 through 1967, but there were no diagnostic rises in antibody.

Sera from 158 cases of equine encephalomyelitis were collected in 1966 and 1967 and tested for HAI antibodies to nine arboviruses. Two cases were diagnosed as WEE by HAI test. Diagnostic rises in HAI antibodies were found in one case each to California encephalitis, Lokern, and Turlock viruses, and in three cases to Main Drain virus. Immunologic conversions to Turlock virus were detected in three cases by neutralization tests.

Serologic evidence was obtained that sheep from Mendocino County had been infected with California encephalitis, Buttonwillow, Lokern, and Main Drain viruses, but not with WEE, SLE, Powassan, Modoc or Turlock virus. At the same locality there was a high prevalence of antibody to Main Drain virus in jackrabbits, and to California encephalitis virus in woodrats. As in other areas of California, there was a high prevalence of Modoc virus antibody in deer mice.

Seventy-five Peromyscus mice were trapped in April 1968 from a locality in Tulare County where a boy probably became infected with Modoc after playing with a sick mouse. No HAI antibody to WEE, SLE, Powassan, Modoc, California encephalitis, Buttonwillow, Lokern, Main Drain, or Turlock virus was found in serum samples from these mice. Unidentified viruses were recovered from in vitro cell cultures of kidneys from one P. maniculatus and one Peromyscus boylei.

Studies were done to determine the prevalence of arboviral antibodies in monkeys that were housed through the summer in outdoor cages at the National Center for Primate Biology, Davis, California. Two species from Southwest Asia, Macaca nemestrina and Macaca speciosa, had high prevalences of HAI antibodies to group B viruses in serum samples taken before their summer's exposure. Sera from 18 of 100 M. nemestrina also reacted by HAI test to California virus. Cercocebus fulliginosus, a species from Africa had a low prevalence of group B antibodies and had no immunologic conversions to nine arboviruses after a summer's exposure. Intensive applications of residual insecticides in and around the cages undoubtedly minimized the monkey's exposures to hematophagous diptera.

We are concerned with at least 13 arboviruses in Kern County and their study requires the development of sensitive and standardized tests for their primary isolation and serologic surveys. Satisfactory hemagglutinins

have been developed for all but three of these arboviruses: Jerry Slough, Hart Park, and Kern Canyon. Neutralization tests in Vero cell cultures are more sensitive than HAI tests to detect antibodies to Buttonwillow, Lokern, and Main Drain viruses. Suckling mice are still the only sensitive assay system applicable to all viruses, but Vero cell plaquing systems are equally sensitive for all but Turlock and Hart Park viruses. Vero cells are more uniformly susceptible to 11 of 12 arboviruses than seven other cell lines. Turlock virus, the exception, is still best assayed in duck embryo primary cell cultures. Group B arboviruses plaque in Vero cell cultures but, on occasion, the presence or absence of an unidentified factor leads to irregular titrations.

Sonication of arboviral hemagglutinins produced signficant increases in antigen titers for Cache Valley, Lokern, Main Drain, California encephalitis, Buttonwillow, and Turlock viruses. Less satisfactory reagents were produced for Jamestown Canyon or Jerry Slough virus. The sonication technique was adapted to prepare hemagglutinins for the rapid identification of newly isolated strains of Main Drain, Lokern, and Buttonwillow viruses.

A reevaluation of protamine sulfate to remove nonspecific inhibitors of arboviral hemagglutinins indicated that specific antibodies to Modoc and Turlock viruses were removed from avian sera by this treatment.

Buttonwillow virus is a new virus in the Simbu antigenic group. A number of strains were isolated and identified: 37 from C. variipennis, and three each from S. audubonii and L. californicus.

Two new viruses in the Bunyamwera serogroup, Lokern and Main Drain, have been characterized by HAI and CF tests. These are common viruses in Kern County as Main Drain virus was isolated from 21 C. variipennis pools and from three blood samples of L. californicus. Lokern virus was isolated from 19 C. variipennis pools and from blood samples of four L. californicus and one S. audubonii.

Four agents isolated from the blood or organs of squirrels have characteristics of arboviruses but cannot be related to known major serologic groups.

Studies were continued on the pathogenesis of arboviruses in laboratory and suspected native hosts. On the basis of avian and mammalian susceptibility group B viruses were separated into two classes. The first class was represented by SLE virus that infected both birds and mammals. SLE virus produced high titered viremias and HAI antibodies persisted for at least 12 weeks in most birds. Adult P. domesticus were the only birds that did not develop infection. Viremia and antibody responses occurred

in A. nelsoni, D. nitratoides, and S. audubonii. Two mammalian species were refractory, C. beecheyi, and P. maniculatus. The second class of group B viruses (Powassan, Modoc, and Rio Bravo) rarely or never produced viremia or antibody responses in birds but were highly infectious for mammals.

Buttonwillow virus did not produce infection in three avian species: C. mexicanus, P. domesticus, or Z. macroura. Most species of mammals were susceptible. Ammospermophilus nelsoni and C. beecheyi had irregular appearance of viremia but good antibody responses. All L. californicus and S. audubonii became infected and developed viremia but viremia was of short duration, one to two days. HAI antibody became undetectable in L. californicus within four weeks. Only one of six D. nitratoides and 0 of 17 P. maniculatus became infected. A substance in both serum and whole blood samples from rabbits inhibited plaque formation by Buttonwillow virus on Vero cells but did not inhibit infection in suckling mice.

Pathogenesis studies with Main Drain virus indicated that birds were insusceptible. Hamsters and S. audubonii were more susceptible than A. nelsoni, C. beecheyi, D. nitratoides, or P. maniculatus.

Efforts were made to adapt the micro-complement fixation test to identify mosquito blood meals and the system was a limited success when applied to ungulate bloods. However, an inability to produce high titered antisera has limited the application of this procedure. Fluorescein-conjugated antisera from commercial sources were unsatisfactory for mosquito blood meal identification.

Studies on antigenic alterations of blood components by digestion in the mosquito gut revealed changes in the a-2 globulins after 16-20 hours of digestion. Alterations in protein structure could result in nonspecific serologic reactions, and this could explain the high frequency of multiple host feedings by mosquitoes reported by some laboratories.

Collaborative efforts with the American Committee on Arthropod-borne Viruses resulted in the publication of the first edition of the "Catalogue of the Arthropod-borne Viruses of the World."

This report represents the summary of an Annual Progress Report. A limited number of copies of the detailed report are available upon request.

(William C. Reeves)

REPORT FROM THE SOUTH DAKOTA STATE UNIVERSITY, BROOKINGS, SOUTH DAKOTA

Epizootic Hemorrhagic Deer Disease Investigation

I. Epizooticological Survey of Epizootic Hemorrhagic Disease (EHD in Deer and Other Animals)

From 1965 to 1967, 396 white-tailed deer, mule deer and antelope sera were collected from many South Dakota areas for epizooticological survey. Out of 228 white-tailed deer tested, 54 (23.7%) showed EHD antibodies; of 146 mule deer tested, 31 (21.1%) had antibodies; and of 22 antelope, six had EHD antibodies. Of the adult deer (both white-tailed and mule) tested 36 of 131 males (27.5%) had antibodies; whereas of 177 females tested only 26 (14.7%) had antibodies. Thus it seems larger percentage of male deer had infection in the field than female, or death due to hemorrhagic may be more frequent in female deer in the field environment. 1967 serological survey indicated that EHD is found in the South Dakota Black Hills deer. This is the first scientific evidence of the presence of EHD viral agent in the 70,000 Black Hills deer population.

In order to understand where the virus comes from and to where it disappears in the field, different possible reservoirs needed to be studied. Therefore from 45 counties, 1,168 sera samples from swine and cattle were collected and tested for presence of EHD antibodies. Of the swine sera tested 6.5% had EHD antibodies as did 7.3% of the cattle sera tested.

II. Growth of EHD Viral Agent in Deer and Other Cell Cultures

South Dakota # 10 strain of EHD agent produced cytopathogenic effects in (a) deer kidney (b) deer spleen and (c) BHK-21 cell lines. It is felt that in order to isolate various EHD strains of South Dakota, primary deer cell cultures might be better than BHK-21 and vero cell lines, in which the agent also grows. Deer cells were obtained from three month old to 2 1/2 year old animals and cultivated in vitro successfully. These cells were capable of supporting replicants of South Dakota EHD agents. Attempts are now being made in our laboratories to establish deer kidney cells from one to two week-old newborn deer. Deer cells from kidney and spleen grew well in (a) roller culture apparatus and in (b) Bellco Roller apparatus utilizing 2,000 cc round bottles. Deer spleen and kidney cells grew in Hanks balanced salt solution containing 0.5% lactalbumib hydrolysate, 0.1% yeast extract, 0.2 mM% glutamine

and 10% fetal bovine serum. Cells were ready for virus inoculation on the fourth day. The cell concentration used for seeding was 2 X 10 cells/ml.

Preliminary studies for primary virus isolation from infected deer spleen and urine specimens were just as successful as newborn mice have been in such studies. Depending on virus concentration CPE in deer cells is generally observed from 48 hours to 14 days. Deer cell culture chromosome studies showed that it has 70 chromosomes. Thirty-three pairs of chromosomes were acrocentric and two pairs were metacentric. The deer cell culture techniques may also benefit in primary virus isolation of other viruses from wildlife.

Our technique of deer cell culture when employed for virus isolation could also lead to the discovery of newer viruses afflicting deer. Increased lipid formation was detected by Sudan Black dye staining in 14 days infected BHK-21 cells.

(C. C. Parikh)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF BRITISH COLUMBIA, VANCOUVER 8, CANADA

Arbovirus serology, south-eastern British Columbia

Near Cranbrook (50° N, 116° W) between May and July 1968, Group B hemagglutination inhibition (HI) reactions have been demonstrated in 369 of 1,053 sera from small forest rodents including 288 of 747 Citellus columbianus, 22 of 54 Citellus lateralis, 22 of 79 Tamiasciurus hudsonicus, 21 of 100 Eutamias amoenus and 16 of 73 other mammals. Of these 369 HI inhibitors, 109 inhibited hemagglutination by Powassan antigen exclusively at 1:10 or higher serum dilutions, 88 inhibited Murray Valley encephalitis antigen, 22 Modoc antigen and 150 reacted broadly against more than one group B antigen. Sera from 21 of 483 animals tested during May and June neutralized Powassan virus by intracerebral mouse neutralization tests and 39 of 292 neutralized St. Louis encephalitis (SLE) virus. None of 18 sera which inhibited hemagglutination by western equine encephalomyelitis (WEE) antigen neutralized this agent. Only at 3 of 15 test sites were 3 or more animal sera found to neutralize Powassan virus, thus emphasizing the focal distribution of this agent in nature. To date, no virus has been recovered from 31 pools of Dermacentor andersoni ticks collected by dragging the mountainside vegetation, or from 18 pools of nymphs removed

from animals. These results suggest the endemic prevalence of Powassan and SLE viruses at Cranbrook.

(Donald M. McLean)

Growth of Powassan virus in Dermacentor andersoni ticks

Adult, nymph and larval states of D. andersoni ticks have become infected by feeding on rabbits which were injected intravenously with $10^{8.7}$ mouse LD_{50} of Powassan virus 48 hours after the ticks were placed in capsules on the rabbits' skin. Immediately after injection, the viremia titer was $10^{5.7}$ mouse LD_{50} per ml, but it became undetectable 6 hours subsequently. Adult ticks which ingested $10^{5.0}$ mouse LD_{50} per tick contained no detectable virus at 5, 7 and 9 days, minimal infectivity at 11 days, and maximum titers of $10^{4.2}$ mouse LD_{50} per tick were attained after 30 to 40 days. The minimum viremia titer which resulted in infection of 4% of ticks was $10^{3.7}$ mouse LD_{50} per ml. Freshly reared larvae ingested $10^{5.7}$ mouse LD_{50} per tick, yielded no infectivity at 7 days, $10^{4.2}$ at 11 days, and after molting the virus titer in nymphs reached $10^{7.0}$ mouse LD_{50} per tick at 21 days. These results demonstrate the proliferation and trans-stadial transfer of Powassan virus in D. andersoni ticks which were infected by feeding on rabbits rendered instantaneously viremic by intravenous injection of Powassan virus after the ticks had become attached.

(Max A. Chernesky)

REPORT FROM THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH, BRISBANE, AUSTRALIA

During this year the Mitchell River Field Station was staffed during November, December and January, and studies carried out aimed at evaluating the importance of mammals as hosts of arboviruses in the late dry-early wet seasons. An epizootic of ephemeral fever of cattle, the third recorded in Australia, spread through Queensland in January, and the Institute collaborated with several veterinary laboratories in studying it. Virus strains were isolated from cattle, mosquitoes and Culicoides and are under study. Techniques for handling Culicoides were developed in collaboration with Mr. A. L. Dyce, C.S.I.R.O., Division of Animal Health, and will be of

value in future work. Sentinel chickens were exposed in the Charleville area again and gave evidence of the spread of Sindbis virus in that area. Serum samples from over 500 Aboriginal children from 12 settlements were tested to extend previous observations on arbovirus distribution. Other serological studies in this year included the completion of earlier surveys of arbovirus activity in the north-east and western Queensland, and a survey of neutralizing antibody to several ungrouped viruses. Serological tests of patients with various clinical syndromes gave evidence of Ross River virus infection in patients with epidemic polyarthritis at Bowen, Charleville, Brisbane and Moree this year, and of Sindbis virus infection in a child with fever and vesicular rash in northern Victoria in early 1967.

The acarology section completed a major study of nasal mites in Queensland birds, and presents new observations and a general analysis of their taxonomy, phylogeny and host-parasite relations in the annual report.

The group studying the biochemistry of arboviruses spent much time this year in calibration of new equipment and exploration of techniques, but preliminary results were obtained on the purification and isotope-labelling of Kunjin virus. The specificity of early (19S) rabbit antibody in haemagglutination-inhibition tests was studies further and used as the basis of a a convenient method for identification of the closely-related members of the Murray Valley encephalitis-Kunjin-Alfuy subgroup of group B. The chemical basis of arbovirus haemagglutination remained under investigation and some previous contradictory findings were re-examined.

(R.L. Doherty)

REPORT FROM THE VIROLOGY DEPARTMENT SCHOOL OF TROPICAL MEDICINE, CALCUTTA, INDIA

Adaptation of Chikungunya virus in adult mouse

One of the strians of chikungunya virus, isolated from cases of 'haemorrhagic fever' in Calcutta in 1963, has been adapted in adult mice, by serial intracerebral passage in mice of gradually increasing age. The task was more difficult than in the case of adaptation of a dengue 2 strain in adult mice in our hands. It seems, this is the second adult mouse adapted strain of chikungunya available, the other being the African strain of chikungunya virus. The characters of this adapted strain, especially its variation from the original unadapted strain, are being studied.

Dengue virus isolated from a case of Encephalitis

Dengue type 2 was isolated from the blood of a patient clinically diagnosed as a case of encephalitis. There was convincing rise of dengue HI and neutralizing antibodies in the convalescent serum. The patients recovered completely. No virus could be isolated from the cerebrospinal fluid of the patient.

Antigenic typing of dengue virus strains isolated from febrile cases

Dengue virus has been isolated from 20 cases of 'short fever' during the last few years in this laboratory. Out of these, 15 have so far been antigenically typed; thirteen belong to type 2 and two belong to type 4.

Serological survey

It was previously reported that haemorrhagic fever broke out in Calcutta in 1963, and that dengue or chikungunya viruses were isolated from many of those cases. Human sera collected in Calcutta in different years, have been tested, and the percentage of sera positive to chikungunya has been 13 percent in 1960, 18 percent in 1964, 32 percent in 1965, 21 percent in 1966 and 11 percent in 1967. Although all the collected sera have not yet been tested, it seems that four years after the outbreak of chikungunya fever epidemic, the percentage of chikungunya positive sera has come down to the same level as it was before the outbreak.

(J. K. Sarkar, S. N. Chatterjee, S. K. Chakravarty and M. S. Chakravarty)

REPORT FROM THE VIRUS RESEARCH CENTRE, POONA, INDIA

Isolation of Kyasanur Forest Disease Virus from Bats

Earlier studies had shown that some species of bats show the presence of neutralizing antibodies to Kyasanur Forest disease (KFD) virus in their sera. Attempts were therefore made to isolate KFD virus from the organs of bats collected in the KFD area. Organs of 517 bats belonging to three species of bats, Rousettus leschenaulti, Cynopterus sphinx and Rhinolophus rouxi, were tested. Four strains of KFD virus could be isolated from the spleens of four Rhinolophus rouxi. All the four bats were collected from a single colony resident in an abandoned dry well situated within the 'hot spot' of KFD infection. One strain of KFD virus could also be isolated

from a pool of five adult Ornithodoros ticks collected from the roosting site of this colony of bats.

Isolation of Viruses of the Phlebotomus Fever Group in India

Further work has been carried out with the two arbovirus strains reported in Information Exchange No. 16, January 1968. The two strains isolated from human sera collected in March 1967 from Aurangabad have been found to be serologically identical and react in HI test with the Phlebotomus fever group hyperimmune ascitic fluid. However, no HI reaction was noticed with eleven individual strain specific immune ascitic fluids of the group. Further work on the identity of the two strains is being carried out. If these strains prove to be a member of the Phlebotomus fever group, they will represent the first reported isolation of this group of viruses in India and will open up a new field of interest for the epidemiological investigations.

Cytopathic Effect of Ganjam Virus in Vero Cell Culture

Ganjam viruses (Arbovirus Catalogue No. 196) have been repeatedly isolated from ticks and once from mosquitoes. They have been found to multiply by parenteral inoculation in mosquitoes and tick and in vitro in mosquito cell cultures. During the course of studies with these viruses in VERO cell cultures, they were found to produce cytopathic effects (granulation, rounding of cells and lysis of the cell sheet) not generally associated with the other well known arboviruses studied by us.

Ganjam viruses induce formation of large syncytial cells with nuclear and cytoplasmic vacuolation. Well defined basophilic intracytoplasmic inclusions can be seen in a number of syncytial formations. The prototype strain of Ganjam virus does not produce any haemagglutinin or haemadsorption in VERO cell culture. These strains do not react either with Herpes simplex or NDV.

I. Use of Conglutinating Complement Absorption Test in Arboviruses

A conglutinating complement absorption test (CCAT) has been applied successfully with arboviruses. The test was found to be very sensitive and highly specific for detecting antibody against viruses of this group in the sera from man and animals.

Antibody response in rabbits and mice

The antibody level studies in serial sera from rabbits and mice after infec-

tion with Japanese encephalitis virus (P 20778 strain) indicated that the perceptible conglutinating antibody activity was first demonstrated between 5-7 days after inoculation, the antibody increased to significant peak level followed by gradual decline. The antibody persisted as long as 101 days of infection. On comparative study with complement fixation test (CFT), it was found that antibody appeared usually after seventh day, rose to low antibody level by 15-21 days followed by very early disappearance. The antibody titres demonstrated with CCAT were usually 2-16 times higher than CFT.

Antibody response in humans

The convalescent human sera taken at varied intervals from twenty-two individuals after Kyasanur Forest disease (KFD) infection, were tested by CCAT and the results compared with those obtained by CFT. The results indicated that out of 40 sera, 29 were positive to KFD antibody by CCAT, and 19 only were positive by CFT. The antibody level studies against various other arboviruses in the sera from humans and animals are now being carried out.

II. A New Serologic Procedure for Detecting Antibody in Chicken Sera

A procedure using specific reactive system consisting of antigen and homologous antiserum and conglutinating indicator system has been developed for testing antibody titres in chickens after infection with Japanese encephalitis virus. The results indicated, in general, that a correlation existed between the antibody response to JE virus as revealed by this procedure and haemagglutination inhibition test. The method was found to be very sensitive and specific (no cross reaction with West Nile antigen) and is sufficiently simple for routine use. The preliminary observations also revealed that the procedure evolved was more sensitive as compared to indirect CFT. The antibody studies with chicken antisera against other arboviruses are in progress.

Mosquito Tissue Culture

I. Primary Isolation of Dengue Viruses in Aedes albopictus Cell Cultures

Aedes albopictus cells are not only sensitive to infection with dengue viruses but the infection can also be easily detected on the basis of a characteristic cytopathic effect produced by these viruses.

To assess the utility of A. alcopictus cell line for primary isolation of dengue viruses from field material, twenty-six samples of human sera

previously collected from cases of dengue-like illness were inoculated in A. albopictus cell cultures for virus isolations. Dengue viruses, types 1, 2, 3 or 4, had been isolated from most of these sera during earlier studies either in Poona or in Vellore.

Dengue viruses types 1, 2 and 4 could be easily detected in A. albopictus cell cultures on the basis of cytopathic effect in cultures inoculated with 1/10 dilution of the sera. No cytopathic effect was noticed in cultures inoculated with two of the sera from which dengue 3 viruses had been isolated earlier.

Further studies on the isolation of dengue viruses from mosquitoes and their identification by CF test using infected TCF as an antigen and by NT in A. albopictus cells are in progress.

11. Comparative sensitivity of mosquito cell lines, VERO cell line and infect mice to infection with arboviruses

The following mosquito-transmitted arboviruses were used in the study. Chikungunya (VRC No. 634029), West Nile (VRC No. G 22886), Japanese encephalitis (VRC No. 20778), dengue 2 (VRC No. 673332), Batai (VRC No. G 20217) and Chandipura (VRC No. 653514).

The titres, expressed as reciprocals of the negative $\log_{10}$ LD_{50}, TCD_{50} and $TCID_{50}$, of various viruses in mice and different cell lines, are given in the table. These results show that Aedes aegypti (ATC-10) cell line is much less sensitive to infection with arboviruses than Aedes albopictus (ATC-15) cell line.

A. albopictus cell line is equally or slightly more sensitive to infection with chikungunya, West Nile and Japanese encephalitis viruses than infant mice and VERO cells. It is slightly less sensitive than mice and VERO cells to infection with Batai and Chandipura viruses. However, it is 100 times more sensitive than VERO cells and 40 times more sensitive than infant mice to infection with dengue 2 virus strain used in this study.

VIRUS TITRES IN VARIOUS CELL LINES AND MICE

Virus	Infant mouse titre LD_{50}	VERO titre TCD_{50}	ATC-15 titre $TCID_{50}$	ATC-10 titre $TCID_{50}$
Chikungunya	8.2*	8.17	8.25	6.6
West Nile	8.6	9.0	9.0**	7.0
Japanese encephalitis	8.4	9.0	9.5**	Not tested
Dengue 2	6.9	6.5	8.5**	Not tested
Batai	7.2	6.83	6.0	Not tested
Chandipura	9.5	9.3	9.0	7.66

* Reciprocal of the negative log 10 endpoint titre

** Cytopathic endpoints (TCD_{50}) of these viruses corresponded to their infective endpoints ($TCID_{50}$) given in the table

III. Loss of mouse virulence in chikungunya virus from the carrier culture Aedes albopictus cell line

The development of a carrier culture state of the Aedes albopictus cell line infected with chikungunya virus has been reported earlier. The tissue culture fluids from the serial passages were harvested and virus assayed in two-three day old mice and in tube cultures of VERO cells. Virus was detectable in both the systems up to the sixth passage level, with a gradual lowering of the titre in mice in the successive passages. In the seventh passage the virus in the TCF failed to produce mortality in mice, though in the VERO cells the titre as calculated by the cytopathic effect was about 10^6 $TCID_{50}$/0.1 ml. Mice inoculated with TCF of the carrier cultures harvested from further passages did not show mortality. The virus in the stock made in VERO cells by inoculating the virus harvested from the 16th passage of the carrier culture was indistinguishable from the mouse passaged virus in neutralization test.

Comparative growth curve studies in mouse brain were made with the mouse passaged virus and the virus stock prepared in VERO cells and tested in neutralization test. The former killed all the mice within 60 hours, and the virus titre in the mouse brain went up to $10^{6.8}$ $TCID_{50}$/0.1 ml., while with the latter, the mice showed a low virus level in the brain never exceeding $10^{4.0}$ $TCID_{50}$/0.1 ml., up to the end of the observation period which was 144 hours, without any mortality.

IV. Anopheles stephensi larval tissue culture

Tissues for cultures were obtained from fully formed larvae inside the eggs. The eggs were surface sterilized by washing twice with acetone and then immersing for 15-20 minutes in White's solution. Eggs after washing with sterile distilled water, were transferred to a small test tube with 0.25 per cent trypsin-salt solution. The eggs were then cut into small pieces and were incubated at 37° C. The tissues and cells after removing the trypsin-salt solution by centrifugation, were suspended in 3 ml. of the culture medium and the entire volume transferred into a 3 oz. bottle.

Twenty-four hours after the cultures were set up, large number of protaplasmic processes were observed around each attached tissue mass. Within the next two days, the protoplasmic processes increased in number and length, which was followed by the migration of nuclei into these cytoplasmic processes. Although a complete layer of cells was formed within a week, no cell division was observed in these cells. Most probably the cell sheet was formed by the migration of the cells from the tissue masses rather than by the multiplication of the cells.

The cells in Anopheles stephensi cultures look very much like muscle cells with large number of thin fibrils and light and dark cross striations in them. In the freshly set up primary cultures rhythmic pulsation of these cells was also observed.

Efforts to subculture these cells were not successful as on each passage decreasing number of cells attached to the glass surface without showing any increase in cell number. The cells in primary cultures remained healthy for a long time if fresh culture medium was added regularly at an interval of eight days.

V. Adaptation of Aedes albopictus and Aedes aegypti cells to media with calf and goat serum

Fetal bovine serum in the medium used for the cultures of Aedes albopictus and Aedes aegypti cells has been successfully replaced with calf and goat serum. A. aegypti and A. albopictus cells are now in 26th and 18th passage respectively in a medium with 20 percent calf serum and 11th passage in a medium with goat serum.

General morphology of cells of the both cultures adapted to the medium with calf serum is similar to the parent cells whereas the cells of the cultures adapted to the medium with goat serum show some changes. No change in chromosome numbers was noticed in any of the adapted cultures.

VI. Adaptation of cell line derived from Aedes albopictus to minimum essential medium (Eagle)

In an effort to adapt A. albopictus cell line to chemically more defined media, studies were undertaken primarily to replace lactalbumin hydrolysate and yeastolate in the original medium with minimum essential medium (MEM) of Eagle.

MEM was prepared in two different salt solution bases, one medium contained Rinaldini's salt solution as base and the second Hanks'salt solution without $NaHCO_3$.

In MEM with Rinaldini's salt solution, the growth of the cells was very poor and the cultures were discontinued after third passage. The growth in the second medium, i.e. MEM with Hanks'salt solution was satisfactory and only one type (small round) of cells grew predominantly in this medium. The cells were serially subcultured ten times.

Susceptibility of the cells adapted to the MEM with Hanks' salt solution to arboviruses was tested at the tenth passage. The adapted cell cultures were found to be as susceptible to infection with arboviruses as the cultures in the original medium.

Aedes aegypti surveys

Four separate field trips made to the towns of west coast of the Peninsular India, south of Bombay, have shown that Aedes aegypti is found only in two of the townships out of over 40 searched. Comparative studies on the prevalence of Aedes aegypti in the west and east coasts of India are in progress.

REPORT FROM THE ARBOVIRUS RESEARCH UNIT, SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH, JOHANNESBURG

Experiments on the transmission of chikungunya virus by mosquitoes

Recently experiments have been conducted with five silvan mosquito species to test their ability to transmit chikungunya virus between vervet monkeys (Cercopithecus aethiops pygerythrus). Batches of mosquitoes were fed upon monkeys which were viraemic following intramuscular inoculation with virus. Transmission to susceptible monkeys was attempted 18-21 days later, after which the mosquitoes were tested individually for virus to determine an infectivity rate which could be related to the level of viraemia in the infecting monkey.

The table shows the results of the experiments. Culex univittatus and Aedes circumluteolus were insusceptible at the infectivity doses used, while Aedes calceatus was barely susceptible. Aedes simpsoni showed high infectivity rates at both 5.5 and 3.5 logs/0.02 ml and Aedes aegypti formosus had a high infectivity rate after infection of 5.3 logs but was refractory after attempted infection at 3.0 logs/0.02 ml. C. univittatus, A. circumluteolus and A. simpsoni failed to transmit the virus, while successful transmissions were recorded for the two batches of A. aegypti formosus infected at 5.3 logs/0.02 ml.

The failure of C. univittatus and A. circumluteolus to transmit chikungunya under these laboratory conditions is of interest to us because these two species are among the most prevalent mosquitoes at Ndumu, Northern Natal, where we have obtained serological evidence for the activity of the virus.

The negative result obtained with A. calceatus also deserves comment.

Previously we have shown a 100% infectivity rate in this species after feeding on a virus suspension of 7.9 logs/0.02 ml and transmission to mice was obtained on the 8th day through the agency of one mosquito (McIntosh et al., 1963). In the same paper it was reported that baboons do not usually show a level of viraemia above 6.4 logs, while the monkeys infected in the present experiments did not show one above 5.6 logs/0.02 ml (see table). It appears therefore, from the results obtained at the infectivity doses used in the present experiments that transmission tests carried out with particularly high infecting doses give an unrealistic picture of the vectorial capacity of A. calceatus.

A. simpsoni is a common species in the forests and tree-veld of Southern Africa. It is noteworthy that this mosquito, a silvan vector of yellow fever, could not be expected to act as a vector of chikungunya in a similar way in monkey populations.

It is well known that A. aegypti is an efficient vector of chikungunya. Our experiments confirm this but indicate that the threshold of infectivity (dose infecting 10% of mosquitoes) is between 3.5 and 5.3 logs/0.02 ml.

It is hoped to extend these experiments so that all the mosquitoes which, on ecological grounds, are important candidates as vectors of the virus in Southern Africa are screened.

Reference: McIntosh et al., (1953). S. Afr. J. Med. Sci. 28: 45-52.

Table

Results of infectivity and transmission tests with 5 mosquito species and chikungunya virus

Mosquito species	titre of infecting monkey blood x	Mosquito infectivity		Transmission attempts			
		Days * on or between which test was made	Rate ‡	Day*	No. mosqs. that fed	No. infected mosqs. that fed	Result
C. univittatus	4.9	21	0/13	20	5	-	Negative
" "	4.5	"	0/13	"	5	-	"
A. circumluteolus	5.0	20-21	0/13	19	10	-	Negative
" "	5.6	11-21	0/11	"	4	-	"
A. calceatus	5.4	21-23	1/25	21	7	-	Negative
" "	4.0	22-23	2/24	"	22	2	"
A. simpsoni	3.5	18-20	18/25	18	6	3	Negative
				"	3	3	"
" "	5.5	19-21	23/24	21	2	2	" ‡‡
				"	4	3	" ‡‡
A. aegypti	5.3	21	14/19	19	20	14 or 15 †	Positive
formosus	5.3	"	22/24	"	25	22 or 23 †	"
	3.0	"	0/25	"	47	-	Negative

* After attempted infection of mosquitoes. x - log mouse LD50 per 0.02 ml.

‡ Numerator = No. mosquitoes infected. Denominator = No. mosquitoes tested.

‡‡ These two results await confirmation.

† One mosquito was untested.

In the case of A. simpsoni the infected mosquitoes were divided into two equal batches each of which was offered a susceptible monkey for the second feed.

REPORT FROM THE VIROLOGICAL SECTION OF THE DUTCH MEDICAL RESEARCH CENTRE, NAIROBI, KENYA

The four virus strains from Culex (Neoc.) rubinotus, Culex (C.) nakuruensis and Mansoni (M.) africana collected on the shore of Lake Naivasha (see No. 16 of the Information Exchange) have been identified as Uganda S virus strains. Those from Culex nakuruensis and Mansonia africana are the first Uganda S isolations reported from these mosquito species. The strain from M. africana was re-isolated.

From about 19,000 mosquitoes collected 15 miles North of Mombasa on the coastal strip of Kenya, seven virus strains have been recovered. So far two strains, from respectively Aedes (S) pembaensis and Eretmapoditis semsiimplicipes, have been identified as Bunyamwera virus. Previous isolations in Africa from E. semisimplicipes have not been reported.

Around Malindi, further North on the coastal strip, isolation experiments have been organized for the second time. About 22,500 mosquitoes were collected, partly on canopy platforms in a forest where in the past serological evidence has been found for circulation of yellow fever virus in primates. No virus was recovered from mosquitoes collected on these platforms, but five strains were isolated from mosquitoes caught on human baits at ground level.

A new method for storage of mosquitoes was successfully tried. After identification, the mosquitoes, pooled to species, are sealed in plastic envelopes and stored in a liquid nitrogen container until inoculation in newborn mice can be done in the main laboratory at Nairobi. This method, worked out by Wolff and Croon for pathogenic enterobacteriaceae, proved to be much easier than storage of mosquitoes in a portable deep freeze and regular shipments of pregnant mice to field laboratories.

Garsen is situated on the Tana River 70 miles north of Malindi and more inland. Here 3,200 mosquitoes were collected and one virus strain was isolated from a pool of An. (An.) coustani. It was identified as a member of the Bwamba group, probably Pongola virus.

Marsabit is a small township situated inland in North Kenya. During a recent serological survey 15 per cent of human sera were positive in the yellow fever mouse protection test (Bull. W.H.O., 38, 1968, 229). Since, three mosquito surveys have been performed in the area and 15,500 mosquitoes have been collected, partly on canopy platforms. The subgenus Stegomyia was represented by small numbers only. Not more than 30

Ae. aegypti were caught on human baits and Ae. keniensis was found only once. Ae. africanus and Ae. simpsoni seem to be absent from the area. Adults of these species were not collected, nor were larvae found in tree-holes or plant axils.

Aedes (Aedimorphus) dentatus group was biting man in the afternoon and evening in relatively large numbers at the forest edge in places frequented by the local population and by primates (Papio doguera and Cercopithecus aethiops). Nine virus strains were isolated from these mosquitoes, which are probably efficient vectors of arboviruses. In relation to the serological results mentioned above, they were considered to have been the most likely vectors of yellow fever in the past, a supposition which was subsequently supported by Serie's communication that yellow fever virus had been isolated from Ae. dentatus in Ethiopia.

Other isolations were done from Mans. (M.) africana (4x), Culex (C.) zombaensis and An. (C.) funestus. None of the isolates from Marsabit behaves in mice like yellow fever virus. One isolate (from M. africana) was identified as a member of the Bwamba group.

The only case in Kenya, accepted by most experts to have been a case of yellow fever, contracted the infection likely in or near Langata Forest in 1943. Langata Forest is on the plateau of Kenya, not far from Nairobi.

Mosquitoes have been collected in the forest, partly on canopy platforms, partly at ground level. Amongst a total of about 4, 200 were 1, 269 specimens of the Aedes (Aed.) dentatus group. One virus strain was isolated from a pool of Ae. dentatus group collected on a platform. It does not behave in mice like yellow fever virus.

All identifications were done at the East African Virus Research Institute at Entebbe.

(D. Metselaar)

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

Following the chikungunya epidemic in Senegal (Nov. Dec. 1966), the 1967 field collections were concentrated in two distinct ecological areas: Bandia Forest (N 14° 35 - W 17° 01), a thorny bush with baobabs and short galleries around permanent water collections, and Saboya area (N 14° 35 - W 16° 05) a mangrove gallery with, along it, a sparse forest with farmed areas.

1. Materials collected

1-1 Vertebrates

1-1-1 Bandia Forest

The recapture programme consisted in the trapping, marking and releasing of small mammals, primarily small rodents. Nine-hundred forty-one mammals were released after capture, marking and bleeding for virus isolation and serological studies. Sixty were caught again one to four times. The commonest rodents were: Mastomys sp. (666), Arvicanthis niloticus (91), Tatera guineae (53), Xerus erythropus (44), Taterillus gracilis (40), and Tatera valida (21).

1-1-2 Saboya area

In this study, the captured animals were not released. Among the rodents, there is relative abundance of Tatera valida and Mastomys sp. in the field and Rattus rattus rattus in the villages.

1-2 Arthropods

1-2-1 Culicidae

Collections are summarized in Table 1 by species and place.

1-2-2 Ixodidea

All the ticks processed for virus isolation have been collected in the Bandia Forest. The species are listed in Table 2.

1-2-3 Other Diptera

Phlebotomine flies and Ceratopogonidae (Culicoides) were in great abundance during the dry season. Processings for virus isolation are in progress.

TABLE I

Species		BANDIA		SABOYA	
		N°	Pools	N°	Pools
Anopheles	gambiae	4285	86	3889	81
	funestus	98	9		
	ziemanni	72	2		
	pharoensis	6	1		
Aedes	scatophagoides	54	3		
	aegypti	143	7		
	unilineatus	2	1	5	1
	metallicus	49	3	5	1
	luteocephalus	283	9	5848	121
	argenteopunctatus	9	2		
	punctothoracis	10	1	10	1
	albocephalus	73	4	211	9
	chamboni			190	8
	irritans	3771	77		
	abnormalis	187	5		
	dalzieli	21	1		
	cumminsi	2281	50	7	1
	ochraceus	3	1		
	furcifer-taylori	121	7	45	5
	lineatopennis	3	1		
Mansonia	africana	64	6	210	8
	uniformis	23	4	151	8
Culex	inconspicuosus	7	1		
	thalassius	741	21	2259	46
	tritaeniorhynchus	42	2	61	4
TOTAL		12348	314	12891	294

TABLE 2

	N°	Pools
Amblyomma variegatum	86	9
Aponomma flavomaculatum	6	1
Haemaphysalis houyi	432	37
Rhipicephalus muhsamae	5	1
sulcatus	12	3
Ornithodoros erraticus sonrai	510	17
TOTAL	1051	68

2. Results

2-1 Virus isolations
Virus identified

Code	Source	Place	Virus
PA 2294	Ornithodoros erraticus sonrai	Bandia	Bandia
PM 2795	Aedes (Aedimorphus) irritans	Bandia	chikungunya
PM 2916	Aedes (Stegomyia) luteocephalus	Saboya	chikungunya
PM 2932	Aedes (Stegomyia) luteocephalus	Saboya	chikungunya
RV 2967	Xerus erytropus (liver)	Bandia	chikungunya
RV 3150	Tatera valida (brain)	Saboya	new?

The ability of Aedes (Stegomyia) luteocephalus to transmit chikungunya virus under natural conditions has been proved by the infection of three mosquito catchers.

RV 3150 is unrelated to 50 african arboviruses with which it has been compared and appears to be hitherto undescribed.

2-2 Serologic studies (HI)

There is serologic evidence that various zoological groups were involved during the Chikungunya epidemic.

2-2-1 Primates

	April 1966 (before the epidemic)	Nov. 1966 - June 1967 (after the epidemic)
Galagos	0/18 +	3/26
Monkeys	3/13	24/27

+ Number positive/number tested. (Positive=complete inhibition of hemagglutination by serum at 1:20 with 4 units of antigen)

2-2-2 Rodents

The results of hemagglutination-inhibition test with chikungunya antigen and 118 rodent sera collected from May 1966 through June 1967 support the impression that the human epidemic has been preceded by a murine epizootic between June and August 1966.

	Tested	Positive	Rate
May 1966	29	0	0
June 1966	48	7	15%
August 1966	20	7	35%
Sept. 1966 - June 1967	211	5	2%

It seems also that, following a natural infection with chikungunya virus, HI antibodies are not long lasting in the rodents.

2-2-3 Other animals

	Tested	Positive	Rate
Reptiles	24	5	21%
Bats	225	3	1%
Birds	662	14	2%

These results show the participation of primates (primarily

monkeys), rodents, reptiles, and, at a lower degree, birds to the 1966 Chikungunya epidemic in Senegal. (Arbo. Info. Exchange, no. 16, p. 37).

(Y. Robin and P. Bres (Pasteur Institute), M. Cornet, R. Taufflieb and J. L. Camicas (Orstom))

REPORT FROM THE ARBOVIRUS LABORATORY, UNIVERSITY OF IBADAN, NIGERIA

Wild Animal Studies

The number of wild animals sampled for virus isolation attempts in the years 1965 through 1967 is shown in Table I. In 1965 the animals sampled were all caught within 75 miles of the central laboratory located at the University of Ibadan.

In early 1966 cryogenic containers were acquired for liquid nitrogen which facilitated the collection of samples for virus isolation attempts at greater distances and over longer periods than previously attempted. At this time and until mid-1967 the virus laboratory was afforded the opportunity of sending a staff member on many trips with the field party of the Smithsonian Institution, United States National Museum, which was then collecting mammals in Nigeria.

Virus Isolations

Ten viral types are represented among 24 viral isolates from wild animals. One of these viral types, prototype AN10065 (Fika)*, consisting of eight isolates, is the subject of this report. (Table II) Prototype virus AN10065 was isolated in 1966 from one of 18 Cricetomys gambianus (a giant rat, locally referred to as "rabbit") sampled at Fika in the Sudan savannah vegetation zone. (Annual Report, 1966). Another isolate was obtained in 1967 from one of three specimens of the same species of rat captured at Dada, also in the Sudan savannah zone. Forty-seven specimens of Cricetomys gambianus examined from other areas in the Sudan and Guinea savannah vegetation zones were negative for this virus. However, at Dada, also in 1967, five isolates, apparently identical by CF testing with AN10065

*Suggested name for prototype strain of new virus

were obtained from three additional species of wild vertebrates. These were: one from 32 Arvicanthis niloticus (a large harsh-furred diurnal grass rat); two from 38 Galago senegalensis (bush-baby); and two from 68 Tatara kempii (a large gerbil). The eighth isolate was obtained from one of 65 Mastomys natalensis, (multimammate rat) at Panyam in the northern Guinea Savannah vegetation zone.

By CF testing these eight agents are closely related to each other, but not to other agents from wild vertebrates tested at this laboratory. The log LD_{50} in three-day old mice is 7.3 and sensitivity to chloroform is 4.7 logs. Suckling mice three and 10 days old die with AST of about three days following i.c. inoculation and with AST of five or six days following i.p. injection. Subsequent testing done in May 1968 by a member of this laboratory while at YARU showed this agent to be unrelated to 58 African agents.

(Staff of Ibadan Arbovirus Laboratory)

TABLE I

YEAR	NUMBER ANIMALS SAMPLED
1965	438
1966	1212
1967	2883
TOTAL	4533

TABLE II

Isolates of Ibadan Prototype Virus AN10065 (Fika) by Date of Capture, Location, Species of Animal, and Virus Number

Date	Location	Species	Virus Number
19- V - 66	Fika	Cricetomys gambianus	AN10065
11-IV - 67	Panyam	Mastomys natalensis	AN18152
29- V - 67	Dada	Tatara kempii	AN19765
30- V - 67	Dada	Arvicanthis niloticus	AN19819
30- V - 67	Dada	Tatara kempii	AN20126
31- V - 67	Dada	Galago senegalensis	AN20027
31- V - 67	Dada	Galago senegalensis	AN20168
1-VI - 67	Dada	Cricetomys gambianus	AN19875

REPORT FROM THE MICROBIOLOGY DEPARTMENT, ISTITUTO SUPERIORE DI SANITA, ROME, ITALY

Isolation of Bhanja Virus in Italy

As referred in the sixteenth issue of the Arthropod-borne Virus Information Exchange, four strains of viruses were isolated from pools of adults of Haemaphysalis punctata, collected in Central Italy during the month of September, 1967. Another strain was isolated from adult ticks collected in October, 1967 and four from ticks collected in November, 1967. No virus was recovered from ticks collected between December, 1967 and April, 1968.

Preliminary attempts were directed to identify the prototype (ISS. IR. 205) of the nine isolates as TBE virus, because of a high level of immunity to this virus, present in goats grazing in the area, where H. punctata ticks were collected. Serological tests, however, performed with immune sera demonstrated that TBE virus and the new isolates were unrelated. Some

human and animal sera, collected in the same area, reacted with both viruses, indicating their circulation in the area.

ISS. IR. 205 virus was sent to Dr. J. Casals, International Reference Center, New Haven, U.S.A., for final identification. By CF test ISS. IR. 205 was found indistinguishable from Bhanja virus, a virus isolated in India in 1954 from Ixodid ticks and registered with No 197 of the Catalogue of Arthropod-borne viruses of the World.

Serological examination of human and animal population of Italy is in progress. Preliminary survey performed in the area showed the following percentage of positive sera to the Italian strain of Bhanja virus: humans (2%), bovines (5%), sheeps (21%), goats (72%), wild rodents (5%).

(M. Balducci and P. Verani)

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

The plaque forming ability on GMK cell line of 11 strains of the Tahyna virus isolated in various areas of Czechoslovakia, in Austria and Yugoslavia was investigated. The differences in plaque size under untreated and with protamin treated agar overlay were in correlation with the sensitivity rate of the given strain to dextrane sulphate used as model inhibitor. The sensitive and resistant strains showed marked differences in the number of intracerebral mouse passages. The dependence of the rise of sensitivity to inhibitor on repeated mouse passages has been demonstrated on two Tahyna virus strains tested after numerous successive mouse passages.

The propagation of Tahyna virus/extraneural variant of strain 236/in syrian hamsters inoculated intramuscularly showed no selective effect on the virus population. Even after 11 passages the inhibitor sensitivity of the virus has not been changed. This is a further proof of the suitability of this method, imitating the natural ways of virus circulation, for preserving the original character of a virus during the laboratory experiments.

Despite the initial infectivity of the two variants/the extraneutral and the neuroadapted variants/ of the 236 strain of Tahyna virus for human diploid cell strains, the virus could not be passaged in this cell system by the transfer of the free tissue culture fluid. The transfer of a part of infectious medium

from cell cultures 48-72 hours after inoculation onto the fresh homologous cultures led to the successive decrease of values of the virus in medium with its subsequent loss in the 5th - 6th passages. The infected cells showed no morphological alterations as compared with the uninfected controls. When part of the infected cells from the previous passage was used an inoculum, total lysis of the cells was recorded in the 3rd - 4th passages. The titres of the virus in medium reached in the case of the extraneural variant the value 4-5 log mouse i.cer. LD_{50} and in the case of neuroadapted variant values about 1 log mouse i. cer. LD_{50}.

In the study of experimental infection of mice with the Tahyna virus, one virus specimen - the 17th mouse brain passage of the neuroadapted variant of the 236 strain - was observed, which in contrast to other specimens of the same variant of this virus did not produce encephalitis in suckling mice after subcutaneous inoculation. It was not possible to detect the virus in the blood of suckling mice during 14 days following the subcutaneous inoculation of a high virus dose/10.000 mouse i. cer. LD_{50}/. The absence of pathogenic influence of this virus specimen was observed also after four successive intracerebral passages in mouse brains. Serological identification of the "subcutaneoulsy non pathogenic" virus variant of the Tahyna virus was confirmed by the virus-neutralization test. The pathogenicity of this variant after intracerebral inoculation has been preserved.

As a part of the experimental pathogenetical studies in primates virological and clinical observations on two young chimpanzees exposed to the Tahyna virus aerosol were performed. For the air-borne infection the extraneural variant of Tahyna virus strain 236 - in high doses/of 36.570 and 41.500 i.c. mouse LD_{50} respectively/ were used. The health status of chimpanzees was checked by virological and physical examinations, hematologic and serologic test prior to the inhalation experiment and in various time intervals after aerosol exposition. None of both chimpanzees manifested any clinical disease or infection. No virus could be demonstrated in either blood or on nasal mucosa of primates, at any time during the course of the experiment, which lasted two months. The results of physical, hematological and serological observations on chimpanzees - when compared with the pre-exposition date were without changes.

The negative results obtained in our previous studies in Rhesus monkeys as well as the present results make it possible to assume, that the upper and lower respiratory tract is not a portal of entry of the mosquito-transmitted Tahyna virus infection.

(L. Sefcovicova, I. Schwanzerova, V. Schwanzer, A. Simkova)

REPORT FROM THE NATIONAL INSTITUTE FOR MEDICAL RESEARCH, MILL HILL, LONDON, UNITED KINGDOM

A simple micro-culture method has been developed and is being applied to study Group B arboviruses and their antisera. The method is carried out in standard perspex haemagglutination trays and uses the PS line of stable pig kidney cells, which are incubated in air and in a conventional laboratory incubator without added CO_2. Of the 39 different Group B viruses listed in the WHO Technical Report No. 369, only two, Bukalasa Bat and Dakar Bat viruses, have failed to form clear plaques, and work is continuing on these two viruses. Antisera have been made against all 39 group B viruses and cross neutralization tests are in progress. A simple screening procedure involving only two trays for each virus has been used to test all the available antisera at a single dilution together with dilutions of homologous immune serum and cell and virus controls. Of 22 sera for which satisfactory homologous serum-virus neutralization has been demonstrated, 14 have given completely specific reactions, two show reciprocal cross reactions, ans six react with one or more viruses in addition to the homologous.

The method should be useful for the identification of fresh virus strains, and may also be applied to measure antibodies in field samples of serum.

(Mrs. A. T. de Madrid and Dr. J. S. Porterfield)

REPORT FROM THE BELEM VIRUS LABORATORY, BELEM, PARA, BRAZIL

The period covered by this report is largely restricted to the Second Quarter (April, May, June), 1968. The data are summarized in Tables I to VII which are more or less self-explanatory, however, a few comments are in order.

A small outbreak of sylvan yellow fever was uncovered in June along the boundary separating the municipalities of Abaetetuba and Barcarena about 40 km S.W. of Belem. Virus was isolated from the blood of two persons, from the viscera of four shot marmosets and from a pool of 39 Haemagogus mosquitoes.

Oropouche virus, in epidemic form, reappeared in the city of Belem early in 1968. This represents the third observed outbreak of this virus, the first epidemic occurring in Belem in 1961 and the second during 1967 in the Braganca area, some 200 km east of Belem. There was a total of 101 virus isolations made from the blood of febrile patients and an additional 67 patients demon-

strated converting CF antibodies in convalescent sera, making a total of 168 proven cases. In addition, there were two isolations of Oropouche virus from Culex quinquefasciatus (fatigans) mosquitoes collected in infected houses and held 48 hours to permit digestion of engorged blood. Neither virus nor antibodies (nt) were obtained from 36 chickens, 320 domestic rats and 60 marsupials (Didelphis) taken around infected houses.

The sentinel mouse isolates (Table IV & V) are of interest in that two of the stations are in trees, one 16 meters above ground and the other 32 meters. The former yielded six virus isolations from baby mice and the latter (operated more frequently) yielded 24 isolations. Of the 15 strains of the new Guama Group agent AN-109303 isolated during the second quarter from sentinel mice, 11 (73%) strains came from the canopy-located traps and only four came from terrestrially based traps. The same picture was seen last year, so that it would appear as if this is primarily an arboreally-transmitted agent. It will be noted from Table VII that Culex (Melanoconion) spp., designated B 19 complex, is involved. Other viruses which seem to be forest-canopy oriented, based on observations over the past two years, are Oriboca, Marituba and Apeu. Isolations of the rodent-associated viruses (Caraparu, Itaqui, Nepuyo and Guama) were concentrated in the swamp forest (varzea) stations.

Virus isolations from birds are not easily come by, nevertheless the Second Quarter produced two EEE isolates in sentinel chickens (one based at ground level and the other 32 meters up in the canopy) (Table VI) and a third isolate from the Yellow-rumped Cacique (an oriole caught in a canopy-net). Itaporanga from an Antbird represents the first isolation of this relatively unknown virus from an avian source. And it would appear that a new agent has been recovered from the White-shouldered Antshrike, another Antbird.

One other point of general interest was the isolation of Guajara (3 strains), EEE and a Group C agent from mosquitoes and sentinel mice in the Bosque Rodrigues Alves. This is a patch of natural forest which has been preserved as a public park within the city of Belem (Table VII).

From the 23rd of June to mid July, Drs. Woodall and Boshell joined the Royal Society Expedition camp in the Serra do Roncador area of eastern Mato Grosso (Rio das Mortes) where they availed themselves of the opportunity to collect vertebrate and arthropod specimens for virus studies.

In August of this year, the Utinga Forest small mammal recapture program, which had been in operation for six years (since mid 1962), was transferred to the Guama Forest Reserve (APEG). This was in line with the policy to centralize routine laboratory field activities in the one forest area. Trapping

at 16 stations (32 traps) commenced 12 August and in traps on 10 tree platforms 18 August. Also the Utinga Forest sentinel monkey stations was transferred to APEG 10 August.

(Prepared by the Staff, Belem Virus Laboratory 14/9/68)

Table I

Human arbovirus isolations, 2nd Quarter, 1968

Belém, Brazil

Virus	April	May	June	Total
Mayaro		1		1
Yellow fever			2	2
Oriboca			1§	1
Guama	2§			2
Oropouche	53	26	3	82
TOTAL	55	27	6	88

§ Not confirmed as yet by convalescent serum conversion

Table II

Oropouche virus epidemic, Belém, Brazil, 1968

(Human cases)

Cases confirmed by:	Feb	Mar	Apr	May	Jun	Jul	Total
Virus isolation	1	17	53	26	3	1	101
Convalescent serum§		13	36	16	2		67
TOTAL cases	1	30	89	42	5	1	168

§ Complement fixation conversion

Table III

Arbovirus isolations from animals, 2nd Quarter, 1968

(Belém region)

Virus and host	April	May	June	Total
Yellow fever (Saguinus ursulus)			4 (viscera)	4
Oriboca (Didelphis marsupialis)		1 (blood & viscera)		1
Group C (Marmosa murina)		1 (skin) §		1
Amaparí (Oryzomys capito)	1 (blood & viscera)	3 (3 blood, 2 urine)	1 (urine)	5
TOTAL	1	5	5	11

Note: Yellow fever from Abaetetuba & Barcarena; Oriboca and Group C from Utinga Forest, Belém: Amaparí from Serra do Navio, Amapá Territory. § Leishmannia-positive tail lesion.

Table IV

Arboviruses isolated from "sentinel" mice by month, 2nd Quarter

Belém, Brazil§

Group	Virus	April	May	June	Total
A	EEE	2		1	3
B	Bussuquara		1		1
C	Oriboca	1	4	1	6
	Murutucú	1	2		3
	Marituba		2		2
	Apeú			1	1
	Caraparú	9	11	5	25
	Itaquí	1	2	1	4
	Nepuyo	4	2		6
	Unidentified	1		2	3
Guamá	Catú	5	3		8
	Guamá	10	11	3	24
	Mojú	1		2	3
	AN 109303	6	6	3	15
	Unidentified	1		1	2
Capim	Capim	2	1		3
	Guajará	5	2		7
	Acará	4	2		6
	AN 84381		1		1
Mirim	Mirim		1		1
TOTAL		53	51	20	124

§ Note: All isolations from the Guamá Forest Reserve (APEG) except one Guajará (April) from the Bosque Rodrigues Alves city park, Belém

Table V

Arboviruses isolated from "sentinel mice" by source, 2nd Quarter, 1968

Belém, Brazil

Virus	CAPOEIRA	MATA TERRA-FIRME				VARZEA		Total
	Stat.L-1 P103/26 Ground	Stat.L-5 P103/89 Tree (32m)	P105/47 Ground	P105/47 Tree (16m)	P125/26 Ground	P301/90 Ground	Stat.L-6 P302/60 Ground	
EEE			1		2			3
Bussuquara		1						1
Oriboca	1	3		2				6
Murutucú	1		1				1	3
Marituba		1		1				2
Apeú		1						1
Caraparú	3	2			6	10	4	25
Itaquí						3	1	4
Nepuyo	1				1	4		6
Group C		1				2		3
Catú	1	2			5			8
Guamá	1	4		1	7	8	3	24
Mojú	1					2		3
AN-109303	1	9	2	2		1		15
Group Guamá					1	1		2
Capim					3			3
Guajará	1				3		2	6
Acará	3					2	1	6
AN-8431						1		1
Mirim					1			1
TOTAL	14	24	4	6	29	34	12	123

Note: One additional Guajará strain came from mice exposed in the Bosque Rodrigues Alves in Belém city.
Capoeira = secondary forest (high ground)
Mata terra-firme = primary high ground forest
Varzea = swamp forest subject to tidal inundation

Table VI

Arboviruses isolated from sentinel chickens and wild birds, 2nd Quarter, 1968

Belém, Brazil

Virus and host	April	May	June
EEE (Sentinel chickens Nº284 & 289, Cacicus cela §)	1	1§	1
Itaporanga (Thamnophilus aethiops)	1		
New virus ? (Pyriglena leucoptera)		1	
TOTAL	2	2	1

Note: All from forests on outskirts of Belém

Table VII

Arboviruses isolated from mosquitoes, 2nd Quarter

Belém, Brazil

V i r u s	April	May	June	Total
EEE			1-Culex taeniopus	1
Yellow Fever			1-Haemagogus	1
Bussuquara		1-Culex B 22	1-Culex B 22	2
Oriboca		1-Culex portesi		1
Apeú		1-Culex B 19 complex		1
Group C			1-Culex coronator	1
Guamá	1-Culex B 19 complex			1
AN-109303		2-Culex B 19 complex	1-Culex B19 complex	3
Guajará	1-Culex B 8			2
	1-Culex spp.			
Wyeomyia			1-Aedes fulvus	1
Orepouche	2-Culex fatigans			2
Lukuni	1-Aedes scapularis			1
T O T A L	6	5	6	17

Note: Viruses in mosquitoes from Belém forests except as follows: EEE, Group C, Guajará (Bosque Rodrigues Alves city park), Lukuni (Bragança)

REPORT FROM THE VIRUS DEPARTMENT OF THE CENTRAAL LABORATORIUM, PARAMARIBO, SURINAM

In the second quarter of 1968 the Arbovirus investigation program was restricted to the investigation of mosquitoes, caught by hand suction on human bait.

Only three places were selected as a source for mosquitoes; for number and species caught see table underneath.

Five viruses were isolated in 1968 and have been sent to the Regional Virus Laboratory in Trinidad.—Three of them from sentinel baby mice set out at the Troelikreek and two of them from mosquitoes.

One from a batch of 93 Culex portesi caught on human bait near Troelikreek. (Matta forest).

One from a batch of 63 Psorophora ferox caught on human bait at Leiding 16 A.

Arbo-virus catches second quarter 1968

	A.	A.G.	AT.	Total
1. Aëdes serratus	2426	1	86	2513
3. A. scapularis	246	1	162	409
4. A. terrens			16	16
5. A. taeniorrhynchus	1			1
7. Psorophora ferox	1050	1	884	1935
8. Ps. albipes	415		664	1069
10. Culex fatigans	5			5
15. Mansonia titillans	925	1392	1	2318
16. M. venezuelensis	338	2256	11	2605
19. Wyeomyia sp.	109		121	230
20. Anopheles aquasalis	28	13		41
21. An sp.			117	117
23. Trichoprosopon longipes	5		3	8
24. Trichoprosopon sp.			6	6
25. Haemagogus capricornii			61	61
27. Uranotaenia sp.			2	2
36. Aëdes fulvus	12		18	30
37. Culex 1(Crybda)	4		704	708
38. " 2(caudelli)			50	50
39. " 3			20	20
42. " portesi	1		1492	1493
43. " spissipes			479	479
44. Anopheles apicimacula	7			7
53. Mansonia pseudo titillans			59	59
54. Limatus durhami			1	1
55. Sabethes chloropterus			22	22
56. Sabethes cyaneus			7	7
59. Limatus flavisetosus			129	129
60. Trichoprosopon leucopus	2			2
63. Culex virgultus			72	72
65. Sabethes tarsopus			5	5
TOTAL	5574	3664	5192	14430

A = Leiding 16A, caught by hand suction on human bait
AG = Garnizoenspad, caught by handsuction on human bait
AT = Troelikreek, caught by handsuction on human bait

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

Bush Bush

The recovery of the rodent population continued. Despite high population densities of Culex portesi no virus activity was discovered in the area.

Turure Forest

The intense virus activity of the 1967 rainy season (May-December) stopped abruptly in early January. At that time susceptible rodents and vector mosquitoes were still abundantly available. The reason for this abrupt cessation of virus activity is at present still poorly understood. No agents were isolated in February, March and April despite continued efforts to achieve this. Activity of EEE virus was found by means of HI testing of sera from recaptured birds. The EEE activity lasted throughout the first half of 1968 but during this period the agent was not isolated. This was done only once, in August, from sentinel mice. Of 42 bird species captured Manacus manacus was the one most frequently caught and recaptured. It appeared that essentially the same information about EEE virus could be obtained by concentrating on this species only. This is now being done.

During 1968 the predominant rodent species of previous years, Proechimys guyamensis was replaced by Oryzomys laticeps. This shift may have been partly responsible for the intense activity of rodent-associated viruses from May, 1968 onwards. An intense VEE epizootic started in mid July and was still in progress two months later. As far as we know this epizootic has so far been limited to the sylvatic environment.

Mayaro

Limited investigations in this southeastern district of Trinidad started in May with monthly expeditions of one week's duration. So far no virus activity has been discovered in the area.

Laboratory

Virus circulation studies in Proechimys rats with Caraparu, Oriboca, Restan, Catu and Guama viruses showed that this species circulated Caraparu virus not at all and the others less abundantly and regularly than Oryzomys and Zygodontomys.

An isolate from Turure sentinels made in December, 1967 was identified

as Cocal virus. Serological studies with this agent showed that Cocal virus had been infecting rodents in Turure in the latter parts of 1966 and 1967. It appeared that young Proechimys which were repeatedly recaptured lost their neutralizing antibodies when about three months old. Presumably these antibodies had been of maternal origin.

(A. H. Jonkers, E. S. Tikasingh and J. B. Davies)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY,
CORNELL UNIVERSITY MEDICAL COLLEGE,
NEW YORK, NEW YORK

During the past year research included investigations of arthropod-borne viruses in the southeastern coastal tropics of Mexico and along the Atlantic lowlands of Guatemala, British Honduras and Honduras, and selected studies of Venezuelan encephalitis, dengue, Nodamura and Tsuruse viruses. VE virus in Mexico seemed to involve Culex, Aedes, and Deinocerites mosquitoes. VE viremia in hamsters was defined and related to virus feedback from sentinel hamsters to mosquitoes in nature. Contact spread of VE virus among cotton rats by urine and feces and the naso or oropharynx was demonstrated thus revealing a possible new transmission cycle of this virus in nature. A field study using sentinel equines, showed that VE virus in Veracruz, Mexico, usually inapparently infected horses and burros. A group C arbovirus from Mexico under study for several years and isolated as numerous strains from mosquitoes, sentinel hamsters and sentinel suckling mice was shown to be Nepuyo virus. Another virus was found to be Patois virus. During 1967, VE virus was recovered from Sentinel hamsters on the Atlantic coast of Central America at Belize, British Honduras and Puerto Cortez, Honduras. Nepuyo-related and Patois-group viruses were also recovered there from sentinel hamsters.

Starch, agarose and agar are of about equal usefulness for plaquing VE virus. Sodium deoxycholate, diethyl ether, chloroform, hydrocarbons and alcohols have different effects on VE viral infection, hemagglutination and complement fixation. Studies of the mechanism of inactivation of VE virus by sodium deoxycholate, diethyl ether and chloroform revealed an effect on the viral surface and ability to adsorb to cells rather than on the capsid with release of infectious ribonucleic acid or destruction of IRNA. A comparative study of virulence and antigenicities of VE virus strains from tropical America have shown them to be uniform in virulence for hamsters inoculated sc except for strains from Florida and Panama and Mucambo strains from the Trinidad Bush Bush habitat. No patterns of

virulence differences were detected with adult mice and cotton rats. Titers of HI antibody produced in cotton rats and mice (upon test against a Mexican strain) were similar with Mexican, Colombian, and Venequelan strains, whereas titers with U.S.A.-Florida, the TC83 vaccine and Trinidad Mucambo strains were lower. Nevertheless, the TC83 vaccine protected adult mice against subcutaneous challenge by strains from all regions suggesting that cross-protective immunologically distinct types of VE virus did not exist among the 41 wild strains examined. Studies with dengue viruses included preparation of seed suspensions in Aedes aegypti, experiments concerning thermal stability, sensitivity and need for protein diluent for type 4 H241 strain, difficulties in plaquing this strain, cross-protection experiments among dengue viruses based on viremia in New World monkeys and attempts to infect animals by surface routes. Information concerning Nodamura virus, an arbovirus from Japan resistant to ether and chloroform was published during the year and further studies of Tsuruse virus from Japan showed it to multiply in Aedes aegypti after inoculation or feeding.

REPORT ON THE HEMORRHAGIC FEVER SECTION OF THE EIGHTH INTERNATIONAL CONGRESS OF TROPICAL MEDICINE AND MALARIA IN TEHERAN 7 - 15 SEPTEMBER, 1968

In spite of problems in communication during the year prior to the Congress, and notwithstanding the absence of several key persons due to the lack of travel funds, sessions on viral hemorrhagic fevers were well attended and highly productive. Three meetings were held, and all scheduled papers were delivered, often in the direct fire of the new Asian influenza virus. The first of these centered on the Cercopithecus monkey hemorrhagic fever, or "Marburg disease". Clinical features were reviewed by Dr. G. A. Martini of Marburg, and etiology and pathogenesis by Drs. R. Siegert and W. Slenczka. After an incubation period of 3 to 9 days, patients experienced high fever, leukopenia and thrombocytopenia. Hemorrhagic exanthemata and enanthemata were frequently seen. Although jaundice was not common, SGOT and SGPT levels were increased, with an average OT:PT ratio of 7:1. There was evidence for 2^{o} contact infection in both Marburg and Belgrade, where two cases were traced to monkeys from the ill-fated German shipment. Mortality was about 20%. Virus was detected in guinea pigs inoculated with human blood, pharyngeal washings, urine and sperm. Virus was also found in many tissues at autopsy.

Infectivity of the agent for guinea pigs, visualization of curved rod-like particles 80 x $\mp$ 500 mu, and preparation of a CF antigen for diagnosis of human infection were reported from laboratories in Germany, Yugoslavia, Great Britain and the Soviet Union.

Results of extensive studies of viral pathogenesis in Cercopithecus and Macaca monkeys, guinea pigs and hamsters were reported by Drs. D. I. H. Simpson and C. E. Gordon Smith, Porton, England. The agent was lethal for all of these species. There was progressive viremia and widespread multiplication of virus in viscera and brain. Although cytopathic effects were not pronounced, several cell lines, especially BHK, supported multiplication of virus as shown by development of cytoplasmic inclusions and by fluorescent antibody staining.

English workers were unable to detect CF antibodies in sera from 200 Ugandan monkeys. However, Stojkovic and associates from Belgrade reported that 36 of 41 Cercopithecus were positive by CF test three months after arrival in Yugoslavia. Someone noted that Dr. R. Kissling, Atlanta, Georgia, had found CF antibodies in sera of African monkeys. A lively discussion followed, which was centered around the clinical horror of this

disease and the urgency of developing safe, reliable methods for testing monkeys for infection and immunity. Although some speakers suggested that serial passage of the agent in animals may already have produced attenuation for man, none was willing to recommend that it could be safely studied in ordinary laboratories.

Dr. Ned Wiebenga, Tokyo, Japan, reported on recent studies of Korean hemorrhagic fever (HF with renal syndrome). Cases continue to occur in Korea with their peculiar November to December and June to July peaks. Despite the use of new hosts and laboratory techniques, no clear evidence for isolation of a causative agent has been obtained. Mycoplasma, giving CPE in cell culture, but difficult to grow on artificial media, were isolated from several tissue specimens. Although no antibodies were detected in convalescent sera, the possibility of a mycoplasma etiology of the disease was not eliminated.

Perhaps the individual highlight of the meeting was the announcement of the isolation of the virus of Crimean hemorrhagic fever (CHF) by Soviet workers at the Institute of Poliomyelitis and Virus Encephalitis in Moscow. In a paper read by Dr. A. M. Butenko, this group reported recovery of several agents pathogenic for infant mice and rats from human blood specimens. By CF test all were indistinguishable and were identical with two strains obtained from patients in Bulgaria. Paired sera showed diagnostic CF responses among cases from Bulgaria, Rostov and Astrakhan regions, and Tajikistan, indicating a single etiology for disease occurring over a large geographic area. Dr. Jordi Casals, New Haven, Connecticut, gave preliminary data indicating that the Drozdov strain of CHF virus is immunologically identical to Congo virus, an agent recovered several times from cattle ticks, humans, mosquitoes and a hedgehog in Africa. The birth date of a new and fascinating international story was clearly evident.

A large 1964 hemorrhagic fever outbreak in Saigon was described by Dr. Vu Qui Dai. Some 1200 patients were studied; 90% of these were less than 10 years of age, and 45% were between 3 to 5 years old. Peak incidence occurred in July to September. Dengue virus antibody (types unspecified) was associated with the majority, although there were many cases with secondary response pattern in acute sera, and a few instances of serologic conversion to Chikungunya antigen. A survey of healthy children showed progressive prevalence of dengue HI antibodies. Thus the picture in Saigon appears to be rather similar to that (high endemnicity of more than one serotype) previously documented in Bangkok. Malaysian workers at Kuala Lumpur (Rudnick, Marchette, Garcia, and MacVean) presented the first evidence for contrasting patterns of dengue virus infection and disease in the same locality. This was Penang, which suffered an epidemic of hemor-

rhagic fever in 1962-64, and another, largely limited to classical dengue fever, in 1967. In the first outbreak, only type 2 viruses were isolated, attack rates were highest in persons of Chinese origin living in densely inhabited portions of the city, and the principal vector was apparently Aedes aegypti. In 1967, however, suburban populations were heavily involved, all four dengue virus types were recovered, and Aedes albopictus was the main vector. Dr. S. B. Halstead, Hawaii, reported on his efforts to develop and test a predictive mathematical model for the occurrence of various dengue-associated clinical syndromes based on Bangkok data and his basic hypothesis that hemorrhagic fever, and especially the shock syndrome, is the result of secondary rather than primary infection. In his view, severe disease and age-specific attack rates are related to the number of serotypes and the degree of endemnicity in a given area. A further corollary is that infection with any two serotypes confers protection against further disease. Available clinical data from many cities suggested a good, but not perfect, fit between hypothesis and observation. In the event that it were wanted (and in view of the second infection or immune disease hypothesis, the time is perhaps not ripe), Drs. Kitaoka, Ogata and Tuchinda, Tokyo and Bangkok, reported that formalin-killed dengue antigens prepared in infant mouse brain gave good protection against modest doses of live virus given IC to adult mice. They suggested that combined use of types 1 and 2 vaccine might protect against all four serotypes, although challenge of animals with their type 3 strain was not always satisfactory since death was irregular in controls.

Much new work was reported on the American group of Tacaribe viruses. Two new serotypes were described from Florida (Tamiami virus, Coleman and Bond) and Paraguay (Paraná virus, Webb et al.). Both were recovered only from cricetine rodents, Sigmodon hispidus and Oryzomys buccinatus respectively, and neither has been associated with human disease. Ecological studies of Amaparí and Junín viruses were presented by two young Latin scientists, F. Pinheiro, Belem, Brazil, and M. Sabattini, Cordoba, Argentina. More than 200 isolations of Amaparí virus were made from two species of sylvan rodents, Oryzomys capito and Neacomys guianae. Other animals were completely negative. The virus was obtained from viscera, blood, and urine, and there was strong evidence for chronic infection. An unexplained observation was the disappearance of virus from Neacomys during a three year study period. Although a few recoveries were made from laelaptid mites parasitizing the two rodents, no other evidence for arthropod transmission was obtained. In Cordoba, Junin virus was recovered from about 12 per cent of Calomys rodents, and a small number of Akodon. There was marked seasonal fluctuation in rodent populations, and isolation frequently varied to some degree with numbers of rodents. Peak populations were found at the time of corn harvests, when

human disease was most common. Several lines of evidence indicated that infection in rodents was chronic, and this was confirmed in experimental studies. Animals infected as newborns showed persistent viremia and poor antibody response.

Dr. N. Mettler, New Haven, summarized two well-studied cases of clinically diagnosed Argentine hemorrhagic fever in which isolation and serologic data showed that St. Louis virus was the cause. She observed that such confusion was likely in Buenos Aires province, where evidence now exists for the presence of the group B agent, when it is remembered that many Junín infections are accompanied by little or no clinical evidence of hemorrhage.

Dr. A. Parodi, Buenos Aires, contributed a stimulating paper on the effect of thymectomy on virus infection of infant mice. Removal of this organ within 24 hours after birth completely abolished the lethal effect of Junín, Machupo, and Tacaribe viruses for infant mice. Illness and death ensued if spleen cells from immune, isologous, adult mice were given at 8 days. These patterns were not correlated with differences in virus content in brains of mice. Titers of $10^7 TCID_{50}$ were found 11 days after inoculation in both types of animal. No "tolerant" sparing was observed, however, in thymectomized mice infected with Amapari virus. The data suggest that disease in newborns is related to cellular immunological function, and that Tacaribe viruses may not be uniform in this property. Experimental studies with Machupo virus were reviewed by Johnson et al., Panama. In the natural reservoir host, Calomys callosus, inoculation of newborns produced tolerant infection manifested by prolonged viremia, viruria and widespread tissue multiplication of virus. Although there was no overt disease, growth was retarded and fertility, especially in females, was severely impaired. No neutralizing antibodies could be detected. Infection of adult Calomys led to two patterns of chronic infection; tolerance similar to that of infants, and infection with development of neutralizing antibodies. The latter animals were fertile, shed virus into oral cavity and urine, but had no detectable viremia. Infants born to viremic females were all infected, but this occurred via milk after birth rather than in utero. Infants born to females with antibodies were completely protected against maternal infection.

This review provides ample evidence of the active interest in the study of diverse hemorrhagic fevers in many parts of the world, and gives further promise that some of the results of continued effort will have biological significance beyond the immediate considerations of the specific diseases involved. Thanks are due to all who spoke and participated in discussions at the meetings, and to Dr. Ch. M. H. Mofidi and his staff of the Congress,

whose patience never wavered as the lumbering section emerged from uncertain mists. But most of all, Prof. M. P. Chumakov, Moscow, and Prof. S. B. Halstead, Honolulu, deserve the real credit for assembling the program and seeing that it ran.

(K. M. Johnson, M. D.)