ARBOVIRAL DISEASE SECTION
ECOLOGICAL INVESTIGATIONS PROGRAM
P. O. BOX 551
FORT COLLINS, COLORADO 80521

ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

Number 19 July 1969

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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 SUBCOMMITTEE ON ARBOVIRUS INFORMATION EXCHANGE

Arbovirus Catalogue. As of this date, 143 Catalogue sets and associated abstract files have been assigned to active participants. These include 12 in Africa, 19 in Asia, 7 in Australasia, 24 in Europe, 65 in North America (including 60 within the continental United States) and 16 in South America.

The working Catalogue contained descriptions of 227 registered arboviruses at the end of the second Quarter (30 June 1969). Thirteen additional registrations have since been submitted and it is anticipated that new cards for most of these will be ready for distribution with the 15 October shipment of materials. The Catalogue editor will welcome additional voluntary registration of previously undescribed agents tentatively accepted as arboviruses, or inquiries concerning procedures for registration of "new" viruses.

In order better to promote the rapid exchange of information among qualified participants, the editor requests that reprints of publications be sent as soon as available directly to the Catalogue office:

Dr. Trygve O. Berge, Curator
Collection of Animal Viruses and Rickettsiae
American Type Culture Collection
12301 Parklawn Drive
Rockville, Maryland 20852, U.S.A.

The same plea is made in regard to Annual Reports from all participating institutes or organizations. These latter should be accompanied by a statement from the Director giving authorization to circulate pertinent information contained in these Reports to Catalogue participants on a confidential basis.

Infoexchange Mailing List. Some participants in the working Catalogue program and in the Infoexchange Newsletter have neither acknowledge receipt of material nor contributed information for two years or more. A revised mailing list is being prepared, and it is essential that individuals who wish to continue to receive this material signify their interest by submitting a short statement to this effect and verifying their current addresses. This is not necessary for those who have recently shown evidence of continued participation in these activities.

SPECIAL REPORT ON ARBOVIRUSES IN VIRAL NOMENCLATURE

Sir Christopher H. Andrewes, Chairman of the "Subcommittee on Viruses of Vertebrates", ICNV, appointed in 1967 a Study Group on Arboviruses with the request that they advise the Subcommittee on a choice of names for these viruses, that would conform with names used in other viral sets.

The members of the Study Group were: Drs. J. S. Porterfield, Chairman, J. Casals, M. P. Chumakov, C. Hannoun and M. Mussgay.

A report was drafted by the Study Group and discussed informally at a meeting of arbovirologists during the sessions of the Eighth International Congresses of Tropical Medicine and Malaria, Teheran. Valuable suggestions were made and incorporated in the report.

The draft of the report as submitted to Dr. Andrewes follows.

DRAFT OF A REPORT FROM THE ARBOVIRUS STUDY GROUP TO THE CHAIRMAN OF THE SUBCOMMITTEE ON VIRUSES OF VERTEBRATES

The Arbovirus Study Group recommends that the term "Arbovirus" be used only in an ecological sense, as defined in the Report WHO 1967; "Arboviruses are viruses which are maintained in nature principally, or to an important extent, through biological transmission between susceptible vertebrate hosts by haematophagous arthropods".

The term arbovirus has been incorrectly used in universal systems of classification to designate those viruses which contain RNA, possess an envelope and have a non-helical, possibly cubical symmetry, thus causing confusion. The Study Group strongly urges that this confusion be avoided, and suggests that a new term be used to designate those viruses which, in a universal system of classification, contain RNA, possess an envelope and have a non-helical presumably cubical symmetry (designated X-viruses for the purposes of this document).

The following names are suggested:

1) TOGAVIRUS

2) RENHEVIRUS

3) TOGARNAVIRUS

4) TOGARNHEVIRUS

As it is realized that the ICNV is not ready to adopt the use of family names, ending in-idae, the names given above are suggested as candidates from which a provisional name may be selected.

The charge given to the Study Group was to propose names, ending-virus, for those arboviruses that contain RNA, an envelope and have non-helical, symmetry, in sets equivalent to "genera", such sets being based upon the current antigenic groups. The names suggested by us are given in Table I.

We propose that Sindbis virus be accepted as the type X-virus, recognizing that although this was not the first to be isolated, it is one of the few X-viruses on which reliable information is available on basic properties.

Sindbis virus is also proposed as the type virus for its group, Sindbivirus. For Flavivirus, the type suggested is yellow fever virus.

The recommendation of names is made with the full knowledge that reliable information on fundamental properties, particularly symmetry and type of nucleic acid is known in only about 20; the symmetry in hardly any. For this reason, it is strongly urged that viruses be not placed within the remaining sets in Table I until sufficient knowledge about them is available.

The Study Group was also asked to suggest a provisional list of member X-viruses. Table I lists the names of the main Arbovirus Groups which are also X-viruses sets. The list of arboviruses in the WHO report No. 369 (1967) includes X-viruses given in Table I, and also viruses belonging to other taxa, like reoviruses (AHS, BTV), rhabdoviruses (VSV) and picornaviruses (Nodamura). Within the category of X-viruses are included some arboviruses, rubella virus and possibly murine leukaemias and avian leukosis viruses (Table II and Figure I).

Table I

PRESENT NAME USED BY ARBOVIROLOGISTS		SUGGESTED NAME IN A UNIVERSAL CLASSIFICATION	RNA established
Group A	19	Sindbivirus	+
Group B	39	Flavivirus	+
Group C	10	Marivirus	
Bunyamwera Group	14	Bunyivirus	
Bwamba Group	2	Bwambivirus	
California Group	10	Calivirus	
Capim Group	9	Capivirus	
Guama Group	5	Guamivirus	
Kemerovo Group	4	Kemrivirus	+
Phlebotomus Group	12	Phlebivirus	
Rift Valley Fever	1	Riftivirus	
Simbu Group	11	Simivirus	
Tacaribe Group	6	Tacrivirus	+
Turlock Group	2	Turlivirus	
	144		

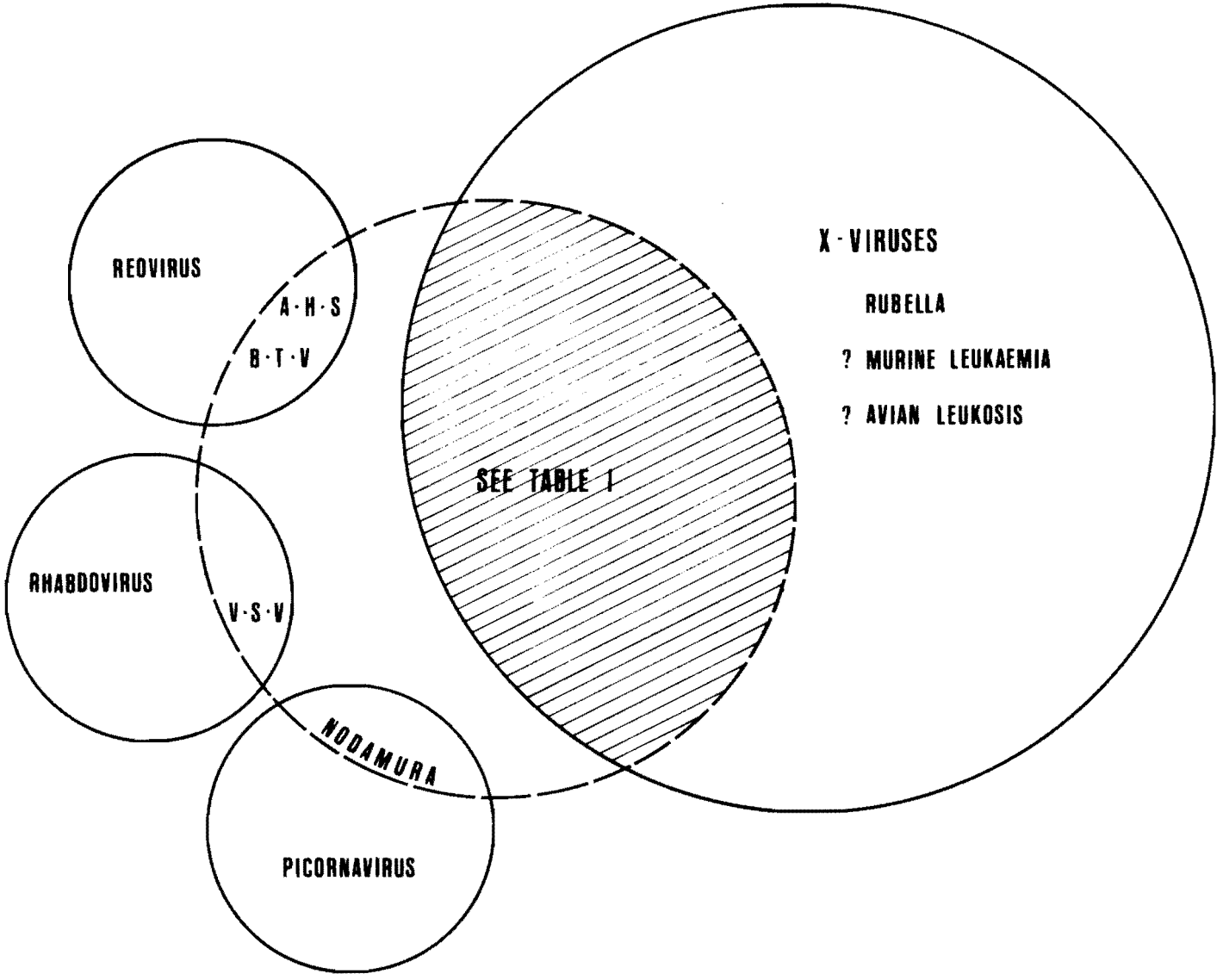
Table II

Arboviruses: comprise members, but not necessarily all members, from the following sets established on basic properties:

- 1) X-viruses (see Table I)
- 3) Reoviruses (African horse sickness, blue tongue virus)
- 3) Rhabdoviruses (VSV Group, Flanders)
- 4) Picornaviruses (Nodamura)

X-viruses: comprise members, but not necessarily all members, from the following sets based on biological properties:

1) Arboviruses
2) Rubella
3) Avian leukosis
4) Murine Leukaemias



ARBOVIRUSES

REPORT FROM THE ARBOVIRUS REAGENT PROGRAM OF NIAID: RELATIONSHIP TO OTHER PROGRAMS IN ARBOVIRUS REAGENTS IN THE UNITED STATES

The Arbovirus Reagent Program of NIH's National Institute of Allergy and Infectious Diseases was initiated as a result of a specific recommendation from the American Committee on Arthropod-borne Viruses. The program is managed by the Research Reference Reagents Branch (RRRB), Collaborative Research Programs. The RRRB Program was established to fill the critical need for uniform reference standards in the form of virus antisera and corresponding seeds. Investigators are furnished with reference reagents of uniform potency and are thus assured of utilizing materials identical to those made available to other researchers in the same field.

The Staff with guidance from the Arboviruses Committee has set priorities regarding selection of viruses for reagent production. Priority one includes arboviruses of public health significance. Priority two is given to viruses indigenous to the North American continent and of potential public health importance. The third priority includes agents useful in the laboratory as virus group representatives. Priority four includes arboviruses found in areas of major political, economic, or military importance to the United States. It is estimated that reagents to approximately 100 individual arboviruses will be produced under the program. In addition, approximately 30 grouping reagents are being produced.

The attached table lists the reagents already produced, those in production, and those assigned for production under existing contracts.

After due consideration of the technical problems involved, RRRB staff and advisors decided to produce arbovirus antibody in the form of immune ascitic fluid in mice with corresponding seed viruses being produced in suckling mouse brain. Also, control ascitic fluids are made in parallel with the immune fluids using mice from the same population. Standards call for immune fluids having a minimum CF titer of 1:64 and where feasible a minimum HI titer of 1:64 and a neutralization index log NI of 3.0.

Contracts presently in effect include eight for the production of specific reagents, one for the production of grouping reagents, and one for the certification of all arbovirus reagents produced for the program. In addition, the Program supports the up-dating of the Catalogue of Arthropod Borne Viruses of the World. This work is presently under the direction of Dr. T. O. Berge at the American Type Culture Collection (ATCC). The

Program is also supporting contracts to produce antisera for the purpose of identifying mosquito blood meals. These reagents include antisera to the mammalian, avian and reptile groups and specific antisera for nine of the most commonly known vertebrate hosts.

In general, a virus reagent set includes 250 ml of seed virus, three liters of immune ascitic fluid and 100 ml of control ascitic fluid. Seed virus and reference antisera are packaged in 0.5 ml quantities in flame-sealed glass ampules under vacuum.

The entire production, testing, and distribution program has been carried out in close liaison with the National Communicable Disease Center (NCDC) and with World Health Organization (WHO) evaluation laboratories in the United States. Close liaison has also been maintained with the American Type Culture Collection. The ATCC is under contract to lyophilize the seed viruses and furnish initial starting seed virus materials to other production contractors. The NIAID and NCDC programs complement one another in that the mission of the NIAID program is to supply research reference material and the mission of NCDC is to produce material for diagnostic and control purposes. NCDC is also one of the production laboratories for the NIAID program. Distribution of arbovirus reagents from the WHO Arbovirus Reference Laboratory (YARU) is generally limited to distribution to WHO regional laboratories and not to researchers in general.

Reagents can be obtained by completing and submitting Form NIH-381-2 to the Research Reference Reagents Branch and can usually be provided within 7 to 10 days after the request is received.

For further information on the availability of arbovirus reference reagents write to the Chief, Research Reference Reagents Branch, National Institute of Allergy and Infectious Diseases, Building 31, Room 7 A 23, Bethesda, Maryland.

STATUS OF ARBOVIRUS REAGENTS

Completed and Available	In Production or Final Testing	Assigned for Production	
Oropouch	Modoc	Oriboca	Sindbis
Bwamba	Hughes	Turlock	Bussuquara
California Encephalitis	Silverwater	SLE	Tacaribe
Group C	Manzanilla	Patois	Tensaw
Group Guama	Buttonwillow	Powassan	WEE
Group VSV	MML	Caraparu	EHD
Group Tacaribe	Hart Park	Anopheles A	YF
Mayaro	EEE	Anopheles B	Sandfly Fever
Sicilian SFF	Bimiti	Rio Bravo	Bluetongue
Colorado TF	Melao	Chagres	Guaroa
Ilheus	Group B	JBE	VEE
Guama	Polyvalent Quaranfil	Wyeomyia	Semliki Forest
Group A	Polyvalent Anopheles A	Nepuyo	Candiru
Group Bunyamwera	Group California	Sawgrass	VSV
Group Simbu	Group Capim	Kern Canyon	Changuinola
	Polyvalent Bwamba	Wesselsbron	Cowbone Ridge
	Polyvalent Patois	Zegla	Main Drain
	Polyvalent Panama	Tamiami	Catu
	Group Phlebotomus	Zika	M 1056
		Bunyamwera	WN
		Cocal	Tembusu
		Punta Toro	Congo
		Junin	RSSE
		Ross River	Chikungunya
		Twenty four other grouping fluids	

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF OTAGO
DUNEDIN, NEW ZEALAND

Growth of Whataroa Virus in Vertebrates

Suckling mice

Since suckling mice are used to detect transmission of Whataroa virus by infected mosquitoes, it is important to know the least amount of virus which can be detected, and the best method of doing this.

Ten-fold dilutions of virus were injected sub-cutaneously into groups of mice less than 96 hours old. Two days later a blood sample was taken from each mouse and tested qualitatively for Whataroa virus. The mice were observed daily for 21 days and deaths recorded. The brains of mice dying later than seven days after inoculation were tested for the presence of virus. Twenty-one days after injection surviving mice were bled and the sera tested for Whataroa virus plaque neutralizing antibodies.

Conclusions:

1. Six plaque-forming-units (PFU) of Whataroa virus injected subcutaneously infected 50 per cent of the mice.

2. All infected mice were viraemic on the second day after injection.

3. Not all infected mice died.

4. Most mice died on the fifth or sixth day.

5. Virus was not detectable in the brain of a mouse which died on the thirteenth day although it had been circulating virus on the second day.

6. Infected mice which survived developed plaque neutralizing antibodies.

7. The most rapid and reliable method of using suckling mice to detect transmission of Whataroa virus is to test them for viraemia two days after they have been bitten by mosquitoes.

Guinea pigs

A study of the virulence of viruses has reached a stage where it is important to be able to detect antibody at the earliest possible time in the course of an infection. Whataroa virus was injected subcutaneously into young guinea-pigs to see if this would provide a suitable system for this investigation.

The first group of animals were each injected with 8,000 pfu. of virus. Five guinea-pigs were bled daily to follow the course of viraemia, while three others were injected but not bled. On the sixteenth day all guinea-pigs were bled to provide sera for antibody tests.

None of the guinea-pigs died or developed signs of disease during the experiment. The five which were bled daily all developed viraemia. The mean levels of virus each day showed that there was little virus circulation for the first two days, but there was then an increase to a peak of $10^{3.9}$ pfu/ml of blood on the fourth day. No virus was detectable on the seventh day. On the sixteenth day after inoculation the sera of all animals contained plaque neutralizing (P.N.), complement fixing (C.F.) and haemagglutination inhibiting (H.I.) Whataroa virus antibodies.

A further nine guinea-pigs were injected in the same way with the same dose of virus. On the fifth day, which was during the terminal stages of viraemia, serum samples were taken and tested for P.N., C.F., and H.I. antibodies. None contained C.F. or H.I. antibodies, but P.N. antibodies were demonstrated in three of the sera, even in the presence of low levels of circulating virus.

Future work will involve the examination of other serological tests for their sensitivity, and dissociation of virus-antibody complexes.

Whataroa virus in Culiseta tonnoiri

Two of the original isolations of Whataroa virus were made from the mosquito Culiseta tonnoiri in South Westland. Difficulty has been experienced in the transporting of C. tonnoiri from South Westland to Dunedin in the past, but recently it has been found that if these mosquitoes are allowed a blood meal before transportation they stand the journey much better.

A study was made therefore of the multiplication of this virus in C. tonnoiri adults captured in Westland and transported to Dunedin. Both intrathoracic injection and feeding on M78 infected mice were used, and virus survival measured by grinding whole mosquitoes at intervals after infection. The

virus content of the suspensions was assayed by inoculating primary duck embryo monolayers and looking for plaque production.

In the first experiment, mosquitoes were inoculated intrathoracically and then assayed for virus at Day 0 (control), Days 2, 6, 7 and 8. In the second experiment, the mosquitoes were again inoculated intrathoracically, both the samples were taken at Hour 0 (control), 6, 12 1/2, 24, 30, 36 and 48 hours.

In experiment 1 the dose per mosquito was about 100 pfu. which rose to about $10^{6.5}$ pfu. in 48 hours. In experiment 2 the dose was ten times higher, but the level reached after 48 hours was about the same as in experiment 1. The observation, which has been made already with Whataroa virus in Aedes australis, that the virus level falls noticeably immediately after inoculation was also made with C. tonnoiri. The average pfu. count per mosquito fell from 1×10^3 pfu./mosq. at 0 hours to 3.4×10^2 pfu./mosq. at 6 hours. The virus did not completely disappear from any of the insects tested.

Having shown that Whataroa virus could multiply to quite high titres after intrathoracic injection, an attempt was then made to infect by feeding field-caught C. tonnoiri on suckling mice 48 hours after the mice had been infected with Whataroa virus. Since C. tonnoiri is a very difficult species to rear or maintain in the laboratory, the feeding was carried out in Westland in the field and the engorged mosquitoes were transported back to the laboratory in individual vials. The survival rates using this method were very good. Mosquitoes were frozen down at intervals for virus assay, and transmission to fresh uninfected sucklings was attempted at intervals on and after the 10th day. Before each feeding attempt, the insects were deprived of moisture for 2-3 days.

Although 136 engorged mosquitoes were originally obtained, it was found that very few take a second blood meal. However, many did probe and it was possible virus could have been transmitted, so all mice which had been exposed were observed carefully and in some cases bled out 48 hours after exposure. Testing of blood for virus had been found to be more sensitive for the detection of virus, than illness or death was.

Virus multiplication and survival in blood-fed C. tonnoiri was measured by titrating all mosquitoes dying at the various times during the experiment. Several mosquitoes with obvious blood contained no virus, but, in general, the virus rose to between 10^5 and 10^7 pfu./mosq. within 24-48 hours of the blood meal. The average intake was calculated to be only 2000 pfu.

The level in mosquitoes killed 35 days after the initial meal was just under 10^7 pfu.

Only one of the five mosquitoes which engorged a second time transmitted virus - this was 24 days after the original meal. The mosquito was found to contain 1.0×10^5 pfu. when killed just after the second feed. Two other transmissions were obtained, one on the tenth post-feeding day by a mosquito which contained 5×10^4 pfu. and one on the 16th day by a mosquito containing 5.6×10^5 pfu. Neither of these latter two had obvious blood in them when they were ground, but both had obviously probed. The fact that three transmissions were obtained with such small numbers of second feedings under laboratory conditions suggests that in nature C. tonnoiri could be an efficient vector for this virus.

(F. J. Austin, T. Maguire and J. A. R. Miles)

REPORT FROM THE ARBOVIRUS RESEARCH UNIT HOOPER FOUNDATION, UNIVERSITY OF CALIFORNIA MEDICAL CENTER, SAN FRANCISCO, CALIFORNIA, AND THE FACULTY OF MEDICINE, UNIVERSITY OF MALAYA KUALA LUMPUR, MALAYSIA

Mosquitoes

A check list of Malaysian mosquitoes has been prepared and a limited number of copies is available to interested persons. It includes 19 genera, 48 subgenera (one undescribed), and 345 species, subspecies, and varieties (21 undescribed). Aedes (Stegomyia), which includes the known vectors of dengue viruses, is represented by 10 species.

The first record for the mosquito genus Culiseta Felt in Southeast Asia was made in our 1968 collections. The distribution of this genus is predominantly holarctic and Australian with Ethiopian and Oriental representation. The species collected in Malaysia is previously undescribed; a full description has been prepared and is in press. The new species is placed in the subgenus Climacura Howard, Dyer and Knab. This subgenus is represented by only five species with widely disjunct distributions -- North America, Australia, New Zealand and Malaysia.

During several years of field work, numerous observations were made of mosquitoes attacking and feeding on fish (Periophthalamus spp., mudskippers) in mangrove swamps. Mudskippers spend a considerable time out of water or partly submerged in their holes at low tide. While out of water, they are frequently and viciously attacked by mosquitoes of the subgenus Aedes (Rhinoskusea). These mosquitoes use crabholes as resting sites both before and after taking a blood meal. As many as 15 or more mosquitoes have been observed attacking a large fish at one time. Blood meal analyses have confirmed the field observations. Thousands of Aedes (Rhinoskusea) mosquitoes have been processed for virus isolation without success.

Human Disease in Malaysia in 1968

Specimens from 776 cases of human illness of suspected viral etiology were processed for virus isolation and/or serology during 1968. Virus infection was confirmed in 208 cases: 171 dengue, 33 Japanese encephalitis, 1 Herpes simplex, and 3 unidentified viruses.

The average age of the confirmed JE cases was 9.6 years; 66.7% were males and 33.3% were females. Analysis of distribution by ethnic group

indicates that Indians and foreigners were at greater risk in developing encephalitis due to JE virus than were Chinese or Malays. Cases occurred throughout the year with no distinct seasonal activity peak.

The strain of Herpes simplex virus was isolated from the brain of a fatal case of encephalitis. The three unidentified viruses were recovered from the serum of a fever of undetermined origin and from cerebrospinal fluid of two cases diagnosed as encephalitis.

The average age of the 171 laboratory confirmed cases of dengue was 24.2 years. 64% were males and 36% females; 40% Chinese, 20% Malays, 20% Caucasion (of foreign origin and temporary residence), 15% Indian and 5% were of other groups.

Epidemic Dengue in Malaysia

The 1966-1968 dengue epidemic in Malaysia was the most extensive on record for that country. The disease was relatively mild, with very few of the severe symptoms seen in dengue hemorrhagic fever. The total number of cases was undoubtedly many times the 483 in which laboratory confirmation of the clinical diagnosis was made. No deaths were recorded due to dengue or dengue-like disease during the epidemic.

Inoculation of acute serum specimens and mosquito suspensions into suckling mice resulted in the isolation of 101 strains of dengue virus; 92 were from human sera, 5 from Aedes aegypti and 4 from Ae. albopictus.

To date, 51 strains have been typed by plaque reduction neutralization test in vero cells:

Dengue II:	Human origin	10
	Ae. aegypti origin	2
Dengue III:	Human origin	1
Dengue IV:	Human origin	35
	Ae. aegypti origin	2
	Ae. albopictus origin	1

Of the 50 strains yet to be typed, 46 are from human serum, 1 from Aedes aegypti, and 3 from Ae. albopictus.

Previous to this work, only dengue I and dengue II had been isolated from peninsular Malaya.

Endemic Dengue in Malaysia

Carey Island Study Area

An intensive study of two relatively remote areas has been initiated. One site, located on Carey Island, is a producing rubber and oil palm plantation bordered by mangrove swamp. At the study site, there is a small population of 299 Tamil laborers living in a single group of 62 quarters (labor lines) with associated clinic, school, and latex collection depot. The population is very sedentary and only rarely do individuals leave the island. Monkeys (macaques and leaf monkeys) inhabit the mangrove swamp and forage in the rubber.

Sentinel monkeys and chickens are stationed at ground to canopy level in the labor lines, rubber, mangrove forest, and rubber-mangrove fringe. A 75 foot tower has been constructed in the rubber-mangrove fringe to allow adequate sampling of canopy mosquito populations. Routine mosquito collections are made by a variety of methods at all levels.

The human population is being utilized as a sentinel population and the inhabitants bled at 6-month intervals. Preliminary analysis of HI results on 187 serum samples collected on the initial bleeding showed the following:

33	negative for group B antibody
16	positive for dengue antibody only
3	" " JE only
1	" " Tembusu only
0	" " Zika only
134	" " two or more group B antibodies

The second bleeding, in which 124 samples were obtained, have not been tested yet.

Intensive search has failed to recover any Aedes aegypti in the study site. Aedes albopictus is very numerous and small numbers of other Ae. (Stegomyia) mosquitoes are present.

Mosquito collections made from the tower at various elevations indicate that A. albopictus moves from ground level to canopy level at dusk and

will feed on the way up.

Two viruses which may be strains of dengue but have not been completely identified yet have been isolated from A. albopictus mosquitoes collected in the mangrove swamp forest and in rubber adjacent to the swamp.

Additional isolations of dengue-like viruses have been made from A. albopictus collected in an A. aegypti-free coconut grove on the opposite side of Carey Island from the study site, and in a relatively isolated, A. aegypti-free, rubber estate on the mainland.

Nineteen sentinel monkeys have been stationed permanently in the Carey Island study site. Monkey #256 stationed for 10 months in rubber-mangrove fringe 40 feet above ground converted serologically for group B antibody. The HI results suggest this may have been a Zika virus infection. Further study is under way.

Monkey #178 in the mangrove swamp stationed 33 feet above ground for 8 months converted serologically for group B antibody. The HI results suggest a dengue infection. Further work is in progress.

Chicken #214 stationed in the labor lines converted serologically for group B. The HI results suggest a Tembusu infection.

A trap-release-recapture program has netted 34 wild Macaca irus monkeys from the mangrove swamp. A number of recaptures have been made, but no serological conversions have occurred.

Carey Island has provided the first confirmed cases of dengue in a rural area of Malaysia. The first case was found in May 1968 followed by another 10 cases in various parts of the island. The serology of the patients and the behavior of the virus isolates in mice suggest that dengue type 4 was the agent involved.

Tanjong Rabok study area

A second intensive study site has been established at Kampong Tanjong Rabok, a small isolated aborigine village located in disused rubber at the tip of a tongue of land that extends into an extensive area of freshwater peat-swamp forest. Mosquito surveys demonstrated the presence of A. albopictus but no A. aegypti mosquitoes.

A study similar to that on Carey Island has been initiated.

The population of 59 persons was bled initially and will be bled again at 6-month intervals for serological study. Eleven sentinel monkeys are stationed permanently in the area.

Monkey #5 (which has been on sentinel duty in various forest habitats since November 1965) converted serologically to group B four months after being stationed 20 feet above ground at Tanjong Rabok. A dengue virus was isolated from a blood sample taken prior to conversion. The isolation has been validated by reisolation from the original sample, but it has not been typed yet.

Monkey #18 stationed in a pole house 7 feet above ground converted serologically to group B after 6 months.

Zika virus in Malaysia

The isolation of Zika virus from Aedes aegypti collected in shop houses in Bentong was reported previously. Analysis of sera from over 2,000 inhabitants in Malaysia is not complete, but preliminary results indicate that Zika virus is being transmitted among urban and rural populations. In a sample of sera from 206 forest-dwelling aborigines with group B HI antibody, 27% had highest titer to Zika virus. Sixteen percent of the 1- to 4- year- olds had higher titer to Zika than to JE, Tembusu, Langat, dengue types 1, 2, 3, and 4. Eleven samples had Zika antibody at a significant titer in the complete absence of measurable antibody to any other group B virus tested. (Twenty-two samples had dengue antibody only and four had JE antibody only). These results suggest that Zika virus occurs naturally in the Malaysian forest and involves mosquitoes that bite man. No illness attributable to Zika virus, however, has been unequivocally demonstrated.

Similar serological results on a large number of wild monkeys from a variety of habitats strongly indicates the existence of natural cycles of Zika virus activity in Malaysian forest mosquitoes and primates.

Philippine Hemorrhagic Dengue Epidemic of 1966

Typing of over 50 strains of dengue virus isolated from acute sera of Manila patients was essentially completed. Of the 49 strains typed by mouse neutralization tests and/or PRNT in Vero cells, 39 were type III, 6 type IV and 4 were type II.

The virus identifications confirm the interpretation of serological results

that dengue type III was the principal etiological agent involved in the 1966 epidemic.

Malaysian Virus Isolates

Characterization and identification of virus strains has been complicated by the apparent presence of a passenger virus (minute virus of mice - MVM) in some stocks. It is not yet known if MVM was picked up in the mouse colony in Kuala Lumpur or in the one in San Francisco.

A strain of Q-fever rickettsiae (Coxiella burneti) was isolated from a pool of Haemaphysalis nadchatrami ticks. A strain of Rickettsia typhi was recovered from Rattus r. diardi collected in a rural area.

Lanjan virus, previously isolated from Dermacentor and Haemaphysalis ticks in Malaysia, was recovered from a pool of Ixodes granulatus ticks.

Several strains of a virus resembling Tembusu have been isolated from pools of Culex gelidus mosquitoes. It is closely related to Tembusu, but can be distinguished from it by cross-neutralization tests in mice.

Viruses not dengue nor dengue-like have been isolated from Aedes aegypti, sentinel monkey, wild monkey (P8-1519), Pteropus vampyrus, and human serum.

Tropical Canine Pancytopenia

This disease, first described in 1967, has caused the death of several hundred dogs (mostly pure bred Alsatians) in Malaysia and Singapore. An outbreak of the disease, characterized by pyrexia, epistaxis, hemorrhagic diathesis of all organ systems, bone marrow aplasia and pancytopenia, occurred in Kuala Lumpur in 1968 among locally-bred and imported dogs. Although the disease incidence appeared to be associated with heavy tick infestations in Singapore, such a correlation could not definitely be demonstrated in the Kuala Lumpur cases. Hundreds of ticks (Rhipicephalus sanguineus) found on infected dogs or in their kennels have been inoculated into suckling mice without isolating any disease agent. The etiology remains unknown, but it has been experimentally transmitted by inoculation of whole blood from acutely ill dogs to healthy puppies. The incubation period in naturally infected dogs is not known, but the subclinical stage of the disease, as indicated by pancytopenia, may last for months. The naturally acquired disease appears to be almost 100% fatal, although some dogs with distinct pancytopenia have survived for a year or longer after an attack of epistaxis. One local mixed-breed puppy experi-

mentally infected at this laboratory developed a pancytopenia within three weeks of inoculation and the blood cell counts returned to normal three months after infection. Transmission studies, epidemiologic and histopathologic investigations are continuing.

(Nyven J. Marchette, Elene Dukellis and Eulalia Venzon - San Francisco. Albert Rudnick, Richard Garcia and Duncan MacVean - Kuala Lumpur Malaysia) .

REPORT FROM THE VIRUS LABORATORY, DEPARTMENT OF MEDICAL MICROBIOLOGY, INSTITUTE OF HYGIENE, UNIVERSITY OF THE PHILIPPINES, MANILA, PHILIPPINES

Isolation of Chikungunya Virus from Clinical Cases

During the latter part of 1967 an outbreak of a disease characterized by fever, rash and severe joint pains was observed among the adult population of Manila and environs. While the fever was of short duration and the rash fleeting, the joint pains usually lasted for several months after the acute illness. There were no hemorrhagic manifestations.

Bloods, consisting of acute and convalescent samples were collected from 49 adults with clinical manifestations described above. HI and CF tests were done on these samples using dengue 2 (1751) , dengue 3 (H87) , dengue 4 (H241) , Japanese encephalitis (Nakayama) , chikungunya, Semliki Forest, and sindbis antigens. The antigens were sucrose-acetone preparations from suckling mouse brain. Serum samples were pre-treated with kaolin and goose red blood cells.

Thirty of the 49 paired serum samples or 61% showed significant antibody conversion to chikungunya antigen alone by HI test. Six serum samples collected 7 or more days after onset of illness had high stationary antibody titers (1:640 or greater) to this virus.

Of 46 paired serum samples tested by CF test 30 or 65% showed significant immunologic response to chikungunya antigen. Six had high stationary antibody titer (1:128 or greater) to this virus.

Four paired blood samples showed significant increase in antibody titer to the group B antigens in both tests.

Twenty-six acute serum samples were inoculated in day-old suckling mice by intracerebral and intraperitoneal routes. Illness in the animals was observed to occur 30-48 hours after inoculation. Fifteen isolates were obtained. Early passage brain suspensions were found to be non-hemagglutinating. Hemagglutinins developed after the 5th mouse passage in 8 isolates. These were identified by HI test using antisera to dengue 2, dengue 3, Japanese encephalitis, chikungunya, Semliki Forest, and sindbis viruses and 6 of the isolates.

Seven of the 8 isolates studied were inhibited from agglutinating goose red blood cells by chikungunya antiserum as well as antisera to 6 of the isolates. None of these were inhibited by antisera to D2, D3, JE and SF. One of the isolates, being most likely dengue virus, was inhibited by antisera to D2 and JE. This isolate as well as three others were inhibited in low titer by sindbis antiserum. Three of the antisera prepared from the isolates were likewise inhibitory in low dilutions to the hemagglutinating activity of a strain of sindbis virus isolated locally. The HI activity of D2, JE, Ch, SF antisera were specific.

The isolates obtained in this study are the first strains of chikungunya virus isolated in this country.

It is remarkable that no referrals for virologic studies from children with similar manifestations were obtained during this period. The absence of such cases in children may have been more apparent than real since they may have been clinically diagnosed as other better known illnesses in children.

REPORT FROM THE VAN HEUWELING LABORATORY FOR MICROBIOLOGICAL RESEARCH, SILLIMAN UNIVERSITY MEDICAL CENTER, DUMAGUETE CITY, PHILIPPINES

An epidemic of febrile illness occurred in the coastal town of Amlan, Negros Oriental, Philippines, during 1968. The disease was clinically recognized as Chikungunya Fever, and was characterized mainly by fever, skin rash, arthralgia, and at times frank arthritis. An attack rate of 29% was recorded. All age groups were affected, though not in equal proportions, and no fatalities were reported.

Amlan Study: H I Test Results on Animals and Birds

Source of test Sera	Arbovirus Antigens		
	Chikungunya	Sindbis	JBE
Rats	2/112 •	0/112	102/112
Rats	3/56	2/56	52/55
Birds	0/23	0/23	21/23
Domestic Animals	2/67	1/67	29/67
Pigs	1/34	0/34	6/34
Chickens	0/19	0/19	16/19
Dogs	1/4	1/4	4/4
Geese	0/2	0/2	1/2
Sheep	0/5	0/5	0/5
Ducks	0/2	0/2	2/2
Calf	0/1	0/1	0/1

• Number positive at 1:20 screening dilution/number tested

Sera from the ill and well populations as well as from rodents, birds, and domestic animals were collected for serological tests. The results of HI tests on human sera were as follows (using chikungunya virus antigen): (1) convalescent sera from individuals who manifested febrile illnesses with rash - 93.0% positive; (2) convalescent sera from individuals who had febrile illnesses but no rash - 52.0%; (3) sera from individuals who remained asymptomatic during the epidemic -- 54.0%. Some activity of group B arbovirus was also detected in the study population.

HI results on animal and bird sera are illustrated in Table I. Very few of the sera reacted to chikungunya antigen at the 1:20 screening dilution. The one dog which gave a positive reaction had a titer of 1:80; this serum was also positive to Sindbis antigen at 1:20 dilution. A remarkably high number of sera were found positive to JBE antigen.

(F. F. Macasaet, G. W. Beran, and A. C. Alcala)

REPORT FROM THE DEPARTMENT OF PREVENTIVE MEDICINE, THE RESEARCH INSTITUTE FOR MICROBIAL DISEASES, OSAKA UNIVERSITY, OSAKA, JAPAN

I. An Intracellular Component Associated with Chikungunya Virus - Specific RNA

Monolayer cultures of BHK-21 cell, clone 13, were infected with Chikungunya virus, African strain, which had been adapted to this cell line. After 2 hours' adsorption of the virus, the cell cultures (10^9 cells) were treated with Actinomycin S_3 (1 µg/ml in Eagle's minimal essential medium) for 2 hours and then they were labelled with H^3-uridine (0.4 µc/ml) in the presence of Actinomycin in S_3 until 16 hours after infection.

The infected culture fluids were removed and cell sheets were scraped into 6 ml of STE (0.02 M Tris-HCl, 0.1 M NaCl, 0.001 M EDTA, pH 7.6) and homogenized in a Waring blender. The homogenate was centrifuged at 700xg for 10 min. and the supernatant was analyzed by sucrose gradient sedimentation (10-35% sucrose in STE, 30,000 rpm for 60 min. in a SW-39 rotor of Beckman model L ultracentrifuge). Fractions were collected from the bottom of the tube. Each fraction was assayed for its Chikungunya virus infectivity on VERO cell monolayers and for its acid-insoluble radioactivity and, after RNA extraction by hot-SDS-phenol method, for its RNA

infectivity on VERO cell under hypertonic conditions.

The results (Fig. 1) indicate that the Chikungunya virus infectivity sedimented far toward the bottom, while the main peak of acid-insoluble radioactivity was found near the middle of the gradient (fraction 7). The distribution of RNA-infectivity was almost parallel to that of the radioactivity except the top part of the gradient.

This component associated with Chikungunya virus-specific RNA in fraction 7, which was revealed by infective RNA and virus-specific incorporation of H^3-count, tended to accumulate inside the infected cell at the late stage of infection. This component was scarcely found in the infected tissue culture fluid, in which the main carrier of virus-specific RNA seemed to be the mature virus. Clear-cut relationship between this component and the precursor of the virus has not yet been obtained.

(Akira Igarashi and Konosuke Fukai)

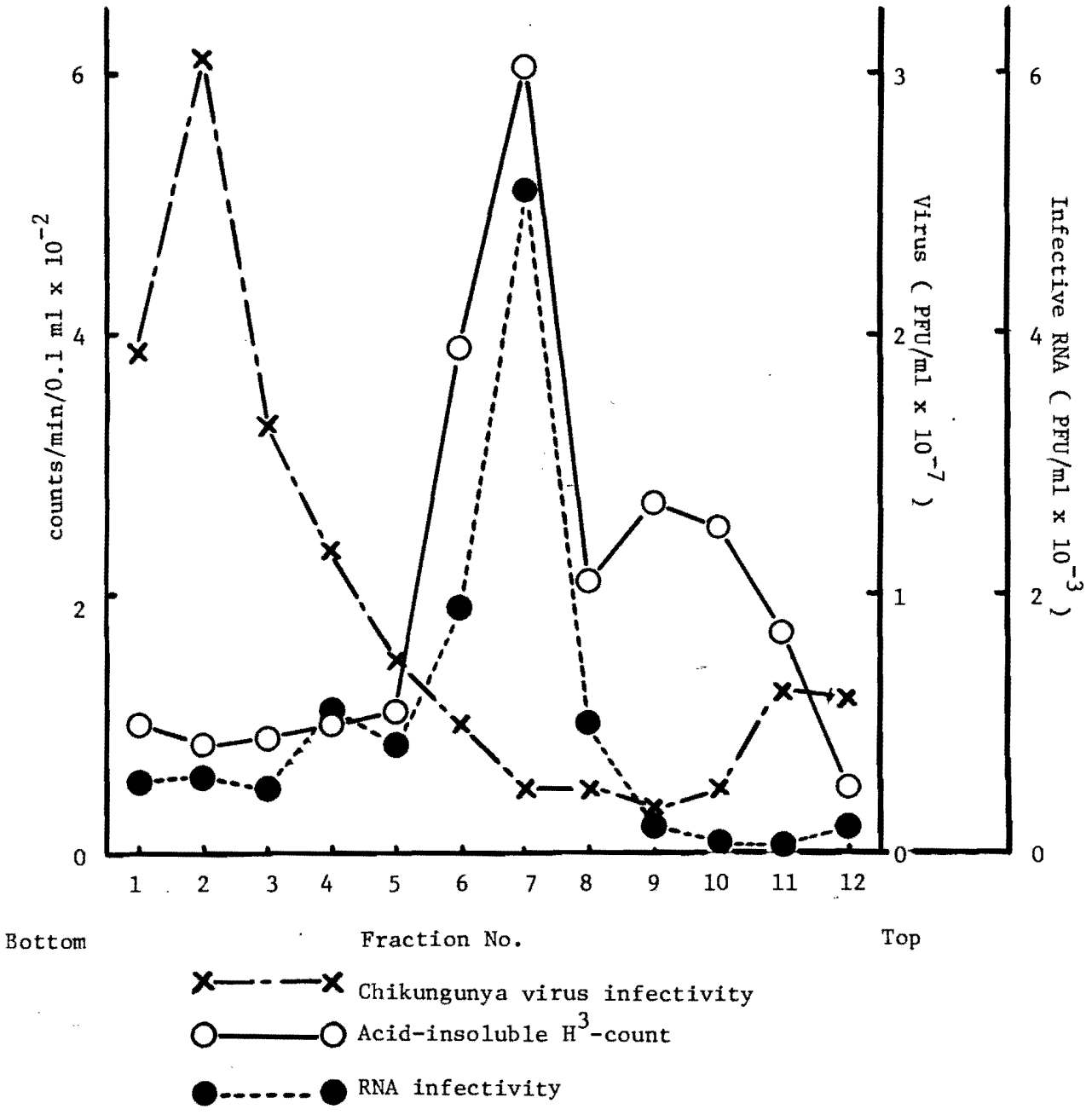
II. The "Ht" Component in Japanese Encephalitis Virus Preparation

As reported before, two forms of Japanese encephalitis virus (Hr and Hs) were identified in infected mouse brain preparation. In the course of studies on the characteristics of these two forms, one more hemagglutinating component has been found in top fraction of density gradient column and temporarily named as the "Ht" component.

In the extract from infected mouse brains centrifuged on a sucrose density column (5-23%), the hemagglutinating activity was separated in three peaks: rapid-sedimenting Hr, slower-sedimenting Hs, and the slowest-sedimenting Ht. While Hr fraction consists of complete virions and Hs fraction consists of partially degraded virions and fragmented envelopes when examined in the electron microscope, observation of the Ht fraction revealed numbers of smaller ring or rosette-shaped particles with a diameter of ca. 160 Å.

The particle morphology suggests the radial symmetry in the particle and the circular arrangement of seven to nine smaller units were shown in rotation analysis of the patterns. One, two, or 0 of the smaller units are enclosed inside the ring-like structure corresponding to the number of the units on the peripheral circle. This does not necessarily reflect the existence of three kinds of the particles but rather to the projections to 2,

Fig. 1


counts/min/0.1 ml x 10^{-2}
Virus (PFU/ml x 10^{-7})
Infective RNA (PFU/ml x 10^{-3})
0
2
4
6
1
2
3
Bottom
Fraction No.
Top
Chikungunya virus infectivity
Acid-insoluble H^3-count
RNA infectivity

3, and 5-fold axes of the particles considered as regular polyhedron (icosahedron or dodecahedron) alike to the case of ferritin molecule.

The buoyant density of Ht determined by CsCl equilibrium density centrifugation (initial density 1.24 - 1.26, SW-39 rotor, 35,000 rpm., 40 hrs.) is 1.248 + 0.004 g/cc, whereas those of Hr and Hs are 1.304 $\pm$ 0.012 g/cc and 1.230 $\pm$ 0.010 g/cc, respectively.

In the cross hemagglutination-inhibition tests among Hr, Hs, and Ht, no significant difference was detected in their antigenicity and the pH dependence of Ht hemagglutination is similar to those of Hr and Hs.

It has not yet been determined if Ht composes some parts of the virion and at present, no chemical degradation of the virion produces Ht particles successfully. The Ht fraction is also recoverable from extracts of JE-infected tissue culture not only from the brain preparations. Particles similar to Ht are also found in the extract from Chikungunya virus (group A) infected tissue cultures.

Several authors have already noticed the association of smaller particles other than virions in preparations of some arboviruses (Cheng, 1961; Mussgay et al., 1963, 1964; Osterrieth et al., 1966; McGee-Russell et al., 1967; Slavik, 1967; Bastardo et al., 1968; and Faulkner, 1968), and Ht-like particles could be found in electron micrographs in several papers, nevertheless the authors did not pay attention to this type of particles (Kitaoka et al., 1963; Klimenko et al., 1967).

Although the origin and the nature of the "Ht" particles has not yet been cleared, it may be suggested that the existence of this type of particles in virus preparations is the common feature throughout the group of arboviruses.

(Konosuke Fukai and Akira Igarashi)

REPORT FROM DEPARTMENT OF MICROBIOLOGY
KOBE UNIVERSITY SCHOOL OF MEDICINE
KOBE, JAPAN

I. Suspected Hemorrhagic Fever Cases in Indonesia

During 1968 suspected HF cases were found in the urban areas of Surabaja, East Djawa. This is perhaps the first medically recognized HF occurrence in Indonesia. A cooperative study on the disease was conducted by medical teams of Airrlanga (Surabaja) and Kobe Universities. Serologic tests of limited scope gave a presumption that dengue virus of the multiple types might be involved. The clinical and laboratory data collected will be published in the future under the co-authorship of Indonesian and Japanese investigators.

II. Combined Immunization of Humans with Tissue-Cultured (Human-Attenuated) Type 1 Dengue (D1) and Yellow Fever (YF) Viruses

Preliminary experiments indicated that D1 (Mochizuki strain) virus multiplied in trypsinized canine kidney tissue cultures (CKTC). While CPE in the infected cells was of slight degree, specific hemagglutination-hemadsorption with goose erythrocytes was positive.

Ten adults were divided into 5 groups, and each group consisting of 2 subjects was inoculated with (1) live D1V, alone, cultivated either in (1a) monkey (M. fuscatus) kidney tissue cultures (MKTC) or in (1b) CKTC; (2) live D1V and YFV (commercial 17D vaccine) simultaneously; (3) live D1V, first, and 2 weeks later with YFV; and (4) YFV, first, and 2 weeks later with live D1V. Intracutaneous injection was done in each instance with 0.25 to 0.3 ml infected culture fluid or vaccine emulsion including active virus of approximately 300 to 3,000 mouse i. c. LD_{50}'s. Two shots for each virus were given at 10 weeks interval. No abnormal clinical signs were recognized after the inoculations. Serum was taken from each subject and HI antibodies were measured. Fractionation of serum by sucrose density gradient centrifugation (Suc-DGC) together with mercaptoethanol (ME) treatment were also performed. Schedule of experiments and excerpted results are shown in Fig. 1 and Table 1.

FIG. 1.

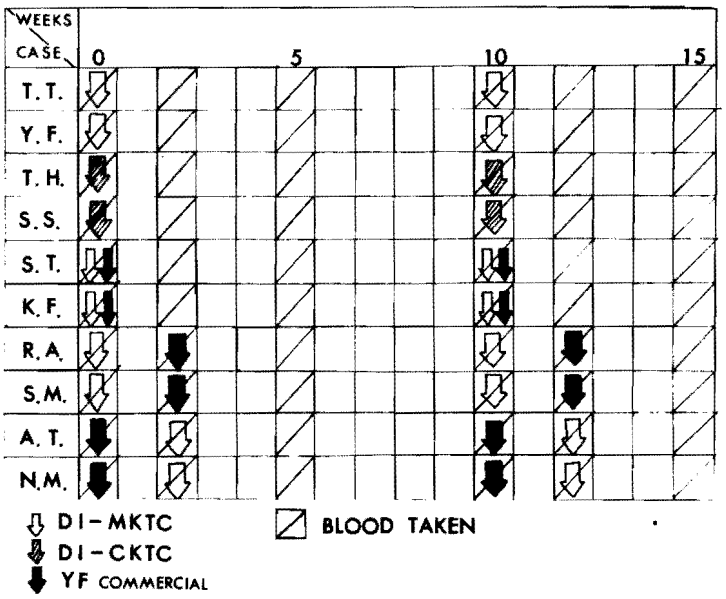

WEEKS
CASE
0
5
10
15
T.T.
Y.F.
T.H.
S.S.
S.T.
K.F.
R.A.
S.M.
A.T.
N.M.
DI-MKTC
DI-CKTC
YF COMMERCIAL
BLOOD TAKEN

Table 1. Anti-D1V and Anti-YFV HI Antibodies in Humans Inoculated with Tissue-Cultured Attenuated D1V and YFV.

Group*	Subject	Time of serum collection	5 weeks		15 weeks	
		Fraction of serum corresponding to	19S	7S	19S	7S
1 a	T. T.	Anti-D1	++	-	+	+++
	Y. F.	,,	++	+	-	+++
1 b	T. H.	Anti-D1	±	-	-	+
	S. S.	,,	++	-	++	++
2	S. T.	Anti-D1	+	-	±	++
		Anti-YF	+	-	+	+
	K. F.	Anti-D1	+	+	+	+
		Anti-YF	+	-	±	+
3	R. A.	Anti-D1	++	+	-	+++
		Anti-YF	+	+	-	++
	S. M.	Anti-D1	++	+	-	+++
		Anti-YF	+	+	-	++
4	A. T.	Anti-D1	+	+	+	++
		Anti-YF	+	+	+	+
	M. N.	Anti-D1	+	-	-	++
		Anti-YF	++	+	±	++

HI titer: +++ ≥ 640; ++ 160-320; + 40-80;
± ≤ 20; - 0

* As to designation, see text.

(1) Intracutaneous inoculation of tissue-cultured, attenuated D1V (of relatively small doses) provoked in human beings anti-D1V HI antibodies of significant titers. During the early stage after the virus inoculation, major portions of the produced antibodies corresponded to 19S globulin (ME-sensitive), while in later stage the antibodies corresponding to 7S globulin (ME-resistant) were predominant.

(2) Combined injection of live D1V and YFV brought about antibody production of the similar pattern as above. However, the following tendencies were noted:

(a) After inoculating D1V and YFV simultaneously, titers of anti-D1V antibodies were somewhat lower than those by inoculating D1V alone.

(b) The similar aspect was shown in the group inoculated with YFV, first, and 2 weeks later with D1V.

(c) In the group inoculated with D1V, first, and 2 weeks later with YFV, titers of anti-D1V antibodies were of the same magnitude to those produced by inoculating D1V alone.

All the volunteers were residents of the Japanese Main Islands and none of them had entered dengue and/or YF endemic areas prior to the present experiments. Some of them had anti-Japanese encephalitis HI antibodies which, however, showed no apparent correlation with the anti-D1 and anti-YF antibodies detected in this study.

III. Some Biological Properties associated with Variation of Japanese Encephalitis Virus

Three strains of JE virus were tested: JaTH strain, of the first mouse brain passage; G1 strain, of the 250th mouse brain passage; and MH30 strain, isolated from a pool of Culex tritaeniorhyncus mosquitoes and passed only in hamster kidney cell cultures incubated at 30° C.

(1) Shifts of infective titers of these strains are shown in Figs. 2, 3, and 4.

FIG. 2.

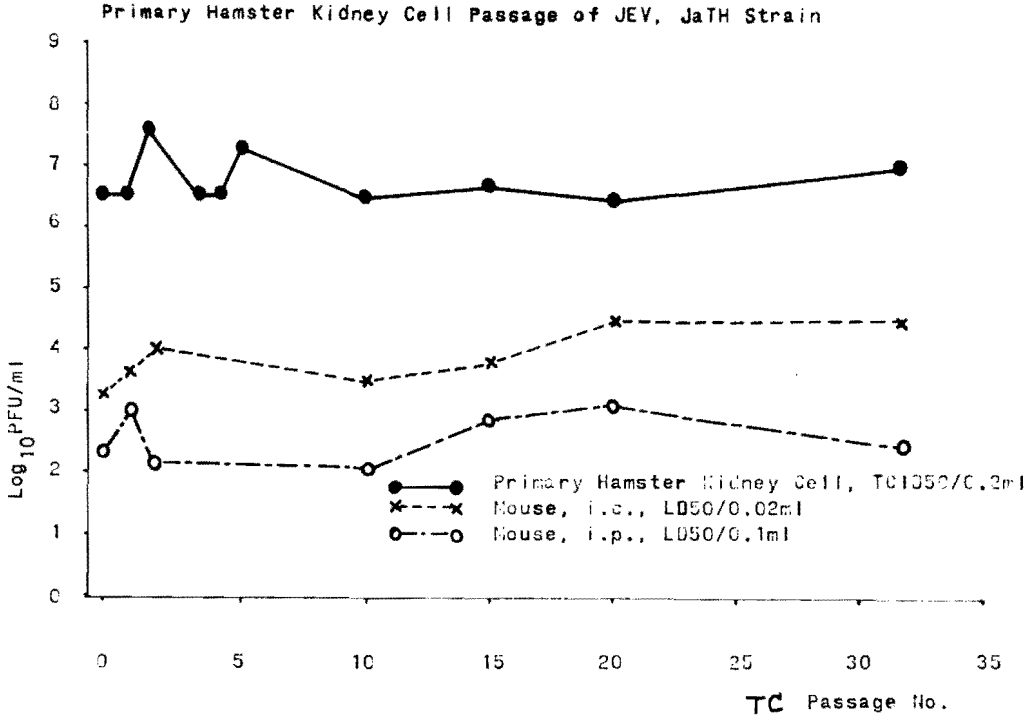

Primary Hamster Kidney Cell Passage of JEV, JaTH Strain
Log10PFU/ml
Primary Hamster Kidney Cell, TCID50/0.2ml
Mouse, i.c., LD50/0.02ml
Mouse, i.p., LD50/0.1ml
TC Passage No.

FIG. 3.

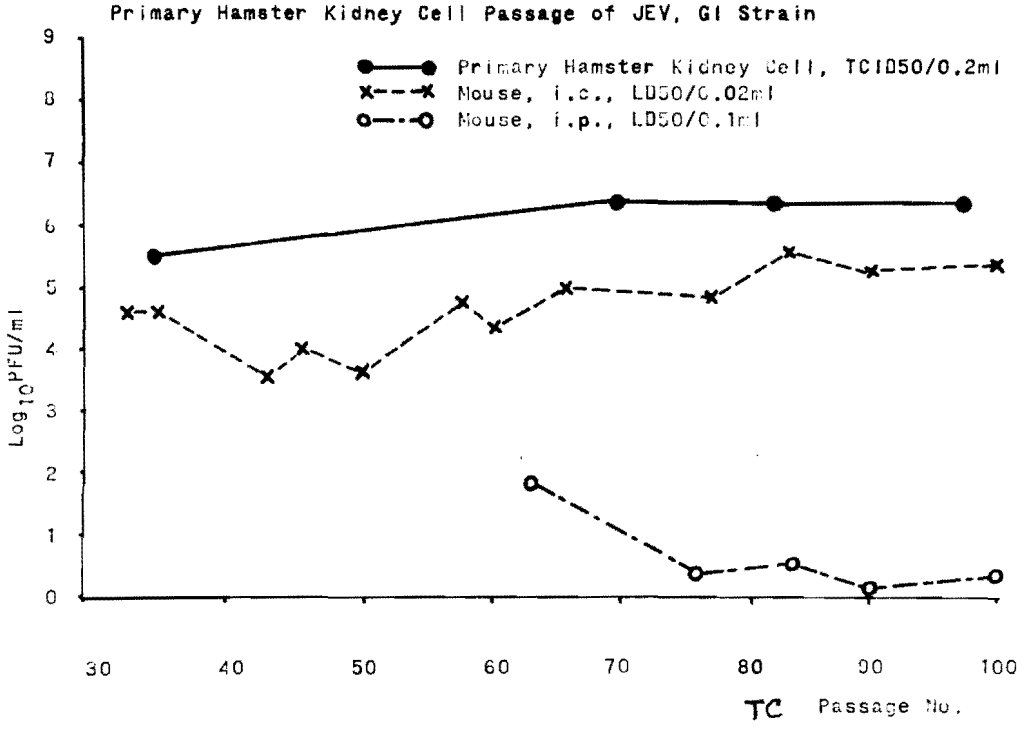
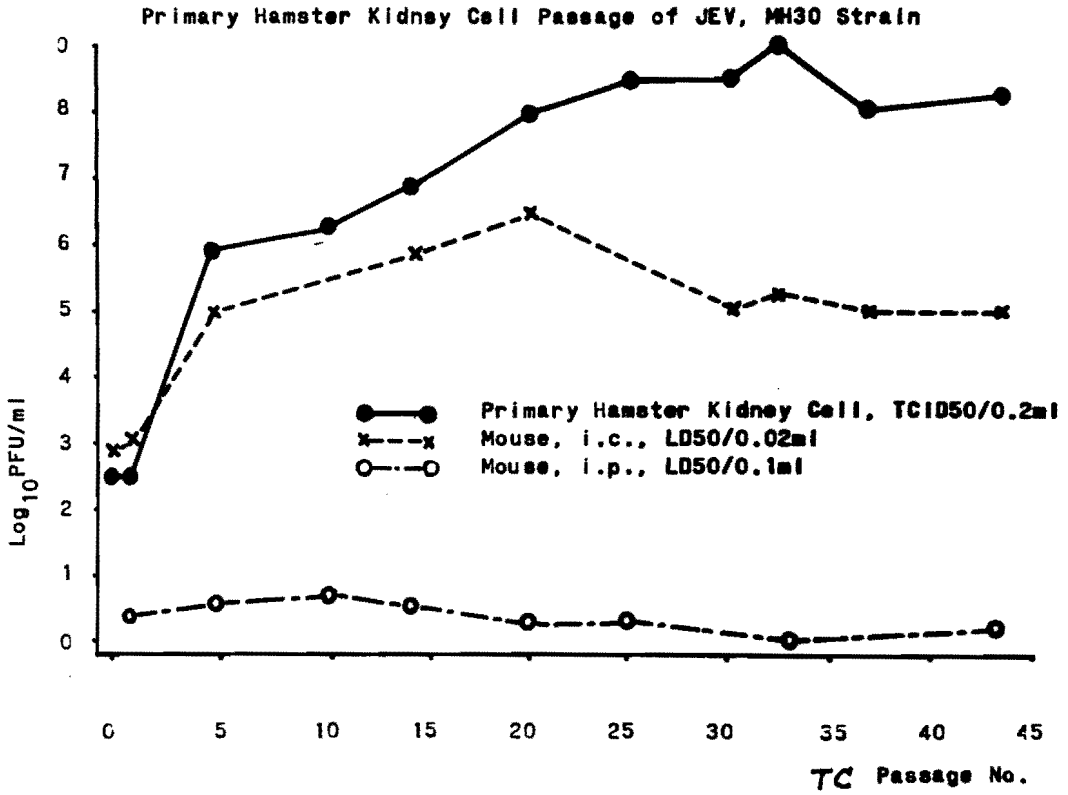

Primary Hamster Kidney Cell Passage of JEV, GI Strain
Log10PFU/ml
Primary Hamster Kidney Cell, TCID50/0.2ml
Mouse, i.c., LD50/0.02ml
Mouse, i.p., LD50/0.1ml
TC Passage No.

FIG. 4.



JaTH strain showed practically no change in its mouse and cell culture infectivities during the observation periods; its mouse i.p. infectivity was comparatively high. G1 strain retained its mouse i.c. pathogenicity and cell culture infectivity fairly constantly, showing certain fluctuation; there was an apparent tendency, however, that the mouse i.p. pathogenicity decreased during the cell culture passages. In contrast to the aspects of JaTH and G1 strains, MH30 strain showed a discrepancy between $TCID_{50}$ and mouse i. c. LD_{50}; its mouse i. p. titers were comparatively low from the beginning and tended to diminish during the course of cell culture passage. This low grade of mouse pathogenicity was not altered after the virus was passed through mouse brains.

(2) DEAE cellulose chromatographic patterns of G1 and MH30 strains are illustrated in Figs. 5 and 6.

FIG. 5.

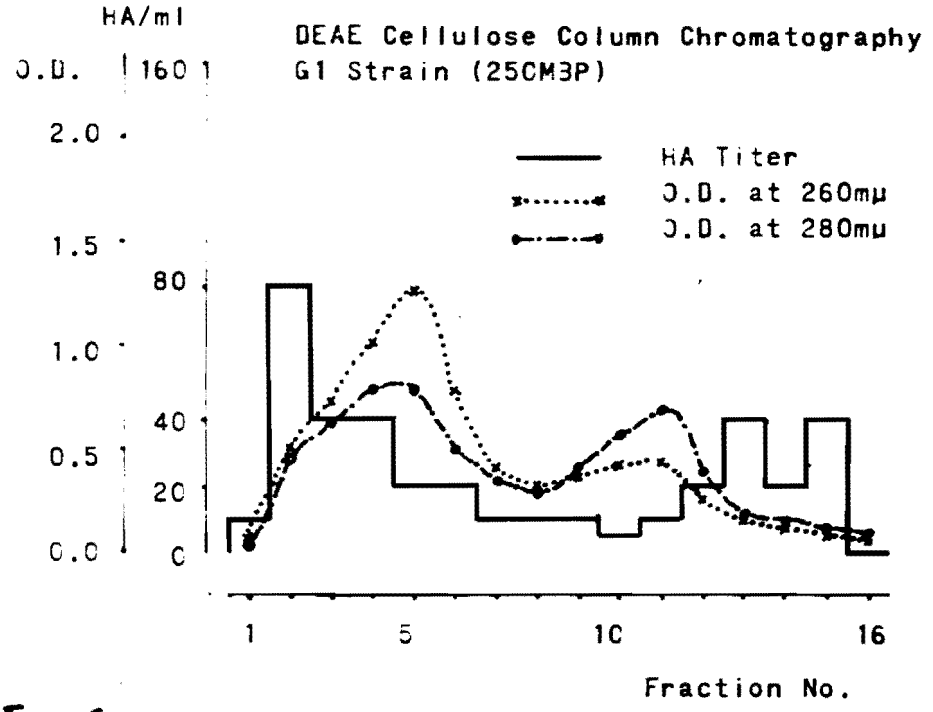
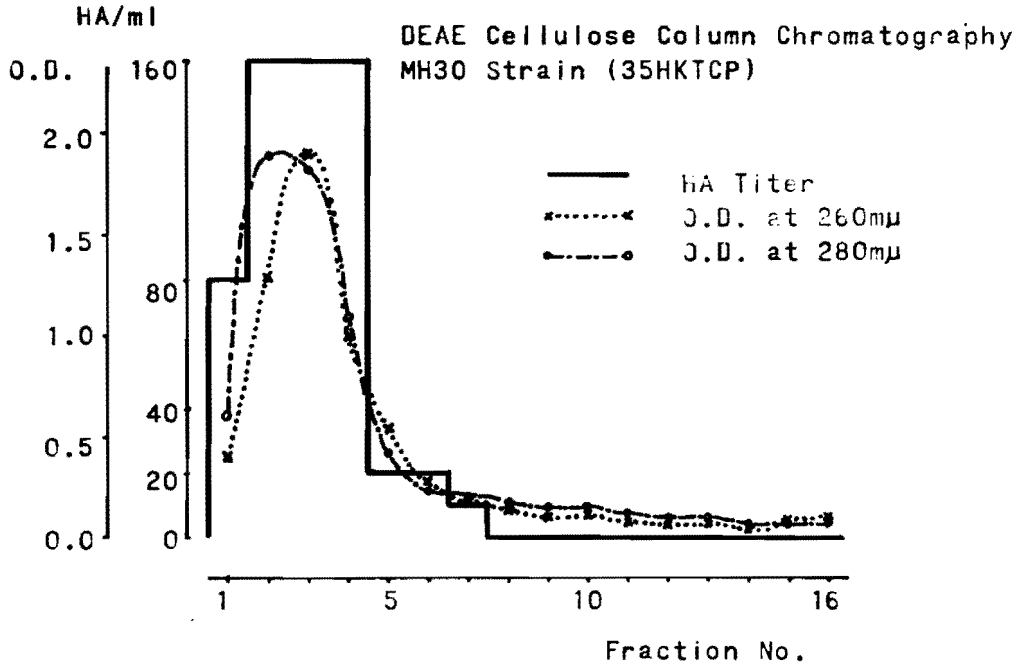


FIG. 6.



It is indicated that G1 strain has multiple (at least 2) peaks, while MH30 is comparatively homogeneous.

(3) The same statement can be made relative to sucrose density gradient centrifugation patterns of both strains (See Figs. 7 and 8).

FIG. 7.

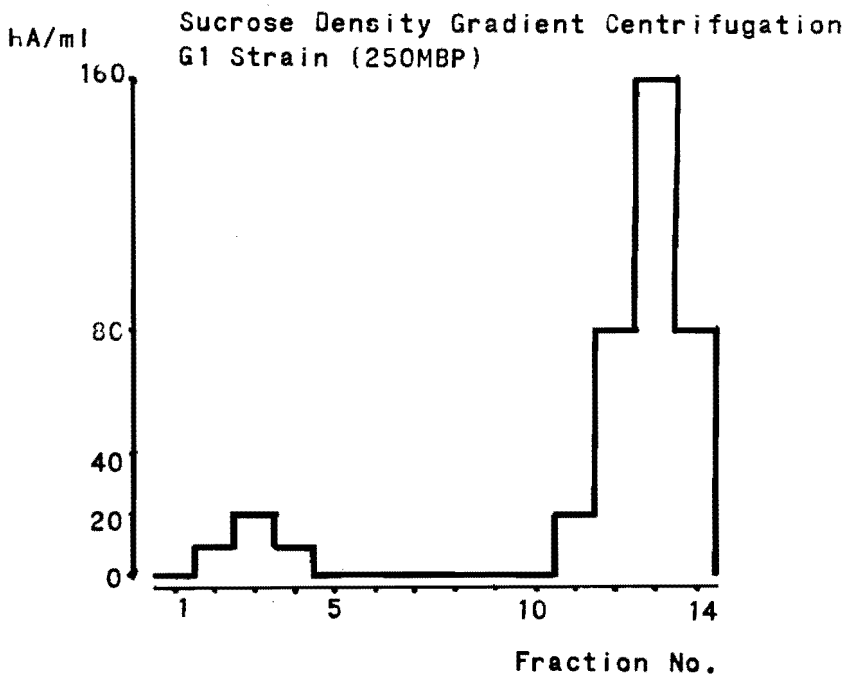
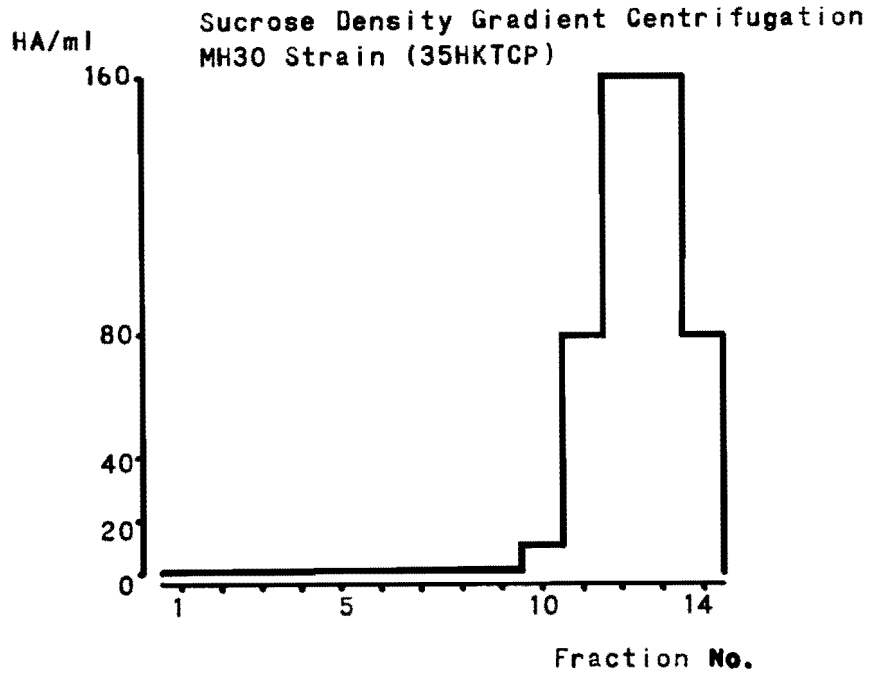


FIG. 8.



(4) MH30 is relatively thermolabile at 37°C, whereas Gl is thermostable.

(5) Plaque reduction neutralization tests using BHK-21 cells indicated that the slopes of linear relationships were essentially the same in each combination of Gl and MH30 viruses and their anti-sera (See Fig. 9). However, relative potency of anti-MH30 serum against Gl virus was higher than that of anti-Gl serum against MH30 virus. (See Table 2, Fig. 10). The results suggest that, from an immunological viewpoint, Gl and MH30 strain viruses are indistinguishable "qualitatively" but have certain "quantitative" difference.

FIG. 9.

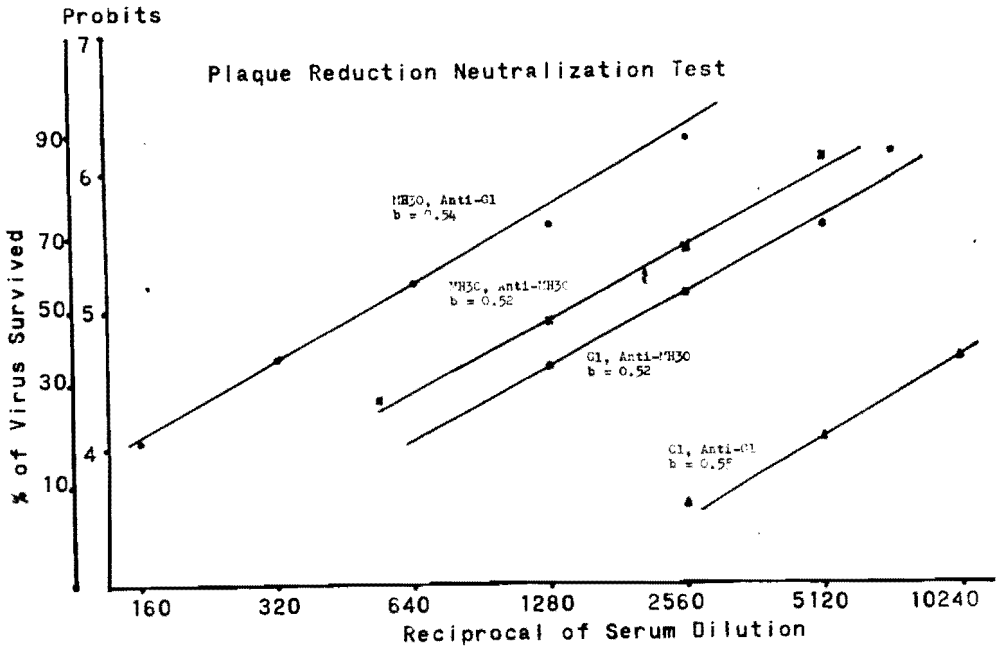


FIG. 10.
Cross Neutralization Test of JEV

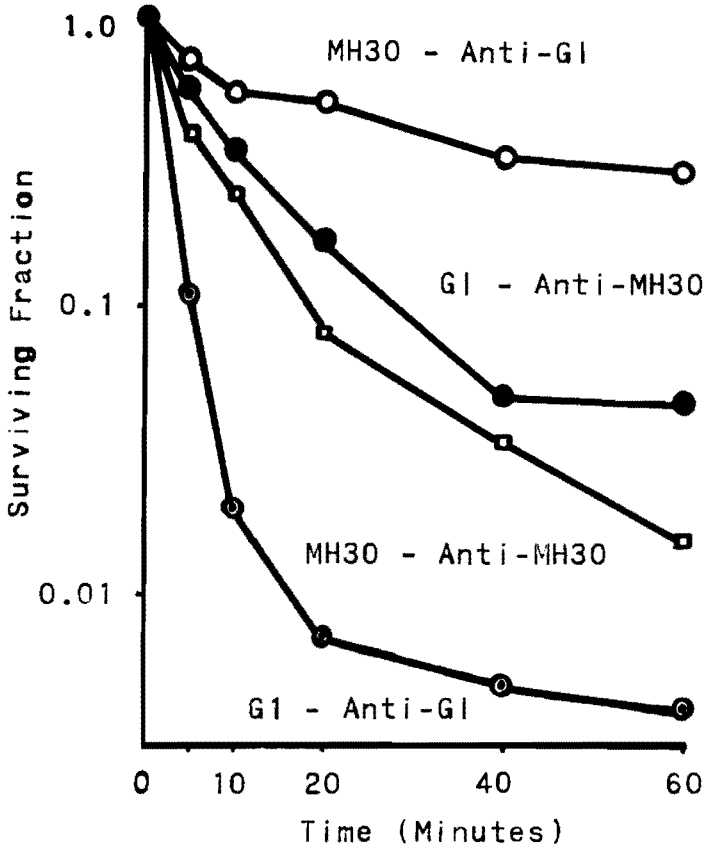


TABLE 2.

Cross Neutralization Test of MH30 and G1 Strain

Serum	JEV, Strain	Reciprocal of ED50	95% CL of R, ED50	RP	95% CL of RP
Anti-MH30	MH30	1390	1165-1650	100.0	
	G1	570	520-620	40.9	33.4-50.0
Anti-G1	G1	14080	12580-17820	100.0	
	MH30	2080	1686-2380	18.9	15.1-23.8
Normal	MH30	24.0	20.9-27.5		
	G1	13.8	11.7-16.3		

ED50; serum dilution of 50% effectiveness
RP; relative potency of the serum against heterologous virus to homologous one
CL; confidence limits

REPORT FROM THE VIRUS RESEARCH CENTRE POONA, INDIA

Complement Fixation Tests for Simultaneous Isolation and Identification of Dengue Viruses Using Tissue Cultures

Aedes albopictus (ATC-15) and Vero cell lines which support the growth of dengue viruses were inoculated with acute sera diluted 1:10 in BAPS. On post-inoculation (PI) day seven or eight and eleven or twelve, two tubes each from the inoculated and uninoculated lots were frozen and thawed thrice to release extra-cellular virus or antigen and the fluids were centrifuged at 300 G for ten minutes and stored at -50° C. for further passage and/or CF test. In case of Vero cell cultures, the presence of phenol red did not interfere with CF reactions but the particular lot of goat serum was found to be slightly anti-complementary. Therefore, two days before harvesting for CF antigens, the medium of these tubes was changed with MEM containing two per cent fetal calf serum.

CF tests were performed using undiluted or diluted (1:2 or 1:4) tissue culture fluids as antigens and hyper-immune sera against the four types of dengue viruses as well as 4-8 units of the standard mouse brain CF antigens. Since the hyper-immune sera did not have similar CF titres for the homologous antigens, comparison had to be made occasionally by calculating the difference in serum titres obtained with the standard and test antigens. The first passage materials of all the specimens which failed to react or yielded low serum titre was passed further and the whole procedure was repeated. By the second and occasionally third passage, all the specimens from which dengue-like viruses could be detected yielded positive CF reactions, and those which were negative did not show any CF reaction even after further additional passages.

By this method more than 20 isolates comprising all the four types of dengue viruses have been identified from acute sera of cases of classical dengue. CF results of a few representative samples are presented in Table 1. From these preliminary results it is clear that the CF antigens could be detected in dengue 1 and dengue 2 viruses earlier than with dengue 3 and dengue 4 viruses. In Vero cultures during the first two passages, the optimum time of harvest for majority of dengue 1 and dengue 2 viruses appeared to be 7-9 PI days while for dengue 3 and dengue 4 it was usually between 10-13 PI days. In ATC-15 cells, CF antigens seemed to develop somewhat earlier.

Table 1

RESULTS OF COMPLEMENT FIXATION TESTS WITH UNDILUTED TISSUE CULTURE ANTIGENS

Source of inoculum	VRC Specimen No.	Cell Culture	Passage level	PI day tissue culture fluid harvested	RESULTS OF CF TESTS: Titres of hyperimmune sera with tissue culture antigens				Identity of the isolate
					HO Titre	D1 32-64	D2 256-512	D3 64-128	D4 128-256	
Acute serum diluted 1:10 (Ceylon)	68911	ATC-15	1	5		<8	<8	<8	<8	Dengue 1
			1	7		16	<8	<8	<8	
Acute serum diluted 1:10 (Vellore)	684970	Vero	1	8 & 12		<8	<8	<8	<8	No dengue-like agent detected
			2	8 & 12		<8	<8	<8	<8	
			3	8 & 12		<8	<8	<8	<8	
Acute serum diluted 1:10 (Vellore)	685000	Vero	1	8 & 12		<4	<8	<8	<8	Dengue 1
			2	7		>64	16	16	32	
Acute serum diluted 1:10 (Vellore)	684980	Vero	1	8		<4	<8	<8	<8	Dengue 2
			2	7		16	>128	16	32	
				Dil. 1:2		8	256	8	16	
Acute serum diluted 1:10 (Vellore)	685003	Vero	1	7		<4	<8	<8	<8	Dengue 3
				12		<4	<8	8	<8	
		Vero	2	7		<4	<8	<8	<8	
				11 & 12		<4	<8	32	<8	
Acute serum diluted 1:10 (Vellore)	684971	Vero	1	7		<8	<8	<8	<8	Dengue 4
			1	12		<8	<8	8	64	

In all instances where dengue 4 virus was isolated specific identification could be made with ease from the first passage material whereas second passage was either necessary or desirable for dengue types 1, 2 and 3. Dengue types 3 and 4 gave almost specific CF reaction with little or no reaction with heterologous sera; dengue 1 and 2 viruses yielded less specific results particularly when harvested later. Furthermore, the titres of dengue 1 and dengue 2 CF tissue culture antigens seemed to be higher because these could be employed at dilution of 1:2 or 1:4 to give more specific results.

Multiplication of Some New and Ungrouped Arboviruses in Aedes Albopictus Cell Culture

Multiplication of following viruses, isolated in India, VRC Nos. G5287, G15534, 68886, G10658, Minnal (G7481) and Wanowrie (G700) was studied in Aedes albopictus cell culture (ATC-15). Of these G5287, G15534, and 68886, isolated from Culex vishnui complex were antigenically related to each other. Virus G10658 was isolated from Anopheles subpictus. Minnal virus 'G7481' (Arbovirus Catalogue No. 200) was isolated from Culex vishnui complex and Wanowrie virus 'G700' (Arbovirus Catalogue No. 198) was isolated from Hyalomma aegypticum as well as Culex fatigans. None of these viruses belong to any of the known arbovirus groups.

Multiplication without producing any visible cytopathic effect was demonstrated with G5287, G15534, 68886 and G10658 viruses. Wanowrie virus did not multiply in this cell culture.

Minnal virus, on the other hand, was not only found to multiply but seemed to produce CPE, which looked different from the type of CPE usually observed in Aedes albopictus cell culture with mosquito-borne group B arboviruses.

REPORT FROM THE UNIVERSITY OF QUEENSLAND VETERINARY SCHOOL, BRISBANE, AUSTRALIA

(1) Bovine Ephemeral Fever

During March and April, 1968, blood samples were taken from 26 cattle that developed clinical disease during an extensive outbreak of ephemeral fever. Buffy coat cells were inoculated intracerebrally into day old mice, and three blind passages were subsequently made. Nineteen agents pathogenic for suckling mice were recovered, usually on the second or third passage. Serum from convalescent animals neutralized one of the isolates while serum from the same animals, collected before the outbreak, did not. The agents were chloroform sensitive. It has not been possible to prepare haemagglutinin from suckling mouse brain.

(2) Studies on the Complement Fixing (CF) and Haemagglutinating (HA) Antigens of Murray Valley Encephalitis (MVE) Virus

Evidence was obtained that CF and HA activity were functions of different parts of the MVE virion.

Treatment with acetone, Tween-ether, and Tween-ether-protamine led to an 8-16 fold increase in HA titre. CF activity increased 4 fold after treatment with acetone or Tween-ether, while Tween-ether-protamine treatment reduced the CF titre. The HA activity of treated or untreated virus was greatly reduced by exposure at 37^{o}C for 60 minutes. CF activity was increased by this treatment and remained after 20 minutes exposure to 65^{o}C. After Tween-ether treatment, the peak of CF activity could be separated from noninfectious HA activity by zonal centrifugation.

(3) The Antibody Response to Arboviruses in Chickens

The antibody response in chickens to MVE and Sindbis viruses was studied, using indirect complement fixation (ICF), haemagglutination inhibition (HI), and neutralization (N) tests. There was no evidence that IgM antibody in chickens could fix complement. The earliest detectable ICF antibody in the primary response was of IgG type and the ICF antibody in the secondary response was all IgG type. There was little production of IgM type or N or HI antibody during the secondary response. In the HI test, IgG antibody produced a degree of cross reaction within the B group viruses, while IgM antibody was relatively specific. Passively transferred HI and N antibodies in newly hatched chickens showed a direct quantitative relationship to anti-

body titres in the hen. The persistence of passively transferred antibodies appeared to be an exponential function of time, and antibodies were no longer detectable by the third week of life.

(Y. Chung and P. Spradbrow)

REPORT FROM THE ARBOVIRUS DIVISION OF THE VIRUS RESEARCH CENTRE PRODUCTION LABORATORIES, CAIRO, EGYPT

Rodent Sera

The following antigens were used in HI test with rodent sera: Sindbis, WEE, W.N. and Bunyamwera. Quaranfil antigen was tested in CF test.

The number of sera with HI antibodies were: one against Sindbis, nine against W.N. and seven against Quaranfil antigen. All sera were negative when tested against WEE and Bunyamwera.

Animal Sera

A total of 934 animal sera (301 goats, 580 donkeys, 32 camels, and 21 sheep) were collected during June 1966-August 1968 from ten governates. The results of HI and CF tests (table 2) showed that the highest rate of antibodies was against WEE and S.F.V., the lowest was against Sindbis. Sera reactive in HI with WEE antigen were tested by NT and none had NT antibodies.

(I. Z. E. Iman)

TABLE 1

ARBOVIRUS ANTIBODIES IN RODENT SERA

Animal	Sera Tested	No. Reactive Sera and Antibodies Titer				
		HI				CF
		Sindbis	WEE	W.N.	Bunyam-wera	Quaranfil
Acomys cabrines cahirinus	36	0	0	1($\frac{1}{10}$)	0	1($\frac{1}{10}$)
Rattus norvegicus	124	0	0	6($\frac{1}{10}$)	0	4($\frac{1}{10}$)
Mus musculus	3	0	0		0	0
Rattus rattus frugivorus	80	1($\frac{1}{10}$)	0	2($\frac{1}{10}$)	0	0
Rattus rattus alexandrinus	49	0	0	0	0	2($\frac{1}{10}$)

TABLE 2

ARBOVIRUS ANTIBODIES IN ANIMAL SERA

Antigen	Titer of reactive Sera				Total Sera Tested	Reactive Sera %
	1:10	1:20	1:40	Total		
W.N.	39	28	8	75	934	8.02
Sindbis	11	8	5	26	934	2.9
WEE	79	30	4	113	934	12.1
Bunyamwera	10	13	7	30	934	3.3
S.F.V.	5	17	4	26	210	12.3
Quaranfil	27	3	0	30	486	6.2

REPORT FROM THE EAST AFRICAN VIRUS RESEARCH INSTITUTE ENTEBBE, UGANDA

The longitudinal studies begun near Kamese forest during 1967 were continued through the year. Last year Ntaya, SIND, Pongola and Kamese viruses were isolated from mosquitoes collected in Kamese forest. This year 3 further strains of Pongola were isolated from pools of M. fuscopennata and M (Mansonioides) spp. mosquitoes. In addition, a total of 230 human blood specimens were collected on a weekly basis at the Butolo aid post and three isolations were made (one of CHIK and 2 of WN).

During the year, 52 weekly routine two-hour post sunset seven-level and one six-level man-baited catches were conducted on the Zika tower. A. africanus was processed separately in 86 pools and the remaining mosquitoes were variously combined in 116 pools. 81 C. centurionis were discarded.

Ten virus strains were isolated from mosquitoes as shown in the first and second columns of the table below. The first isolate MP 7442, came from a mixed pool collected in February, details of which are given in the second column of the table. A series of 8 isolations of CHIK followed from A. africanus collected in March-May, and finally in June a last isolation of CHIK was made from M. fuscopennata.

In March, 3 special man-baited catches for A. ingrami (mostly retained for other studies) were conducted from ground level to the 60-foot platform in the period 16-1900 hours. The totals of the mosquitoes processed in 6 pools from these catches are shown in the third column of the table below. One pool of A. africanus yielded CHIK.

MP 7442

This isolate was obtained from a pool of 140 mixed mosquitoes collected from Zika forest in March, 1968. On initial inoculation all mice were sick on day 4, and after passage the agent titred 5.0 logs/0.02 ml. NBIC, 1.5 logs/0.02 ml. NBIP and was not pathogenic for adult mice IC or IP. No haemagglutinin was produced by fluorocarbon or sucrose acetone extraction. MP 7442 hyperimmune mouse serum and sucrose acetone antigen were tested against antigens and antiserum to 75 arboviruses and no relationship was found. The homologous serum/antigen CF titre was 80/160. The agent was reisolated and is ether sensitive.

Species	Routine Catches		3 Special catches at tower in March
	Year's total	MP 7442	
A. implexus	100	-	1
A. paludis	8	-	-
M. aurites	2329	48	8
M. fuscopennata	3234(1)*	51	35
M. maculipennis	14	-	-
M. metallica	6	-	-
M. pseudoconopas	5	-	-
M. africana	800	12	4
M. uniformis	60	2	1
Uranotaenia sp.	1	-	-
Hodgesia sp. or spp.	2	-	1
A. africana	3	-	-
Eretmapodites sp. or spp.	58	1	9
A. nigerrimus	19	-	-
A. (Mucidus) sp. or spp.	6	-	-
A. ingrami	156	1	4
A. africanus	4228(8)	-	104(1)
A. apicoargenteus	37	-	13
A. abnormalis/tarsalis gp.	3	-	4
A. cumminsii	1	-	-
A. domesticus gp.	3	-	-
A. circumluteolus	2	-	-
C. annulioris	1284	21	14
C. poicilipes	25	-	-
Culex spp.+	159	-	6
Total	12543(9)	136(1)	204(1)

* Figures in brackets denote virus isolates. All but MP 7442 were of CHIK.

\+ Include C. Antennatus, C. guiarti, C. trifilatus, C. univittatus, C. nebulosus, C. rubinotus and probably other Culex species.

During the year samples were collected from 76 patients attending the Institute out-patient dispensary from which 9 viruses were isolated. These included 3 strains of CHIK and 6 strains of RVF.

Beginning in mid-April coincidental outbreaks of CHIK and RVF occurred in the Entebbe area and a total of 13 strains (6 CHIK and 7 RVF) were isolated from humans.

Coincidental with the outbreak of RVF, small mammals were collected from Lunyo forest for attempted virus isolation. Mims (EAVRI, Annual Report No. 6, 1956) had previously reported finding immunity to RVF in a single A. abyssinicus. From May to October, 281 small mammals were trapped, as shown below. Blood and suspensions of liver, spleen and brain were inoculated for attempted virus isolation. Eight virus strains were isolated from 4 species of rodents. These viruses are apparently hitherto undescribed agents. No isolations of RVF were made.

Species	Month					
	May	June	Aug.	Sept.	Oct.	Total
L. flavopunctatus	41 (3)	8	15	6	2	72
sikapusi	3	11	6 (1)	4	1	25
A. abyssinicus	43 (1)	32 (1)	18	3	14	110
R. rattus	10 (2)	5	2	-	-	17
P. morio	5	9	1	-	-	15
L. straitus	3	7	4	2	1	17
A. kaiseri	-	1	1	2	1	5
O. tropicalis	-	3	1	-	-	4
O. incomtus	-	2	1	1	-	4
T. valida	-	2	1	4	5	12
Total	105 (6)	80 (1)	50 (1)	22	24	281 (8)

Studies directed towards appraisal of the present status, distribution and inter-relationship of YF and other arboviruses were continued during this year.

In 1967 experimental viraemia studies in Rhesus monkeys indicated that WESS virus immunization interferes with circulation of YF virus. This year, Zika virus immunization of Rhesus monkeys did not result in decreased circulation of YF after challenge. However, Zika immunized vervet (C. aethiops) monkeys demonstrated reduced viraemia after YF challenge. These studies indicate that cross-protection occurs among African Group B arboviruses and that the selection of the host for experimental study is important. The implications of these findings in relation to the distribution of YF in eastern Africa and Somalia are given below.

In Uganda, YF protection tests were done on sera with HI antibody to any Group B antigen selected out of 1762 sera collected from 8 localities during the past 2 years. Yellow fever protection tests were done on all sera from Bwamba. The overall incidence to YF immunity was 32/1762 (1.3 per cent). All immune sera were from adults. Of particular interest is the absence of YF immunity in children in Bwamba. Previous surveys by Hughes et al. (Trans. R. Soc. Trop. Med. Hyg., 40, 1946), indicated 4.5 per cent immunity rate in children in Bwamba. The results suggest that there has been no recent activity of YF in the areas sampled in Uganda. On the other hand, immunity to Banzi, WESS and WN is widely distributed throughout Uganda.

Coincidental to the human surveys in Uganda, monkey sera were collected from 6 localities including Karamoja, West Nile, Madi and Busoga Districts. Zika HI antibody was detected in sera from monkeys at all localities except Entebbe. A total of 71 per cent of the sera collected were Zika immune in the protection test. Again, the percentage of Zika immunes in juveniles (5 per cent) was lower than that in adults (88 per cent). Only 4 sera from adult monkeys were YF immune a surprisingly low rate of 3 per cent. The classic YF immunity surveys of Haddow et al. (1951, Trans. R. Soc. Trop. Med. Hyg., 45, 189) indicated that YF immunity was widespread in Uganda primates. The present survey indicates that YF immunity is rare, conforming with the results of the human survey. It is possible that the extensive Zika immunity may be one factor that has reduced the incidence of YF immunity.

Samples from all human surveys in Kenya, Tanzania and Somalia were similarly tested against YF, WESS and WN viruses in comparative mouse neutralization tests. The results indicate, as in the case of Uganda, that immunity to WESS and WN is widespread, with the highest incidence on the

coastal area of Kenya and Tanzania. The position with regard to YF immunity is similar to that in Uganda with the exception of the Burgi tribe at Marsabit, Northern Frontier District of Kenya, where protection tests with YF, WESS and WN confirmed the presence of YF immunity in children (18.2 per cent) and adults (22.9 per cent). The youngest immune serum was from a 6 year old child. The levels of YF immunity in the Burgi tribe are the highest ever recorded in East Africa, apart from Bwamba, and indicate either endemic or post epidemic YF on Marsabit mountain. It is also pointed out that the traditional absence of human epidemic YF from the coastal area of Kenya may be related to the presence of other Group B arbovirus immunity.

Turning to the YF vectors, early workers in this Institute found that A. simpsoni is an important vector of YF. In some parts of Uganda, particularly in Bwamba, it was found to bite man readily, but throughout a wide part of Uganda it was found non-anthropophilic; this biting behaviour of the peri-domestic A. simpsoni was thought to be in all probability the most important factor limiting the occurrence of YF throughout a large part of Uganda.

Studies continued during the year at Bwamba and at Bwayise, near Kampala, confirmed that Bwayise A. simpsoni prefers rodents to other hosts and the Bwamba A. simpsoni shows preference for primates. These findings together with others reported in last year's annual report, may indicate that the apparent absence of human YF in a large part of Uganda is due to A. simpsoni in these areas being non-primatophilic.

(G. W. Kafuko, B. E. Henderson, B. G. Kirya, L. G. Mukwaya and S. D. K. Sempala)

REPORT FROM ARBOVIRUS LABORATORY UNIVERSITY OF IBADAN, NIGERIA

During the past several years a number of agents characterized by long mouse incubation periods and low titers have been isolated human bloods. In late 1968, several of these were given additional passages in infant mice following which they were found to react in CF with a group-B immune mouse ascitic fluid. Additional tests with of isolates revealed reactions

with dengue immune sera and ascitic fluids, but not with West Nile. Because dengue antigens were not available at Ibadan, these 8 strains were sent to YARU where subsequent testing of one employing a battery of group-B antigens and immune fluids confirmed the close relation to dengue: typing has not yet been completed. Further studies at Ibadan with acetone extracted antigens and homologous immune mouse sera indicated the presence of at least two serotypes.

In addition to the 8 isolates currently considered to be dengue on the basis of CF testing, 24 other agents are under study as possible dengues. Distribution by year is as follows:

Year	No. isolates
1964	1
1966	10
1967	1
1968	20

Most strains (24) have been recovered from heparinized capillary blood specimens obtained from febrile infants and young children attending the General Out-Patients Clinic of the University College Hospital, Ibadan. The remainder were from sera collected from ill staff and children.

To our knowledge, these strains represent the first to be identified as dengue on the continent of Africa.

(Staff of Ibadan Arbovirus Laboratory)

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

During the year 1968, field collections have been carried on in Bandia forest and Saboya area (Arthropod-borne Virus Information Exchange, No. 18, Dec. 1968).

1. Materials collected

1.1 Human specimens: 48 blood samples were collected at the Bandia Dispensary from febrile children. Work is still in progress and no conclusion yet reportable.

1.2 Vertebrates: 499 specimens were processed for virus isolation.

1.2.1 Bandia forest: The capture and recapture programme of small mammals has been improved so that all the animals trapped one month after their last capture are bled again. 330 specimens have been collected. The recapture rate was 38 per cent. Of 240 trapped and marked animals, 35 were caught again one to three times making a total of 90 recaptures (Table 1).

1.2.2 Saboya area: Rodents and birds constitute the greatest number of specimens obtained in this area. All trapped animals were exsanguinated by heart puncture, followed by removal of brain, liver and spleen for attempts to virus isolation.

1.3 Arthropods: (Table 2)

1.3.1 Culicidae: Owing to a poor rainy season, captures yielded less specimens. Nevertheless 47,000 mosquitoes have been pooled for virus isolation.

1.3.2 Ixodoidea: The species collected are listed in Table 3. Non-engorged adults of Haemaphysalis mushamae were found in burrows of Arvicanthis niloticus and non-engorged adults of Hyalomma truncatum were captured on the ground.

1.3.3 Other diptera: Pooling of Culicoides and Phlebotomine flies for virus isolation was done on the genus as a whole. (Table 2.)

TABLE 1

S P E C I E S	Capture and marking	Recapture
Galago senegalensis	5	-
Erythrocebus patas	1	-
Heliosciurus gambianus	1	2
Xerus erythropus	15	11
Arvicanthis niloticus	29	8
Rattus rattus	1	-
Myomys sp.	10	4
Mastomys sp.	154	65
Cricetomys gambianus	2	-
Tatera valida	16	-
Taterillus gracilis	6	-
Total	240	90

TABLE 2

SPECIES	BANDIA		SABOYA	
	Pools	N°	Pools	N°
Anopheles ziemanni	5	80	-	-
gambiae (s.l)	67	4078	194	14432
funestus	21	1631	-	-
pharoensis	1	11	1	24
squamosus	-	-	1	3
Uranotaenia annulata	4	46	-	-
balfouri	2	12	-	-
Ficalbia mediolineata	-	-	1	2
Mansonia (M.) uniformis	10	373	8	242
africana	6	188	6	106
Aedes (M.) grahami	-	-	1	2
(St.)aegypti	4	83	-	-
unilineatus	-	-	1	4
metallicus	1	7	-	-
luteocephalus	6	199	89	6897
(A.) punctathoracis	-	-	1	8
albocephalus	1	4	5	227
chamboni	-	-	17	878
irritans	189	11713	-	-
(Dic.) furcifer-taylori	4	39	3	13
Culex (C.) poicilipes	1	11	1	25
tritaeniorrhynchus	11	381	4	94
thalassius	55	2844	48	2478
simpsoni	3	74	-	-
univittatus	1	4	3	15
gr. decens-perfuscus	1	32	2	40
Total Culicidae	393	21810	386	25490
Culicoides spp.	6	4638	17	24845
Phlebotomus spp.	6	612	7	1248
Total	405	27060	410	51583

TABLE 3

Species	BANDIA		SABOYA	
	Nº	Pools	Nº	Pools
Amblyomma nuttalli	-	-	4	1
Amblyomma variegatum	48	7	69	6
Aponomma flavomaculatum	422	24	-	-
Aponomma latum	27	1	-	-
Haemaphysalis houyi	271	18	-	-
Hyalomma truncatum	27	2	65	4
Rhipicephalus mushamae	187	10	-	-
Rhipicephalus sulcatus	33	2	25	2
Ornithodoros erraticus sonrai	832	35	-	-
Total	1847	99	163	13

2. Results

2.1 Virus isolations (Table 4)

- PA 4499, a tick virus, shows irregularities in its pathogenicity for mice making the identification difficult.

- Four isolates of RV 4600 virus were obtained from Tatera valida collected in Saboya area. Prototype RV 4600 belongs to group B, is related to Uganda S virus but is different.

- RV 4611 and RV 5314, two ungrouped viruses are different from each other and seem to be unrelated to the 71 African arboviruses with which they have been compared.

2.2 Vertebrate sera tested for HI antibodies: Samples were taken onto filter paper discs. 1391 discs have been tested (Bandia 1048 and Saboya 343) for antibodies to the following antigens: chikungunya (CHIK), yellow fever (YF), Uganda S (UGS), Dakar Bat (DAK), West-Nile (WN), Zika (ZIK), Bukalasa Bat (BUK), Wesselsbron (WESS) and Bunyamwera (BUN). The positive results are recorded in Table 5. In Saboya, 19 specimens showing antibodies to Uganda S were collected at the time and place of isolation of RV 4600.

Monkeys specimens show serological scars of the 1966 chikungunya epidemic.

2.3 Experimental transmission studies: Preliminary viremia studies on suckling mice have been conducted to determine the best period for infecting the mosquitoes. Suckling mice inoculated i-p with 3.3 log LD50 of chikungunya virus (local strain SH 2807) were all found to circulate virus from the 12th hour to the death on the third day; virus titer ranged from 5.5 to 9 log LD50.

(Y. Robin and P. Brès, Pasteur Institute; R. Taufflieb, M. Cornet and J. L. Camicas, ORSTOM)

TABLE 4

Code	Source	Place	Virus
PM 3913	Aedes (A.) cumminsi	Bandia	Middelburg
PM 3925	Culex (C.) thalassius	Bandia	BA 209 (sub-type of Ntaya)
PM 3964	Anopheles gambiae	Saboya	Chikungunya
PA 4499	Amblyomma variegatum	Bandia	Not identified
RV 4600	Tatera valida (blood)	Saboya	New, Group B
RV 4606	Tatera valida (blood)	Saboya	Identical to RV 4600
RV 4611	Tatera valida (liver-spleen)	Saboya	New, ungrouped
RV 4615	Tatera valida (blood)	Saboya	Identical to RV 4600
RV 4633	Tatera valida (blood)	Saboya	Identical to RV 4600
RV 5314	Tatera valida (liver-spleen)	Saboya	New, ungrouped

TABLE 5

Place	Discs N°	Species	Date of capture	ANTIGENS CHIK	Y-F	UGS	DAK	W-N	ZIK	BUK	WESS
BANDIA	3733	Taphozous perforatus	5-06-68	0(1)	0	0	0	0	0	40(2)	0
	2872	Crocidura sp.	2-12-67	0	0	0	0	20	0	0	0
	3103	Agama agama	15-02-68	0	0	20	0	0	0	0	0
	3069	Mastomys sp.	9-02-68	0	0	±20	0	0	0	0	0
	2951	Xerus erythropus	29-12-67	0	0	0	40	0	0	0	0
	3188	Ploceus vitellinus	7-03-68	0	0	0	0	20	0	0	0
SABOYA	3749	Cercopithecus aethiops	12-06-68	40	0	0	0	0	0	0	0
	3751	Cercopithecus aethiops	12-06-68	20	0	0	0	0	0	0	0
	3752	Erythrocebus patas	12-06-68	20	0	0	0	0	0	0	0
	3886	Erythrocebus patas	3-07-68	40	0	0	0	0	0	0	0
	4084	Cercopithecus aethiops	29-08-68	20	0	0	0	0	0	0	0
	4104	Cercopithecus aethiops	17-09-68	⩾40	0	0	0	0	0	0	0
	3894	Felis domesticus	3-07-68	0	0	20	0	0	0	0	40
	2945	Tatera valida	22-12-67	20	0	0	20	0	0	0	0
	2947	Tatera valida	22-12-67	0	0	0	20	0	0	0	0
	3293	Tatera valida	15-03-68	0	0	0	0	20	0	0	0
	3549	Tatera valida	24-04-68	0	320	160	80	80	640	160	320
	3566	Tatera valida	24-04-68	0	0	0	0	40	0	0	0
	3571	Tatera valida	24-04-68	0	0	40	20	20	0	0	160
	3590	Tatera valida	24-04-68	0	80	20	20	20	0	0	80
	3765	Tatera valida	12-06-68	0	0	40	0	0	0	0	0
	3769	Tatera valida	12-06-68	0	0	80	20	20	0	0	160
	3914	Tatera valida	4-07-68	0	0	20	0	0	0	0	0
	3915	Tatera valida	4-07-68	0	0	40	20	0	0	0	20
	3918	Tatera valida	4-07-68	0	0	40	20	0	0	0	40
	4024	Tatera valida	8-08-68	0	⩾40	⩾40	20	0	0	0	⩾40
	4026	Tatera valida	8-08-68	0	0	20	0	0	0	0	0
	3591	Taterillus gracilis	24-04-68	0	0	40	20	40	0	0	320
	3767	Taterillus gracilis	12-06-68	0	320	80	40	40	320	80	320
	3922	Mastomys sp.	4-07-68	0	0	20	0	0	0	0	0
	3923	Mastomys sp.	4-07-68	0	0	20	0	0	0	0	20
	4031	Mastomys sp.	8-08-68	0	0	0	±20	0	0	0	20
	2761	Heliosciurus gambianus	3-10-67	0	0	20	0	0	40	20	20
	2763	Heliosciurus gambianus	3-10-67	0	0	20	0	0	0	0	0
	2786	Heliosciurus gambianus	15-11-67	0	0	20	0	0	0	0	0
	3533	Nectarinidae	24-04-68	0	0	40	0	0	0	0	0
	4142	Lissotis melanogaster	9-10-68	0	0	0	±20	20	0	0	20
		Total		7	4	21	14	11	3	4	14

(1) 0 = négative at 1:20

(2) 40 = inverse of the dilution giving complete inhibition.

REPORT FROM THE INSTITUTE OF POLIOMYELITIS AND VIRUS ENCEPHALITIDES OF THE USSR ACADEMY OF MEDICAL SCIENCES, MOSCOW, USSR

Relationship Between Strains of Virus of Crimean Hemorrhagic Fever (CHF) and Congo Virus

Recently Dr. J. Casals of the Rockefeller Foundation established that antigens of the Astrakhan strain "Drozdov" of CHF virus (USSR) reacted well in the complement fixation test and in the test of diffuse precipitation in agar gel (DPAT) with immune sera against two strains of Congo virus, and that antigens of these two Congo virus strains reacted well with a human immune serum of CHF convalescent (USSR) as well as with mouse immune serum against "Drozdov" strain. These observations first raised the question of the possible etiological identity of human diseases in the USSR and Bulgaria of the type of Crimean HF and in some countries of Africa and Asia known under various names.

This paper presents the results of comparative investigations of CHF and Congo viruses with other than Dr. Casals' strains and immune sera from USSR Middle Asia, European part of USSR and Bulgaria. The studies were performed using cross CFT, DPAT and neutralization tests of virus with immune sera in hamster kidney tissue cell culture by interference against cytopathic vesicular stomatitis virus. The evidence obtained confirms and supplements Dr. Casals' conclusions of the immunologic relationship of CHF and Congo viruses.

Materials and Methods

Congo virus - Thanks to kind assistance of Dr. J. Woodall of arbovirus laboratory in Entebbe, Uganda (Director Dr. G. W. Kafuko), through the mediation of WHO representative Dr. D. A. Henderson, our Laboratory received in lyophilized form 4 strains of Congo viruses: (1) Congo No. 3010, pass 75; (2) Congo An. 7620 tick orig. of June 7, 1968; (3) Nakiwogo (Ellice) pass .6 of June 6, 1963; (4) K 67/67, pass .2 of August 18, 1967.

All the 4 strains were successfully restored in the very first inoculation of newborn white mice. They produce after a 4-7-day incubation period paralytic disease in the great majority of newborn white mice, inoculated intracerebrally.

CHF virus - In the studies we used 3 strains of the virus isolated from the blood of patients with typical clinical picture of CHF, including: (1) "Khodzha" strain (23rd passage) isolated in June 1967 in Samarkand region, the Uzbek SSR; (2) "Kash" strain (18 passage) isolated in May 1967 in Rostov-on-the-Don region, RSFSR; (3) "Sud" strain (14 passage) isolated in June 1968 in Rostov-on-the-Don region, RSFSR. All strains have characteristics quite typical of CHF virus and antigenically are identical with each other and with strains isolated in other regions of the USSR and in Bulgaria.

Antigens for CFT and DPAT - Antigens for CFT and DPAT were prepared from brains of inoculated sick newborn white mice by the conventional methods of borate-saline and sucrose-acetone extraction. Higher titers of antigens were obtained in preparations of the latter treatment.

The complement-fixation test was performed with 2 complete units of complement, fixation at 37° C for one hour.

The test of diffuse precipitation in agar gel (DPAT) was performed on slides, using some modifications according to Ouchterlony.

The neutralization test of virus by immune globulins was performed in primary cultures of kidney cells of young Syrian hamsters using the phenomenon of interference with a cytopathic strain of vesicular stomatitis virus. The procedure of this test was developed at our Institute and published (Tkachenko. Chumakov, and others, 1968).

Antibody - In immunologic tests we used: immune sera of patients convalescing after CHF, typical cases occurring in Tajikistan, (patients Valiev and Khalilov), Astrakhan region (patient Gushchina), Bulgaria (mixture of human immune sera); also ascitic fluids of mice hyperimmunized with Kash strain, and sera from white rats immunized with Khodzha strain.

Unfortunately, two immune sera (lyophilized) sent from Entebbe against Congo 3010 and Congo Nakiwogo strains were found to be inactive in tests by DPAT with all antigens including homologous strains.

Results

In the complement fixation test quite definite results were obtained (see Tables 1, 2) indicating similarly high activity of complement fixation with antigens prepared with 4 Congo virus strains and 2 strains of CHF virus from Rostov and Samarkand foci of disease and immune sera containing antibody for CHF virus. These data seem to indicate immunologic identity

of strains of CHF and Congo viruses from different foci of disease in Africa, Asia and Europe.

Table 1

Complement-fixation test. Relationship between Congo K67/67 strain and Middle Asian strain of CHF virus

Sera: / Antigens (borate-saline extraction)	Immune serum of white rats for Khodzha strain	Human immune serum(conval. after CHF), Valiev, Tajik., 1967	Controls		
			Normal human serum	Normal white rat serum	No serum (control of antigen)
Congo K67/67 (2919) (3 pass. + 1)	32/320[x)]	16/160	0/0	0/0	0
CHF, Khodzha strain, 23 pass. (2702)	32/160	32/160	0/0	0/0	0
Serum control (without antigen)	0	0	0	0	0

x) reciprocal serum titer/reciprocal antigen titer

Table 2

Complement fixation test. Interaction between 4 Congo virus strains with immune sera for CHF virus from Tajikistan, Rostov region and Bulgaria.

Antigens	Sera: "Kash" (ascit.)	Human conval. serum Rostov region	Human conval. serum Bulgaria	Controls		
				Normal human serum	Normal ascit. fluid	Antigen without serum
Congo, K67/67 (4 pass. sucrose-acetone)	16/1280 x)	16/1280	8/1280	0/0	0/0	0
Congo, 3010 str. (77 pass. sucrose-acetone)	16/1280	16/1280	8/1280	0/0	0/0	0
Congo, Nakiwogo strain (8 pass. sucrose-acetone)	16/640	16/1280	8/1280	0/0	0/0	0
Congo, An7620 strain (6 pass. sucrose-acetone)	16/320	16/640	8/640	0/0	0/0	0
CHF, Kash strain (Rostove, borate)	16/640	16/640	16/640	0/0	0/0	0
CHF, Khodzha strain (Samarkand, borate)	16/320	16/320	8/320	0/0	0/0	0
Normal brain (borate antigen) newborn w.m.	0/0	0/0	0/0	0/0	0/0	0
Serum control (without antigen)	0	0	0	0	0	0

x) Reciprocal serum titer/reciprocal antigen titer

0/0 - Negative results with dilutions 1:8 and higher

In the test of diffuse precipitation in agar gel (Table 3) preliminary results were obtained also clearly demonstrating the capacity of the Congo virus strains tested to interact with immune sera containing antibody for CHF virus. These data confirm the results obtained in the CFT. The question of interaction of CHF virus strains studied with immune globulins for Congo virus requires further study because the 2 available antisera were inactive with homologous viruses (3010 and Nakiwogo).

TABLE 3

Diffuse precipitation in agar gel. Interaction of 4 Congo virus strains with immune sera for CHF virus.

Sera Antigens	Congo Nakiwogo	Congo 3010	CHF Khodzha white rat immune serum	CHF Kash ascit.	CHF Human Conval. Serum Tajikist.	Control Normal Human Serum
Congo, K67/67	0	0	+	+	+	0
Congo, 3010	0	0	+	+	+	0
Congo, Nakiwogo	0	0	+	+	+	0
Congo, An7620	0	0	+	+	+	0
CHF, Khodzha	0	0	+	+	+	0
Control (normal antigen, ascit. white mice)	0	0	0	0	0	

Antigens prepared by sucrose-acetone extraction; antigens and sera used undiluted.

The Congo virus, like CHF virus does not produce any cytopathic destruction in tissue culture and multiplies in cells asymptomatically. Therefore we successfully applied the method previously developed at our Institute using the phenomenon of interference against 100 units of vesicular stomatitis virus in Syrian hamster kidney tissue culture.

Table 4 presents the results of neutralization test with strains of Congo and CHF virus by immune sera interacting with these viruses for 1 hour at 37° C.

TABLE 4

NEUTRALIZATION OF VIRUS in hamster kidney tissue culture by interference with the cytopathic effect of 100 doses of vesicular stomatitis virus (VSV). Neutralization at 37° C for one hour. Incubation time of inoculated cultures for 5 days.

	Sera:				
Virus:	Control. Normal human serum + virus	Nakiwogo antiserum (Congo)	CHF, Khodzha Im. serum	CHF, Kash Im. ascit.	CHF, Valiev conval.sera Tadjikistan
Congo, Nakiwogo strain, 10% susp.	++	00	00	00	00
CHF, Sudarkina strain, Rostov 10^{-2}	++	00	00	00	00

+ = interference positive
0 = inhibition of fenomen interference by immune globulins
2 cultures use in each test system.

Conclusion

According to the results of CFT, DPAT and NT Congo virus and CHF virus proved to be immunologically identical.

(M.P. Chumakov, S.E. Smirnova, E.A. Tkachenko, A.M. Butenko)

REPORT FROM THE STATE RESEARCH INSTITUTE OF INFLUENZA, LENINGRAD, U.S.S.R.

During 1966-1968 investigations were carried out to study immunogenic and reactogenic properties of the attenuated M-pk mutant of Japanese encephalitis virus obtained by Dr. Kanda Inoue (Kyoto, Japan). This strain was characterized by a significantly decreased pathogenicity for monkeys and swines (Y. K. Inoue) and was successfully used by this author as a live vaccine to immunize swines in certain districts of Japan.

Study of biological properties of the attenuated M-pk mutant established the following markers differentiating it from routinally used laboratory strains:

(i) low pathogenicity for albino mice inoculated per os or intraperitoneally;

(ii) significantly decreased pathogenicity for Mac. rhesus monkeys.

The animals developed clinical symptoms of encephalitis only in cases when more than 5.0 lg LD_{50}/0.5 ml of the virus were injected into the brain. Smaller doses of the virus induced febrile reaction and appearance of mild histopathological lesions in the brains. The virus appeared to be highly sensitive to heating at 50^{o}.

On the basis of significantly reduced virulence of the M-pk strain for albino mice and monkeys the study of reactogenic and immunogenic properties of this virus was carried out on 76 adult volunteers ranged in age from 18 to 50 years.

The vaccine used for experimental procedure was prepared from the virus developed in the brains of suckling mice and injected subcutaneously in 1 ml amounts. The dose of the virus administered varied from 1.0 to 7.0 lg LD_{50}/1 ml.

The volunteers were observed by a physician in the Neurological Clinic for 4-6 weeks after vaccination. Clinical evaluation of subjects included daily taken temperature, regular blood and urine analyses, neurological and electroencephalographic examinations. No febrile reactions or other symptoms were observed among volunteers in response to the live virus injection.

Complete areactogenicity of the vaccine correlated with moderate immunogenic activity of the preparation. Single injection of the virus stimulated

rise in virus-neutralizing antibodies in 41% of vaccinees. Repeated vaccination increased the average number of subjects developing blood antibodies up to 68%. Effectiveness of revaccination depended on the time interval between primary and secondary immunization.

The second injection of the vaccine 10 days after the first vaccination resulted in an increase of antibodies in 56% of volunteers and with the interval of 2-3 months in 88%.

REPORT FROM THE MICROBIOLOGY DEPARTMENT,
ISTITUTO SUPERIORE DE SANITA'
ROME, ITALY

Experimental infection of some laboratory animals with Bhanja virus

Following the isolation of Bhanja virus in Italy, as referred in the eighteenth issue of the Arthropod-Borne Virus Information Exchange, we are determining some biological characteristics of our strains. The host-range was evaluated on the following species:

Mouse. One-day-old Swiss mice were susceptible to infection with Bhanja virus by the intracerebral (i.c.), the intraperitoneal (i.p.) and the subcutaneous (s.c.) routes; survival times were 3 to 4 days for i.c. route and 7 to 9 days for i.p. and s.c. routes.

The titers of a 10% mouse brain suspension in one-day-old mice were respectively $10^{5.8}$ LD_{50}/0.01 ml by i.c., $10^{4.5}$ LD_{50}/0.01 ml by i.p. and $10^{5.4}$ LD_{50}/0.01 ml by s.c. routes.

To determine whether viremia was present, animals from 4 out of 12 litters of one-day-old mice, each inoculated i.c. with 0.01 ml of a 10% mouse brain suspension of the virus, were exsanguinated on the 1st, 2nd and 3rd day after inoculation, and the heparinized bloods pooled and tested. The virus titer in the blood was $10^{2.7}$ LD_{50}/0.01 ml on day 1, $10^{2.6}$ LD_{50}/0.01 ml on day 2 and $10^{1.1}$ LD_{50}/0.01 ml on day 3. Weanling (3-4 weeks old) mice were susceptible to infection when inoculated i.c. with a 10% mouse brain suspension, but no deaths occurred when the virus was inoculated i.p. No attempt has been made to adapt the virus to weanling mice.

Guinea-pig. Fifteen guinea-pigs were inoculated i.c. with 0.1 ml of a 10% mouse brain suspension containing about 10^5 suckling mice LD_{50}. None of the animals fell ill. Afterwards they were inoculated four times i.p. at weekly intervals and were exsanguinated for immune sera production one week after the last inoculation. The HI titer of the immune serum against Bhanja antigen was 1:2,560.

Hamster. Three adult hamsters (Cricetus auratus) were inoculated i.c. with 0.1 ml of the same suspension used for guinea-pigs. The animals did not show any sign of illness during a three-week observation period.

Rabbit. Three adult rabbits were inoculated i.c. with 0.3 ml of the same suspension as above; none out of them fell ill during a three-week observation period.

Monkey. Two monkeys (M. mulatta) were inoculated intrathalamically with $10^{5.8}$ suckling mice LD_{50}. All the monkeys showed a temperature increase on days 5-9, followed by a drop on days 10-11, when the animals died.

Chick embryo. When 7-day-old chick embryos were inoculated with 10% mouse brain suspension by the yolk sac route, the embryos died. The titer was about 10^3 EID_{50}/0.5 ml. The virus was recovered by inoculation of the yolk sac membranes into one-day-old mice. 10-day-old chick embryos inoculated by the allantoic route did not die, and no evidence of propagation by this route was obtained in one-day-old mice. No pocks were produced when the virus was inoculated on the chorioallantoic membranes of 12-day-old chick embryos.

(M. Balducci and P. Verani)

REPORT FROM THE INSTITUTE OF HYGIENE, UNIVERSITY OF VIENNA, AUSTRIA

Studies on Marburg virus (Vervet monkey disease agent)

Marburg virus was successfully propagated in Aedes aegypti using the guinea pig adapted strain F. which had initially been isolated by us from a patient's blood in baby mice. Replication of the virus in the mosquitoes which were artificially infected by the thoracic route was assayed in guinea pigs using the immunofluorescent method for the identification of the virus in liver imprints. The virus failed to replicate in artificially infected Anopheles maculipennis and in Ixodes ricinus.

VSV, Cocal and Hart Park viruses were investigated for their ability to produce interferon in baby mouse brain. In addition, Marburg virus which presumably is also a virus of this group was incorporated in the study. Infected brains were suspended in distilled water and dialysed against citrate buffer pH 2, phosphate buffer pH 7,5 and distilled water. EAGLE's medium of 5-fold concentration was added 1:4. Two-fold dilutions

of this preparation were tested to prevent infection of 100-300 $TCID_{50}$ of EMC virus in L cells.

No or only a low production of interferon was seen after infection with VSV, Cocal and Marburg virus. By contrast, in brains infected with Hart Park virus interferon was detectable up to a dilution of 1:640.

Studies on TBE virus

The interferon inducing compound Poly I:C was tested for its ability to prevent or inhibit the infection with TBE virus in baby mice. Mice (10g) were given intraperitoneally 100γ Poly I:C 3 hours before and again 18 hours after infection with 7 LD_{50} of TBE virus. 94 percent of the treated mice survived as compared with 26 percent of the controls. A similar protective effect was obtained with the same doses of the substance given 3 and 18 hours after infection with 10 LD_{50} of TBE virus. When Poly I:C was injected 24 and 48 hours after infection no such inhibitory effect was seen.

3 young foxes (Vulpes vulpes), 2 weasels (Mustela nivalis) and 4 young polecats (Putorius putorius) were infected with TBE virus by having viraemic nymphs and females of Ixodes ricinus suck on them. All animals developed viremia which lasted up to 4 days. The maximum titer of virus in blood was 10^5 ic LD_{50} (assayed in baby mice). Three of the foxes showed clinical symptoms of encephalitis within approximately 2 weeks after infection. The other animals showed no signs of disease. From the results of our study it can be concluded that all the vertebrates tested can act as host for TBE virus in nature.

(Drs. Ch. Kunz, H. Hofmann and A. Radda)

REPORT FROM THE DEPARTMENT OF VIROLOGY
STATE INSTITUTE OF HYGIENE
BUDAPEST, HUNGARY

Isolation of Uukuniemi virus in Hungary

In 1968 in the south-western part of Hungary 3445 Ixodes ricinus and 342 Haemaphysalis concinna ticks were collected. Virus isolation was attempted from 53 I. ricinus and 7 H. concinna groups in suckling mice.

From a group consisting of 31 I. ricinus males a virus strain was isolated. According to the HI and CF tests carried out by Dr. M. Grešíková (WHO Regional Reference Laboratory for Arboviruses, Institute of Virology, Bratislava, Czechoslovakia), it seems to belong to the Uukuniemi group.

The identification of two further tick-borne arbovirus strains different from TBE virus, pathogenic only for suckling mice but not for adult ones, is still in progress. One of them was isolated from a group consisting of 22 I. ricinus females and the other from a group consisting of 27 H. concinna females.

From a group consisting of 100 I. ricinus nymphs a TBE virus strain was isolated.

In the same area blood samples of 153 humans and 149 cows were examined for HI antibodies to TBE virus. 18.3 per cent of the human sera and 12.7 per cent of the cow sera tested was found to be positive against TBE virus.

(E. Molnár, T. Kubászova, L. Kubinyi, J. B. Szabó)

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

Experimental Ťahyňa virus infections in primates were continued. To approach as closely as possible to the conditions occurring in the nature we used in the present study mosquitoes for inducing the infection in young chimpanzees and we worked with an extraneural variant of the Ťahyňa virus at a low passage level not inoculated intracerebrally during passaging.

Chimpanzees (Pan troglodytes) which have been found suitable for experimental subcutaneous studies with this virus (Šimková, Bárdoš 1966) were exposed to infected mosquitoes - species Theobaldia annulata (Schrk) in which the winter survival of Tahyna virus was proved experimentally (Danielová, Minář 1969).

Four from five chimpanzees exposed to infected mosquitoes developed illness. Infection of chimpanzee could be caused by as few as three infected mosquitoes. The course of the Ťahyňa virus infection in chimpanzees has been characterised by the rise of body temperature, viremia and the virus neutralizing, hemagglutinin inhibiting and complement fixing antibody formation. In two chimpanzees also the acceleration of erythrocyte sedimentation was observed. As for the relation between viremia and the febrile period, we found interesting and important that in all four chimpanzees the development of viremia (lasting eight days, with peak titres of $10^{2.4}$ LD_{50}/0.03 of blood) was preceded by the rise of body temperature (up to 38.5° C). The temporal appearance and persistence of Ťahyňa virus neutralizing, hemagglutinin inhibiting and complement fixing antibodies in the mosquito infected chimpanzees closely resembles those seen in human Ťahyňa virus infections.

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REPORT FROM THE REGIONAL REFERENCE LABORATORY FOR ARBOVIRUSES, INSTITUTE OF VIROLOGY BRATISLAVA, CZECHOSLOVAKIA

Relationship of TBE virus to the wild duck, Anas platyrhynchos

Ten subadult wild ducks were infected by infectious nymphs of Ixodes ricinus. The nymphs came from a laboratory breed and had previous contact with viremic white mice infected with TBE virus. The blood for virus assay was taken at two-day intervals starting from the second or third day until the 14th day and then every seven days for 20 weeks. The organs from ducks dead in the course of the experiment and those obtained from ducks killed 10, 15 and 20 weeks after tick bite were used for virus isolation experiments.

The nymphs of I. ricinus sucked differently and only from one duck we obtained all six. The virus content in engorged nymphs examined individually was from threshold values to $10^{3.2}$ mouse ic $LD_{50}/0.03$ ml. Viraemia in ducks was proved from the second to the thirteenth day with individual differences. The period of viraemia was also different, but it lasted 2-4 days. The maximal virus titre was 10^2 mouse ic $LD_{50}/0.03$ ml on the 4th and 5th day after tick bite.

From the 17th day to the 20th day, four ducks died; two showed the symptoms of meningoencephalitis, another two ducks died without any symptoms. From the cerebellum of a duck (died on the 19th day with meningoencephalitis symptoms) and from the spleen and liver of a duck (died on the 20th day without symptoms) TBE viruses were isolated in a titre of 10^1.

TBE neutralizing antibodies were demonstrated in five ducks on the 7th day; their titre was 1:16 - 1:32 and raised until the 21st day. Five ducks did not develop virus neutralizing antibodies. The levels of antibodies followed until the 20th week differed in the course of the experiment and they were from 1:2 to 1:64.

From the blood of ducks TBE virus was isolated at irregular intervals (on the 9th, 11th, 14th and 16th week) and at the same time we demonstrated neutralizing antibodies. The virus titres in blood of ducks reached only threshold values. From the blood of three ducks we did not isolate the virus.

On the 37th day after bite of ticks one duck died. The examination of spleen and mesenteric lymph node showed titres of $10^{1.5}$ and $10^{1.2}$ mouse ic $LD_{50}/0.03$ ml, but we found no antibodies in its serum until it died. From the organs of other ducks killed after 10, 15 and 20 weeks we did not isolate the virus.

The identification of isolates was carried out by the neutralization test with hyperimmune TBE goat serum, using intracerebral inoculation to mice. TBE virus was confirmed in all cases.

Owing to a limited contact between Anas platyrhynchos and I. ricinus we can consider the wild duck as an occasional host of TBE virus.

(E. Ernek, O. Kožuch and J. Nosek)

Surveillance - virus identification and serological studies

Uukuniemi* virus isolated from Ixodes ricinus ticks in Slovakia

Ticks for isolation attempts were collected from April to May 1967, in the localities Žirany, Topoľčianky (Tribeč region) and Lamač (Bratislava district, in horn beam - oak woods). Pools for isolation attempts were prepared according to the stage of ticks and washed for 1 hour in saline containing penicillin and streptomycin. The suspensions were made in Earle's solution containing 5% heated calf serum with added penicillin and streptomycin. After clarification, each pool was inoculated into one litter of suckling mice 1-4 days old and into chick embryo cell (CEC) cultures. The presence of virus was detected in the second passage by interference method and in the first or second mouse passage, respectively.

Virus isolation was attempted from 392 nymphs, 85 females and 80 males of Ixodes ricinus ticks. Three of 145 pools yielded the virus as shown in

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* Poteplí virus is identical with Uukuniemi.

Table 1. Of the positive pools, 3 were positive in CEC cultures and 2 in suckling mice. All isolates in CEC cultures were accompanied by a partial CPE, appearing 5 days after inoculation of virus dilutions from 10^1 - 10^2. The interference was observed in CEC cultures in the titre of 10^7 IF_{50} per ml. The virus titre in suckling mice was $10^{8.5}$ LD_{50} per ml.

The identification study was carried out mainly by complement-fixation (CF) tests, as the antigen prepared from fresh isolates according to the method of Clarke and Casals (1958) failed to agglutinate goose erythrocytes in the routine procedures.

Immune sera for a number of viruses with CF homologous titres in the range from 1:32 - 1:256, including group B polyvalent sera, were tested against the sucrose-acetone antigens prepared from the new isolates. Under the conditions of these tests, the new isolates were found to be unrelated to any of the following arboviruses: Semliki, tick-borne encephalitis, West Nile, Tribeč; nor was a relation found with LCM virus. Subsequent studies carried out with Potepli antiserum, showed the isolates Ir 268, Ir 293 and Ir 301, indistinguishable from Potepli virus in the CF test.

Our results confirmed that Ixodes ricinus ticks may yield variety of arbovirus strains. In the area under study, tick-borne encephalitis virus (belonging to B group of arboviruses), Tribeč virus (belonging to the Kemerovo group) and Potepli-Uukuniemi (ungrouped) were isolated from Ixodes ricinus ticks.

(O. Kožuch, M. Grešíková, J. Nosek)

Serological survey - Sera from Turkey

From the Mediterranean area, patients' sera from Izmir (Turkey) were examined in haemagglutination inhibition (HAI) tests against arbovirus antigens. We succeeded to demonstrate a high percentage of sera with antibodies against TBE and West Nile viruses. The results of our experiments are illustrated in Table 2.

Based on the results obtained, sera from apparently healthy residents of different age groups from the same region were examined in HAI tests against 9 different arboviruses. The results are evident from Table 3. Very low antibody levels to group A arboviruses were found. A relatively high percentage of sera reacted with antigens of group B arboviruses, namely with dengue, West Nile and TBE antigens.

Table 1

Isolation of Uukuniemi virus from Ixodes ricinus ticks

Collection of ticks		Nymphs		Females		Males	
Locality	Date	No. of examined ticks	No. of isolated strains	No.of examined ticks	No.of isolated strains	No.of examined ticks	No. of isolated strains
Lamač	22.4.67	133	0	19	0	21	1
Žirany	26.4.67	80	0	42	1	50	0
Topolčianky	3.5.67	179	1	19	0	14	0
Total		392	1	85	1	80	1

Table 2

HAI antibody titers to TBE and West Nile viruses in patients' sera from Izmir area

Serum Number	Name	Antigen, 8 units		
		Tick-borne encephalitis	West Nile	Sindbis
I.	S.I.	320	80	0
II.	S.I.	160	80	0
I.	I.Ö.	640	160	0
II.	I.Ö.	400	200	0
III.	I.Ö.	640	320	0
I.	K.E.	640	80	0
II.	K.E.	640	40	0
I.	T.T.	320	80	0
II.	T.T.	1280	80	0
I.	Z.Y.	160	160	0
I.	E.D.	0	80	0
I.	B.G.	160	80	0
II.	B.G.	2560	80	0
I.	A.C.	80	0	0
II.	A.C.	80	0	0
I.	S.E.	160	0	0
II.	S.E.	1280	40	0
I.	M.S.	0	80	0
II.	M.S.	0	80	0
III.	M.S.	0	80	0
I.	M.D.	160	20	0
II.	M.D.	160	20	0

Table 3

HAI antibodies to arboviruses in 81 human sera from Turkey

Arbo-group	Virus	Per cent of positive sera
A	Western equine encephalomyelitis	0
	Sindbis	1.1
B	Tick-borne encephalitis	7.4
	Yellow fever	6.1
	Dengue I	3.7
	Dengue II	9.8
	Dengue IV	20.9
	West Nile	20.9
California	Tahyna	16.0

As for Bunyamwera supergroup, 16% of the sera tested exhibited antibodies to Ťahyňa antigen. Regarding incidence of antibodies against TBE virus in sera from patients, we may consider TBE virus as a potential etiological agent of aseptic meningitides and meningoencephalitides also in this particular region. Cases of aseptic meningoencephalitis have been reported in Izmir area (Serter 1964).

The present serological surveys allow to draw the following conclusion: tick-borne viruses are distributed all over Europe, but are of importance mainly in the temperate zone. Mosquito-borne viruses are characteristi-

cally distributed in exoglacial regions, but are of importance mainly in the Mediterranean area.

(M. Grešíková, M. Sekeyová)

Approached laboratory methods

The use of rivanol for treatment of arbovirus antisera

Different techniques for the preparation of sera for the haemagglutination-inhibition (HAI) test with arboviruses were described. It has been shown (Grešíková and Sekeyová, 1967), that kaolin treatment of heated animal sera is not a convenient method for the HAI test with arboviruses. Therefore the rivanol method was introduced for the extraction of reference arbovirus antisera. Acetone and kaolin treatment of sera was used for comparison.

Animal sera. Sheep antisera against Western equine encephalomyelitis (WEE), Yellow fever (YF) and Čalovo viruses were used in the tests. Eastern equine encephalomyelitis (EEE), Semliki forest, tick-borne encephalitis (TBE) and West Nile (WN) antisera were prepared by immunization of goats. Japanese encephalitis (JBE) antiserum was prepared by immunization of rabbits. All antisera were collected after 3 subcutaneous injections. The viruses were applied in the form of a 10% baby mouse brain suspension, centrifuged at 10,000 g for 20 minutes at 4° C. The intervals between injections were 7 and 14 days respectively; the sera were prepared on the 10th day after the third injection.

From Table 4 it is evident that untreated sera showed an inhibitory activity against the haemagglutinin of WEE, Semliki, Y. fever, West Nile and Čalovo antigens. The most sensitive antigen to the inhibitor was Semliki forest virus.

There were differences in HAI titre after rivanol and acetone extraction. The same antibody titre was found in WEE, EEE, Semliki, YF, JBE and Čalovo antisera, treated with either acetone or rivanol. In TBE and West Nile antisera the antibody titre was by 1 dilution lower after rivanol treatment than after acetone treatment.

Table 4

Comparison in HAI tests of different methods for extraction of reference arbovirus antisera

Antiserum		Methods of extraction			
		Kaolin	Acetone	Rivanol	Not treated
Against virus	Origin of serum	HAI titre			
WEE	Sheep	2.560	1.280	1.280	10.240
EEE	Goat	320	160	160	320
Semliki	Goat	40.960	40.960	40.960	16,000.000
TBE	Goat	2.560	2.560	1.280	2.560
Y.fever	Sheep	20.480	10.240	10.240	40.960
W.N.	Goat	640	640	320	1.280
Jap.Enc.	Rabbit	5.120	5.120	5.120	5.120
Čalovo	Sheep	320	320	320	640

WEE = Western equine encephalomyelitis virus
EEE = Eastern equine encephalomyelitis virus
Semliki = Semliki forest virus
TBE = Tick-borne encephalitis virus
Y. fever = Yellow fever virus
WN = West Nile virus
Jap.Enc. = Japanese encephalitis virus

By comparing different methods for extraction of animal sera, it has been shown that rivanol treatment is also convenient for removing nonspecific inhibitors.

(M. Sekeyová, M. Grešíková)

The use of carbon dioxide for establishment of population density of ticks

The establishment of population density of vector in certain biotopes is the first presumption of any ecological vector studies. For establishment of the population density of ticks we used a small trapping area of 1.5 - 2.5 square meters. Five pieces of dry ice, each weighing 0.5 kg were placed in a mosaic-like pattern into forest litter and covered by a white cotton blanket of the same dimension as the trapping area.

The first ticks appeared on the blanket after 5 to 10 minutes. Usually, during the first 30 minutes we obtained two thirds of the ticks from the population. As a rule, one hour was sufficient for each location.

For establishment of population density of ticks it is necessary to know the radius of location and the population density of the trapping area. The population density for the area may be calculated similarly as for small mammals.

It is necessary to add that the results obtained by this method are 4-5 times higher than those of collecting ticks by usual methods.

(J. Nosek)

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REPORT FROM THE VIRUS LABORATORY PRINS LEOPOLD INSTITUUT VOOR TROPISCHE GENEESKUNDE ANTWERPEN, BELGIUM

Formerly we found that through feeding, the large plaque strains of Middelburg, Sindbis and WEE viruses were more infectious for laboratory bred Aedes aegypti than the corresponding small plaque strains.

We now have tested also SF virus: a laboratory strain with a high, unknown number of I.C. mouse brain passages was compared with a small and large plaque strain obtained from Nicholas H. Acheson, Rockefeller University.

The results (Table 1) show that our "wild" laboratory strain is not particularly infectious for A. aegypti when compared with results mentioned in the literature; this may be the result of numerous mouse brain passages of this strain.

The large and small plaque strains were still less infectious for A. aegypti; it may be that here also the large plaque strain is more infectious than the small plaque strain, however results are not as clearcut as with the first 3 viruses mentioned.

Next the influence of DEAE-dextran on mosquito infection by Sindbis virus was studied, since it was found (see Arthropod-Borne Virus Information Exchange No. 18) that this compound enhances plaque titers of Sindbis-1 virus for chick embryo tissue cultures.

Table 2 shows the results. DEAE-dextran not only enhances considerably the infectiousness of the large plaque strain (as in CETC) but also of the small plaque strain (which is not enhanced by DEAE-dextran in CETC).

For the dextran to be effective in mosquitoes it has to be administered in toxic levels, for in observations not related in Table 2, higher doses of DEAE-dextran from an old stock solution that had apparently deteriorated were given to mosquitoes, no toxicity was observed but also no enhancing effect.

TABLE 1 : INFECTIOUSNESS OF SF VIRUS STRAINS FOR AEDES AEGYPTI .

	Virus Titer in infecting meal PFU/ ml	Number of pos.mosquitoes /mosquitoes tested		%
		4 weeks	5 weeks	
SF	$1.4\ 10^7$	6/19	7/20	33
	$1.4\ 10^6$	1/15	4/19	14
	$1.4\ 10^5$	0/15	N.T	0
SF-1	$1.5\ 10^9$	4/10	3/10	35
	$1.5\ 10^8$	1/9	0/10	5
SF-s	$3.5\ 10^8$	0/9	0/5	0
	$3.5\ 10^7$	2/16	0/9	8
	$3.5\ 10^6$	0/8	0/12	0

TABLE 2: Enhancing effect of DEAE-dextran on infection of A.aegypti by Sindbis viruses .

	INOCULUM PFU/ml	Composition of infectious meal				% mortality at 24 hours	Positive mosquitoes / total tested	%
		Dextran 2%	BGT	Virus	Rabbit blood			
Exp.1 Si L	$1.8\ 10^7$	0.75	0.25	2	7	13/90	39/60	65
		0.60	0.40	2	7	0/26	6/20	30
		-	1	2	7	2/50	2/39	5
Exp.2 Si L	$5.\ 10^7$	1	-	2	8	15/60	31/31	100
		0.1	0.9	2	8	2/70	13/45	28
		-	1	2	8	6/70	11/47	23
Exp.3 Si s	$1.3\ 10^8$	1	1	1.2	6.8	40/54	4/11	36
		0.75	1.25	1.2	6.8	20/80	16/47	34
		-	2	1.2	6.8	1/62	2/56	3.5

Since a mosquito meal is of the order 0.002 to 0.003 ml. the amount of DEAE-dextran taken in by the mosquitoes varied from 3.75 to 4.5 to 5 mcg. per specimen.

The enhancing effect of DEAE-dextran for Sindbis virus infectiousness for A. aegypti differs in at least 2 aspects from the effect on CETC: the activity is evident both for Sindbis-1 and for Sindbis-s strains (the latter not being influenced in CETC) and it is obtained by a smaller amount of the compound that however is toxic for the mosquitoes.

Furthermore an enhancing effect of DEAE-dextran was also found for Semliki Forest virus, as follows:

Virustiterfed PFU/ml	Composition of blood meal				Pos. mosq.	% pos.
	DXT	Blood	BGT	Virus		
$5.4\ 10^6$	0.9	7	0.5	1.6	13/20	65
	0.6	7	0.8	1.6	40/84	47
	-	7	1.4	1.6	13/69	18

Incomplete observations show that a similar enhancing effect is also produced in the system West Nile virus - Aedes aegypti, while no enhancing effect was observed in the system RSSE - Aedes aegypti.

A more detailed study of the mode of action of DEAE-dextran on Aedes aegypti is presently under way, and results so far obtained - which we hope to complete for the next arbo info exchange issue - point to the mosquito stomach-wall cells as the site of action of DEAE-dextran.

(S. R. Pattyn)

REPORT FROM THE MICROBIOLOGICAL
RESEARCH ESTABLISHMENT, PORTON, SALISBURY
WILTS., ENGLAND

Variations in virulence between 'original' strains of Semliki Forest Virus

Although many arboviruses show population heterogeneity with respect to a number of biological and other markers it appears that this variability has usually been recognised in terms of the differences between original and long-passaged strains and rarely in terms of the differences between original isolations and their very early passages. An attempt has therefore been made to define the pattern of virulence markers for a number of nominally original or low-passage strains of Semliki Forest Virus by quantitative assay of relative LD50 values for suckling mice, adult mice and guinea pigs. Infectivity assays were made by intracerebral (ic) and intraperitoneal (ip) inoculation of these hosts and also by plaque counting in agar suspensions of 12 day old chick embryo cells. Table 1 shows the virulence characteristics of three extreme early strains of Semliki Forest Virus arranged according to the susceptibility of the host/age/route systems employed. These are representative of a group of 'original' strains obtained from several sources. The strain VR67(13) of Table 1 is the present original or prototype strain of the American Type culture collection at the 13th passage in mice since isolation by Smithburn and Haddow (1944). The 4th mouse passage of Semliki Forest Virus by Smithburn and Haddow probably corresponded to a virulence profile between those for strains A(7) and VR67(13). At the 19-99th passage in mice by Smithburn and Haddow the virulence profile was probably that of VR67(13). Although the virulence profile of strain A(7) is preserved during two passages in chick embryo cells it is modified by selection to the virulence of strain VR67(13) within 3 passages in hamster brains.

Although strain A(7) is avirulent for all the hosts tested other than young mice below 18 days old it is nevertheless capable of benign replication in all of these hosts. Benign replication is accompanied by viremia at 2-3 days, antibody production (S.N.I. of about 4 at 12 days) and protection against lethal challenge by 1000 LD50 of strain LS(10) by all of the routes tested. The end-point for benign replication appears to correspond with only 1-10 suckling mouse LD50 or PFU and applies to all hosts and routes below the horizontal bars in Table 1.

These data (Table 1) establish the virulence profile as a quantitative statement of the status of a particular strain and illustrate the relationship between a sequence of host/age/route systems in order of susceptibility

and a sequence of virus strains in order of virulence. The clear and sequential division (horizontal bars) between lethal (above) and protective (below) responses to 100 PFU implies that a sequence of distinct and progressively restrictive physiological and immunological barriers may be penetrated in turn by virus strains showing the appropriate invasive mechanisms. Distinct virus strains showing varying degrees of selection for invasive or virulent subpopulations emerge rapidly during early routine passage in mouse or hamster brains. These selections are accompanied by changes in other characteristics of the population (pH and thermal stability, etc.) and demand extreme caution in the definition of the virus strains employed for further experimentation.

TABLE 1

Virus Strain Designation & (passage level in mice)	A(7)	VR67(13)	LS(10)
Host/age/route system	Log number of PFU required to give 50% lethal response		
2-4 day old suckling mice (ic or ip, 0.025 ml)	0	0	0
25-30 day old mice (ic, 0.025 ml)	5.0	0.7	0.7
25-30 day old mice (ip, 0.025 ml)	> 6.0	1.7	1.4
2-4 week old guinea pigs (ic, 0.10 ml)	>7.0	>9.0	1.8
2-4 week old guinea pigs (ip, 0.10 ml)	>7.0	>9.0	>6.0

Further observations on the transmission of Quaranfil virus to mice

Mice inoculated by the intracerebral route developed clinical symptoms and lesions. The lesions were found to spread out from the needle-track damage to the brain or from a cerebral haemorrhage caused by the injection.

The initial inflammatory reaction is invariably traced to the vicinity of the mechanical damage. Mice inoculated by the intraperitoneal route did not develop clinical symptoms nor lesions in the C.N.S. However, when such mice were challenged after 1 to 8 weeks by the intracerebral route, they remained unaffected. Examination of their brains revealed the initial needle track necrotic lesion, but without any tendency of spread to the adjacent brain tissue.

Arbovirus investigations in Sarawak

As previously reported 57/879 mosquito pools collected in Sarawak in the period September-early December 1966 yielded strains of virus. 36 arbovirus strains have been identified; 8 strains of Japanese encephalitis virus, 9 strains of Tembusu, 16 strains of Sindbis and 3 Group B arboviruses. Seven strains of Japanese encephalitis were isolated from pools of C. tritaeniorhynchus and a single strain from C. gelidus. The Tembusu strains were obtained from C. tritaeniorhynchus, C. gelidus, and C. pseudovishnui. The 3 Group B isolates obtained from C. pseudovishnui have also been under study by Dr. Clara Yuen and Dr. Jordi Casals at Yaru and similarities to Kunjin virus have been demonstrated.

The identity of 22 virus strains isolated only in monolayer cultures of primary chick embryo cells have not yet been established. Attempts to produce an efficient plaque assay system in BHK, L-cells, LLC-MK_2 and vervet monkey kidney cells or in monolayer and suspended cultures of chick embryo cells have been unsuccessful. Small plaques, 1-2 mm in diameter, have recently been produced in the PS-C_1 line of stable pig kidney cells under 0.7% agar. All 22 isolates appear to be identical and are very sensitive to ether and deoxycholate. Complete inhibition of virus growth was achieved with 20 µg/ml of bromodeoxyuridine suggesting a DNA containing virus. Mice, guinea pigs, rabbits and fowls are not susceptible but attempts to produce antisera have been unsuccessful. Neutralization tests with a wide variety of known antisera have yielded no information as to the identity of these viruses.

Studies are continuing in Sarawak in collaboration with the Institute for Medical Research, Kuala Lumpur, Malaysia. 409 mosquito pools collected since October 1968 have been processed for virus isolation. Fourteen virus strains have been isolated in mice and of these 8 have been provisionally identified as Group B arboviruses. A further 20 virus strains have been isolated in primary chick embryo cells but no identification has yet been carried out.

Mosquito surveys carried out in and around Kuching showed that the population of Aedes aegypti has not built up in the seaport capital over the past four years but it was found breeding up to 10 miles from the city. The major species breeding in domestic containers in close association with man was Aedes albopictus. Serological surveys have shown dengue virus antibodies to be present in a high proportion of human sera and it was considered that infection is maintained in the human population by a domesticated strain of A. albopictus. In ricefield habitats Culex tritaeniorhyncus was the dominant species breeding in padi fields and it was found that in flooded conditions larvae were washed from plot to plot in the irrigating flow of water.

Field studies on louping ill in Scotland

No viruses were isolated from ticks and small mammals collected in 1968. The largest numbers of Ixodes ricinus were obtained from habitats containing Juncus and from sheep pens. Louping ill virus was isolated from cerebellar material from each of 8 sick lambs. Sera from 3 of these lambs contained reciprocal louping ill complement fixing antibody levels of 1:320, 1:320 and 1:160 respectively.

Studies on the causative agent of the outbreaks of human disease in Marburg/Frankfurt/Belgrade associated with vervet monkeys

Initial isolations from human acute phase blood samples and post mortem tissues were made in guinea pigs which developed a non-fatal febrile illness. Further guinea pig passage produced a uniformly fatal illness with characteristic pathological lesions irrespective of the route of inoculation. The organs principally affected were the lung, liver and spleen.

Experimental infection of rhesus, vervet and squirrel monkeys produced a uniformly fatal illness in all 3 species of monkey irrespective of the route or dose of the inoculum. In the early stages of infection the animals appeared to be quite healthy but after incubation periods ranging from 6-13 days they rapidly deteriorated. A frequent observation in the later stages of infection was the finding of a petechial skin rash on the flexor surfaces of the arms and thighs and on the face. This was most noticeable in rhesus monkeys. The infectious agent was detected in the monkey's blood following infection and often appeared on the day following inoculation. Saliva and urine often contained considerable quantities of the organisms and amounts up to 10^6 guinea pig LD50/ml were found in urine.

Following nine successive passages in hamsters the agent has become adapted to this host causing characteristic lesions in the liver, spleen and kidney and lesions in the brain.

The agent produces characteristic intracytoplasmic inclusion bodies in BHK cells and will multiply without obvious cytopathic effect in several cell systems. By fluorescent-antibody techniques the agent can readily be identified in the cell cytoplasm as early as 24 hours after infection.

Electron-microscopic studies have revealed elongate tubular structures 80 mμ in diameter and up to 4000 mμ in length with associated "head-like" structures 200 mμ in diameter.

The infective agent passes through a 220 mμ gradocol millipore filter and is inactivated by formalin, ether, deoxycholate, methanol and heating at 60° C for one hour. It is not sensitive to trypsin or heating at 37° C for 1 hour. A wide range of antibiotics, antimalarial, antiplasmodial and antiviral substances have no affect on the agent in vivo or in vitro.

(D.I.H. Simpson)

REPORT FROM INSTITUTO DE VIROLOGIA DE CORDOBA, ARGENTINA

In the "Argentinian Hemorrhagic Fever" project, we have analyzed, till now, 18 pools of Acari ectoparasites, (Eubrachylaelaps rotundus, Haemolaelaps glasgowi, Schistolaelaps mazzai and Ornitononyssus bacoti), collected from wild rodents captured in the southeast of Cordoba Province, by isolation attempts, using suckling mice as hosts.

Junin virus strains were recovered from 2 pools of E. rotundus; simultaneously, the blood samples of the carrier rodents were negative in the isolation attempts.

(B. R. de Ferradás)

REPORT FROM THE BELEM VIRUS LABORATORY
INSTITUTO EVANDRO CHAGAS
BELEM, PARA, BRAZIL

The ecology of bird viruses and parasites in tropical forest

Since 1963, more or less intensive investigations of mosquito-borne viruses and antibodies in birds have been carried out in the tropical rain forest on the outskirts of Belem, Brazil, in a cooperative effort by the Belem Virus Laboratory of the Instituto Evandro Chagas, and Dr. Philip S. Humphrey of the U.S. National Museum.

Birds are caught in Japanese mist nets, capture rates being approximately one bird per 2 net/days, and up to 140 nets being set from dawn to afternoon. A small blood sample is taken from the jugular vein and then the birds are banded for identification and released. Through February 1969 the total of birds banded has passed 5000, of 185 species, only one of which is migratory, so that on a given capture day from 50-75% of birds taken in the main study area are already banded. One bird has been recaptured over 30 times, and at least 2 have been recovered more than 4 years after their initial banding.

The studies in 1963 and 1964 resulted in the isolation of western equine encephalitis (WEE) virus for the first time in Amazonia, St. Louis encephalitis (SLE) and Turlock viruses (1). Antibodies were also found for eastern equine encephalitis (EEE). Although the encephalitis viruses are responsible for severe human and equine outbreaks in the United States, where birds have also been implicated in their epidemiology, only equine morbidity and mortality have ever been recorded in Brazil. No human cases have been found, although antibodies to WEE, EEE and SLE are found in the human population.

Antibody rates were found to be much higher in forest birds than in birds from the open field. The studies in 1965 revealed antibodies to other viruses besides the 4 mentioned above, partly because it was possible for the first time to sample the birds of the forest canopy. A simple, cheap device for setting nets at any height up to nearly tree-top level was developed and successfully used (2). Of the 47 species caught in high nets in 1965, 14 were not taken at ground level, indicating a vertical stratification of the avifauna. There was apparently a vertical stratification of virus activity also: antibodies for Itaporanga virus, for instance, were confined to birds netted in the canopy.

During the years 1966 and 1967 no virus isolation tests were made on bird plasmas, but 885 individuals of 106 species were tested for antibody with a battery of 18 arbovirus antigens. Haemagglutination-inhibition titres of 1:20 or more were found in 97 individuals of 44 species, and this antibody was confirmed by neutralization test in some cases. The time of appearance of antibody in birds whose plasma had been negative in previous captures gave an indication of the periodicity of virus infection in the area. The results point to outbreaks of WEE infection in mid-1966 and mid-1967. Virus isolation attempts were restarted in 1968, resulting in the following 12 isolations (through February 1969): SLE 5, EEE 1, Turlock 2, Itaporanga 1, BeAn 141106 (new?) 1, BeAn 157575 (new?) 2.

The question of the importance of the stratification of bird species in relation to their involvement as virus hosts has never been resolved. A current project is using the new high netting device to study this stratification in a detailed and quantitative fashion, and is the first to tackle the question adequately. The project is being directed in the field by Thomas E. Lovejoy III, a Yale University zoology graduate student, and is being funded by the National Institutes of Health ($21,000 over 2 years) and the U.S. Air Force Office of Scientific Research ($40,000 over 2 years). Beginning in late 1967, ground level and high nets have been installed in 3 different types of forest of the Guama Ecological Research Area near Belem. These are terra firme forest, varzea (tidal swamp) and igapo (permanent swamp) forest. Regular captures are made. The data derived will provide a 4-dimensional picture of the distribution of birds and 18 mosquito-borne viruses of tropical rain forest during a complete annual cycle. This information is being correlated with data on mosquitoes and rodents collected simultaneously by the Belem Virus Laboratory in the same locations. These coordinated studies will yield fundamental information needed for the better understanding of the epidemiology of a number of mosquito-borne viruses in the tropical rain forest.

A subsidiary study is being made of the blood parasites in these birds. Dr. Ralph Lainson of the Wellcome Parasitology Unit of the Instituto Evandro Chagas examines blood smears, and has found signs of a higher incidence of parasites in birds frequenting the forest edge than in true forest species.

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(1) Shope, R. E. et al. (1966) Amer. J. Epidemiol. 84:467-477

(2) Humphrey, P.S. et al. (1968) Bird-Banding, 39:43-50

REPORT FROM THE ARBOVIRUS LABORATORY
INSTITUTO NACIONAL DE SALUD
BOGOTA, COLOMBIA

The arbovirus laboratory processed the specimens obtained during a VEE outbreak affecting the upper Magdalena Valley in the Tolima Department, occurring by late 1967. The VEE virus was frequently recovered from patients during the first few days of illness. On the contrary it was seldom isolated from sick equines or from mosquitoes. (See: Weekly Epidemiological Report, Pan American Sanitary Bureau, Vol. XL, No. 18.)

Production and careful study of immune ascitic fluids from mice was continued. Twenty five monovalent and five polivalent of such preparations are now available in no less than 200 ml. each.

Fifteen volunteers were vaccinated with a live modified VEE virus vaccine produced by the National Drug Co. Their antibody reaction was poor when the HI and CF tests were used (bleedings were done until the 21st day post-vaccination) perhaps in relation to antigenic differences between the vaccine virus and that used to prepare the antigen for those tests. Twelve persons clearly showed development of antibodies as judged by the N test IC in mice. It was not possible to demonstrate viremia through daily bleedings in five volunteers.

Experiments on VEE virus inactivation with BPL, ~~in an endeavor to produce~~ a vaccine, have proved unsuccessful. The preparations so far assayed are not infections to suckling mice IC, but they do not develop antibodies in mice, guinea pigs or rabbits after 0.5 ml. inoculated IP.

A serological survey, particularly of equines, was performed in a restricted area in The Eastern Plains (Llanos Orientales, Departamento del Meta) to study possible activity of VEE virus. HI and NT antibodies were found in equines which had never been vaccinated. The HI and NT results were clear cut and correlated, in equines recently vaccinated (1-4 months) against VEE, with a national avianized formalin-inactivated product. Between 1956 and 1958 many bloods from inhabitants from The Eastern Plains had been studied and no antibodies against VEE were found. As the area is becoming a center of rice cultivation during recent years, it was chosen because of the possibility that it could eventually reach an ecologic situation like that observed in Tolima during 1967. It is noteworthy to specify that in The Eastern Plains pesticides are in use only after the beginning of 1968; the entomologic fauna has not yet undergone selection as in Tolima, and the human and equine populations are scarce. These factors apparently pre-

clude VEE outbreaks. Nevertheless, it seems that the virus has reached the area, as judged by the serologic results in equines. It also seems that the ecologic conditions could favor its latent existence and that eventually it could cause serious problems.

By mid-September VEE virus was isolated from a fatal horse case from Valledupar, north of Colombia (Departamento del Cesar). Besides, in the same area several fatal cases in horses occurred, which clinically were diagnosed as VEE infections. On October the 20th an outbreak in humans and horses began in Sinamiaca (Venezuela) and VEE virus was the causative agent. In the neighboring Mid-Guajira (Colombia) soon the alarm spread. Some people stated the occurrence of fatal cases in horses since early October. We inspected the Colombian Guajira from November the 14th to December the 3rd and the information obtained allowed us to conclude that many equines were affected since early November. An intensive vaccination campaign was under way and the epizootic declined by late November. No human cases were reported or seen. As a considerable proportion of the population was affected during the 1962 outbreak (see Infoexchange No. 8-10 Reports from U. del Valle and Inst. Nal. de Salud-Bogotá) it is reasonable to assume that the immunity left precluded an epidemic. On the other hand a faster turnover of the equine population offered many susceptibles. It is not possible to estimate whether the Venezuelan and Colombian Guajira VEE outbreaks in 1968 were due to an extension of the cases in Valledupar, or if they were the end of a wave that swept all Venezuelan since 1962 and came back to die out in the Guajira. As the Caribbean littoral has been in late years the scene of VEE outbreaks, there is another alternative to think of, which is the existence of natural foci, over there, not properly known, from where the virus spreads out from time to time producing outbreaks whenever the conditions prove favorable. This also seems to be in accordance with antigenic variants in the VEE complex being dependent on the geographic procedence rather than on the time and guest under study.

During 1968 the arbovirus laboratory closely collaborated with the Instituto Zooprofilactico Colombiano in the diagnosis of VEE cases and with city and country hospitals in the diagnosis of central nervous or systemic diseases that could be produced by arbovirus infections.

Eight persons (professionals and students) summed up 114 days of training in our laboratory techniques besides the basic theoretic instruction on arbovirus.

(Dr. E. Prías)

REPORT FROM THE VIRUS LABORATORY
FACULTAD DE MEDICINA, UNIVERSIDAD DEL VALLE
CALI, COLOMBIA

VEE Epizoodemic of 1967-68

In August 1967 an epizoodemic of VEE was recognized at Carmelo, a rural community some 18 kilometers from Cali. During the remaining months of 1967 and in early 1968 extension of the epizoodemic in Colombia occurred through the valley of the Río Cauca, the upper basin of the Río Magdalena, the valley of Río Patia, and the Atlantic plain of the north of the country. Epidemiological studies were carried on in some depth at three foci near Cali.

At Carmelo, on the floor of the Cauca valley, the intense outbreak was associated with a country brick and tile industry for which numerous clay pits had been excavated creating a series of excellent mosquito breeding sites. Of 446 horses in a four square kilometer area at Carmelo 181 demonstrated overt illness (40%) and 90 died (20%). Virological and serological evidence was also obtained of infection in domestic rabbits, cattle, pigs, dogs and domestic fowl. Among wild vertebrates, VEE virus was isolated from rats, Rattus rattus (3/37) and Rattus norvegicus (1/2), from opossums, Didelphis marsupialis (6/31) and Caluromys derbianus (1/5), from a bat, Artibeus lituratus (1/18), and from two birds, Jacana jacana (1/8) and Otus choliba (1/2). Blood specimens from 269 acutely ill persons yielded 133 VEE virus isolations. Of the remaining 136 patients, 70 were bled a second time, of which 38 showed a significant rise in HI titer. Based on these data it is estimated that about 76% of all "suspected" cases were in fact due to VEE infection. On the basis of a subsequent serological survey the overall attack rate has been calculated at 11%, with the lowest incidence in urban children of about 6% and the highest incidence in rural adult males of 24%. There were eight cases of frank encephalitis requiring hospitalization, and no deaths. VEE virus was isolated from 49 of 480 pools containing 8,044 mosquitoes. The virus was recovered from all species of which 85 or more specimens were processed except Culex erraticus. Virus isolations in mosquitoes were distributed as follows: Anopheles punctimacula, 1; Mansonia indubitans, 25; Mansonia titillans, 7; Aedes angustivittatus, 1; Culex corniger, 1; Culex quinquefasciatus, 1; Culex aikenii, 7; Culex (Melanoconion) spp., 5; Culex spp., 1.

At two other foci studied in mountainous terrain, ground-pool-breeding, nocturnal-biting mosquitoes were almost completely absent, and virus isolations were made from five species of Simulium black-flies attacking

horses, and the bromeliad-breeding Aedes sexlineatus and Wyeomyia sp. There was also one isolation from Aedes angustivittatus. At one of these localities, Atuncela, of 148 horses, 80 became ill (54%) and 53 died (34%). Virus was not isolated from small mammals or birds. A serological survey of humans, four months after the outbreak, gave an HI positivity rate of 37%.

From calculations based on census data of human and equine populations of the epizoodemic areas affected, and human infection and equine mortality rates determined in selected study areas, it is estimated that throughout Colombia there were 200 to 400 thousand human infections and not less than 40 to 50 thousand (and perhaps in excess of 100 thousand) equine deaths attributable to VEE virus during the 1967-68 episode.

(Pablo Barreto, Ronald B. Mackenzie, Carlos Sanmartín, Harold Trapido)

REPORT FROM THE GORGAS MEMORIAL LABORATORY, PANAMA

Hemagglutinins of Guama Group Viruses in Vero Cell Cultures

At least five arboviruses isolated from mosquitoes in Brazil, Panama, Trinidad and the Florida Everglades are serologically classified in the Guama group. Two of these viruses, Guama and Catu, have been shown to infect man. They have also been recovered repeatedly from sentinel and wild vertebrates. Shope demonstrated the presence of hemagglutinin (HA) of viruses in this group from infected suckling mouse sera which produced relatively low yield. Our experience showed that his method seldom produced HA antigen of workable titers.

This communication describes the production of HA antigens of the Guama group viruses in a stable cell line of green monkey kidney (Vero) and demonstrates the relationship of members of the group by HI tests.

Vero cells were grown in 2-ounce prescription bottles and were inoculated with viruses prepared from suckling mouse brains. Medium 199 plus 1% fetal bovine serum, pH 7.0, was used as a regular maintenance fluid. Five other variations, i.e. medium 199 plus 1 or 2% bovine albumin, 1 or 2% human albumin, and medium 199 without serum, also were used in the

experiments. Fluids from infected cultures, harvested at the time moderate CPE occurred, were used for virus infectivity assays and for HA preparations. Antigen was extracted by double acetone technique from infected fluid and reconstituted to 1 in 10 volumes with borate saline, pH 9.0. HA tests were performed at room temperature using goose erythrocytes. The mixture had a final pH range of 5.75 to 6.0.

Positive HA was obtained for certain viruses tested when cell cultures were maintained in the regular medium. Infected fluid containing bovine or human albumin and those without serum produced lower HA titers. Thus, infected fluid maintained in the regular medium was tried for each virus with different pH values. Results in Table 1 demonstrate that all five viruses and a locally isolated strain of Guama virus (BT 640), tested in Vero cells, produced HA in culture fluid. Optimal titers were secured when pH values in the media were slightly high. HA titers were as high as 1:320 for Bimiti, 1:1280 for Catu, 1:80 for Guama, 1:160 for Mahogany Hammock, 1:40 for Moju and 1:160 for BT 640. Preliminary tests with Bertioga, a recently-isolated Guama group virus from Sao Paulo, Brazil, showed negative results. Without acetone extraction, infected fluids of all 6 viruses mentioned above were negative for HA. Infectivity titers of Catu and Moju infected fluids were 2.5 to 3 $Log_{10}TCID_{50}$ higher than mouse brain material. These two viruses are being serially transferred in Vero cells in order to compare their HA activities during passages.

In addition to Vero cells, hamster kidney and chick embryo cells were inoculated with the six virus strains. Regular maintenance media of pH 7.2, 7.6 and 8.0 were used for each virus. However, none produced HA. Since Vero cells were found to be useful for HA production of the Guama group, other viruses were tried in this cell line as well. So far only Melao virus has produced a low HA titer of 1:40. Changuinola, VSV-Indiana, Bush Bush and Mirim viruses were negative. Other viruses known to occur in Panama are being tried in this cell culture system.

By HI testings, the six antigens of the Guama group prepared by this same technique showed specificity with immune mouse sera. Preliminary tests for cross HI reaction of viruses within the group are given in Table 2. By these tests BT 640 is closely related to both Guama and Moju viruses, although the latter two are clearly distinguishable. Confirmation of these relationships is now in progress by the use of single-injection immune sera prepared in adult hamsters. This animal has been shown to be susceptible to this group of viruses.

Table 1

Hemagglutinins of Guama group viruses in Vero cell cultures

Virus	Reciprocal HA titers in culture fluid maintained at pH				
	6.8	7.0	7.2	7.6	8.0
Bimiti	40	80	320	160	80
Catu	160	1280	1280	1280	1280
Guama	0	0	40	40	80
Mahogany Hammock	0	0	80	160	40
Moju	0	0	40	40	0
BT 640	40	80	160	160	80

Table 2

Cross HI reactions of viruses in Guama group

Serum \ Antigen	Bimiti	Catu	Guama	Mahogany Hammock	Moju	BT 640
Bimiti	160*	20	10	20	10	20
Catu	20	320	20	0	10	20
Guama	20	20	640	40	40	160
Mahogany Hammock	20	0	80	320	40	40
Moju	20	20	40	80	320	80
BT 640	0	0	80	40	80	320

*Reciprocal serum titer against 4 units of antigen.

During the past four years more than 45 strains of Guama virus have been obtained from mosquitoes, sentinel mice, sentinel hamsters and wild rodents in Panama. Two to three isolates from each source were tested for HA activities in Vero cells. Positive HA resulted from all isolates with titers ranging from 1:80 to 1:640.

In CF testing, members of Guama group were strongly reactive indicating this test to be useful only for group specificity. Final identification, therefore, can be completed with ease by HI tests using this simple method of HA antigen preparation. Of the Panamanian Guama group isolates thus far tested, all have been shown to be the same type. Detection of Guama virus transmission in sentinel hamsters also has been demonstrated by HI tests using the HA antigen prepared by this technique. In addition, we have used HA antigen of BT 640 for antibody surveys. In one of the known endemic areas of this virus, 16 positive results were obtained by HI from 26 CF-positive rodents. Some of the HI positives had titers as high as 1:320. A survey of 218 humans indigenous to the same area revealed that 19 had Guama HI antibodies. Neutralization tests are in progress to confirm these antibodies in human populations. No isolates of the Guama group viruses have been obtained from man in Panama.

It is hoped that HA titers of this group of viruses in Vero cells can be improved by further chemical and physical treatment, as has been shown for other agents. In addition to other serological techniques, HI test is useful for identification of new isolates and for antibody surveys in endemic areas of this group of viruses throughout tropical Western Hemisphere.

(Sunthorn Srihongse)

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

In the year 1968 the number of mosquitoes caught totalled up to 5, 623 specimen divided over 41 species.

The bulk belonging to:

Aedes serratus	8445 specimen
Mansonia titillans	4043 "
Mansonia venezuelensis	8557 "
Culex portesi	20440 "
Culex spissipes	3849 "

Special attention was payed to the places frequented by Culex portesi.

Most of the mosquitoes were caught by handsuction on human bait; 49984 specimen. The remainder were caught in Trinidad No. X traps and suction traps baited with:

rats	2948 specimen
albino swiss mice	3390 "
monkeys	201 "

The year 1968 yielded 30 virus isolations; 14 of them resulted from sentinels, for which litters of swiss albino baby mice were used and 16 isolations came from mosquitoes. The following mosquito species participated in this result.

Psorophora ferox	3	isolations,	i.e.,	1	isolation	from	1300	mosquitoes
Psorophora albipes	1	"	"	1	"	"	1700	"
Mansonia venezuelensis	2	"	"	1	"	"	4000	"
Culex portesi	9	"	"	1	"	"	2250	"
Limatus flavisetosus	1	"	"	1	"	"	200 (by accident)	"

From the 30 virus isolations 8 belonged to the Guama group. The remainder, mostly belonging to group A are not yet finished. The Guama group encloses

Guama (prototype)	from	Culex portesi
Bimiti	"	sentinel mice
Catu	"	sentinel mice

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

Bush Bush No further recovery of the rodent population was discovered here. The area has now been declared a wild life sanctuary.

Turure Forest Virus activity was intense in this forest from June through November. It stopped rather abruptly in December. Culex portesi, the main vector of rodent-associated viruses maintained high parous rates throughout the period of high virus activity. EEE virus was recovered 29 times, 17 times from sentinel mice, 11 times from C. taeniopus and once from C. portesi. No isolations were made from 1, 200 bird sera. C. taeniopus is at present the best candidate for the EEE vector role. In the laboratory this mosquito transmitted EEE while C. portesi and C. fatigans failed.

A comparative study of two 400 x 400 feet areas about 1/4 mile apart which was started in July showed promising results. However, these two areas were unexpectedly cleared for farming purposes.

Mayaro No viruses were recovered during the studies in this south-eastern part of Trinidad.

Due to lack of funds and the uncertain future of TRVL activities are at present maintained at greatly reduced levels.

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY UNIVERSITY OF THE WEST INDIES, MONA, KINGSTON JAMAICA, W. I.

Dengue activity continued into the latter half of 1968 with 483 cases reported during this period mainly from the Kingston and St. Andrew area. Sixty-five cases, representing 62% of the paired sera tested, were confirmed by HAI and CF tests. Thirty-four isolates were recovered from sera and Aedes aegypti mosquitoes employing one-day-old Swiss white mice, primary and continuous (BSC-1) African green monkey kidney cells. Five of these isolates have been conformed by the Department of Virus Diseases, Walter Reed Army Institute of Research, as dengue viruses belonging to types I

and 2. This is the first time that dengue virus has been isolated from Jamaica, and the first time that two dengue serotypes have been isolated from a Caribbean community during the same epidemic.

The rapid development of high HAI titres in the sera of patients with dengue symptoms is indicative of anamnestic responses consequent on reinfections. More gastroenteric problems and the appearance of a total rash have been observed during this period of the epidemic.

REPORT OF THE PAHO INSECTICIDES TESTING UNIT, KINGSTON, JAMAICA

The PAHO Insecticides Testing Unit was established in Kingston, in 1962, in collaboration with the Government of Jamaica and the University of the West Indies to investigate the susceptibility of strains of Aedes aegypti from that area to various insecticides and to evaluate new products that might be substituted for chlorinated insecticides in the eradication of this mosquito.

A series of tests to determine the best length of cycle using Abate were initiated at the beginning of the year. In this series, 2 and 3 month cycles were used for perifocal, intradomiciliary and reduced perifocal treatments. In the reduced perifocal, only the important larval sites were treated. The verification surveys should be completed in the first quarter of 1969. Initial results indicate that up to 3 month cycle is satisfactory for intradomiciliary treatments, but a 2 month cycle is required for perifocal treatments.

A proposal has been submitted to the Ministry of Health of Jamaica for the establishment of an Aedes aegypti eradication project as the initial step for the nationwide program.

Preliminary surveys in Gran Cayman indicate that A. aegypti is absent from the island. A more thorough survey will be conducted early in 1969 to verify this finding. If confirmed, the species was probably eradicated by a program of intradomiciliary treatment with DDT designed to protect the population from pest mosquitoes. Conditions on the island are very favorable for the mosquito and some years ago an index of close to 100% was reported.

The determination of the baseline insecticide susceptibility are virtually complete. They show that in almost all areas the species is strongly resistant to chlorinated hydrocarbons and susceptible to organophosphates. The only exception is Cayman Brac where the species shows only reduced susceptibility to chlorinated hydrocarbons.

In 1969 the Unit will carry out field tests to evaluate some compounds as ovicides against A. aegypti eggs. The first ones of such chemicals to be tested on a village basis will be some ethanolamines which, in laboratory and simulated field tests carried out by NCDC Laboratory in Savannah, Georgia, proved to be effective ovicides.

REPORT FROM THE UNIVERSITY OF MIAMI
MIAMI, FLORIDA

Additional studies on the epidemiology of Venezuelan equine encephalitis (VEE) in South Florida area are currently being carried out in cooperation with the Florida State Board of Health (Miss Elsie Buff) and the Arbovirus Infection Unit of the National Communicable Disease Center. A survey of 340 individuals in the Homestead area reveals 5 to have both neutralizing and HI antibodies to VEE. Four of these persons were asymptomatic; one had symptoms that may have represented a recent infection. Three of the five persons along with the symptomatic index case (Arbovirus Info Exchange No. 18) live in an urban area.

(N. Joel Ehrenkranz)

REPORT FROM THE EPIDEMIOLOGY RESEARCH CENTER
STATE BOARD OF HEALTH
TAMPA, FLORIDA

The Encephalitis Research Center was renamed the "Epidemiology Research Center" during 1968. The name change reflected a broadened area of interest including viral diseases other than those caused by arboviruses. Special studies of influenza, mumps, measles, and Tamiami virus characterized these extended interests.

The principal financial support for the center, however, remained with the N.I.H. grant entitled "Arbovirus Epidemiology in Florida". These investigations, therefore, received first priority. The center's staff assisted in the investigation of the first recognized human infection with Venezuelan encephalitis (VE) on the North American continent which occurred in Homestead, Florida, 250 miles south of the Tampa Bay area. Careful surveillance in the Tampa Bay area suggested that there was no evidence of VE activity in central Florida during 1968.

California encephalitis (CE) viruses are still the most prevalent, and therefore, most investigated of the arboviruses in the Tampa Bay area. Fifty-five recoveries were made during 1968, all from mosquitoes. Forty-six of these were from Aedes infirmatus, eight from Aedes atlanticus, and one Anopheles crucians. Biological studies strongly implicated the cotton rat as the vertebrate reservoir for CE viruses in the Tampa Bay area. Marsh rabbits were also shown susceptible to experimental infection in the laboratory. For the third consecutive year we again failed to demonstrate transmission in the laboratory using Aedes infirmatus mosquitoes and Keystone virus. A plaque reduction neutralization test in VERO cell culture was perfected by the laboratory and considerable improved the serologic tools available for study of Keystone virus. The immunodiffusion technique for identifying sub-strains of CE virus was evaluated during 1968. The complement fixation (CF) test remains the cheapest, quickest, and simplest identification procedure.

For the sixth successive year there was no evidence of St. Louis encephalitis (SLE) activity either in humans, vertebrates, or arthropods. Physicians and hospitals in the area referred 589 human cases for viral diagnostic studies. None gave evidence of recent arbovirus infection. Sentinel chickens and pauperized dove flocks were again negative for SLE serologic activity; 31,074 Culex nigripalpus mosquitoes were tested and found negative for SLE virus. Follow-up studies of surviving SLE patients

from the 1959, 1961, and 1962 epidemics were completed in 1968. Survivors maintained hemagglutination-inhibition (HI) and serum neutralizing (SN) antibodies, but most had lost their CF antibody by the fourth or fifth year. Many of the survivors had neuroasthenic complaints which differed from those of their matched controls and there was some minimal difference in their ability to perform finely coordinated movements of the hand and postural balance muscles.

The newly identified Tamiami viruses from cotton rats were successfully grown on VERO cell culture and a plaque reduction technique developed for assaying serum neutralizing antibodies. Laboratory infected rodents were found to produce CF antibody titers. It was concluded the most likely method of transmission between rodents was via virus contaminated urine. Again there was no evidence of human infection with Tamiami viruses. Since Tamiami virus is a member of the larger hemorrhagic fever group which produces serious illness in Central and South America, further careful surveillance for human infection is necessary.

Other arboviruses identified and studied during the year included Tensaw, Western encephalitis (WE), Sawgrass, and Eastern encephalitis (EE). The new study site near the University of South Florida (USF) was unusually productive of Anopheline mosquitoes and a large number of Tensaw virus isolates were recovered from these. A small serologic survey of students revealed less than two per cent reactors with the Tensaw antigen. A newly identified Florida tick virus termed "Sawgrass" was recovered from five tick pools during the year. As in the past, these were either Dermacentor variabilis or Haemaphysalis leporis-palustris.

A pre-medical student performed an experimental infection of water snakes with WE virus. The virus was found to circulate in the snake during the first week after laboratory infection. Artificial hibernation was then produced and the snakes checked again for virus circulation; none was detected. Two other students carried out significant research activities. A cooperative education student from USF performed an experiment to determine the distribution and biological half-life of radioactive iron (Fe^{59}) in the blood of the mourning dove. A pre-medical student from Tulane University participated in an extensive sero-epidemiologic study of mumps in a group of 72 families in Hillsborough County. Important data was obtained which will be useful in the public health indications for live attenuated mumps virus vaccines.

The E.I.S. Officer assigned to the center participated in a large measles vaccination campaign in Hillsborough County, in a virus immunity survey

of 2900 individuals in the Tampa Bay area, assisted in the investigation of an important dual epidemic of meningococcal memingitis and ECHO-9 viral meningitis in Manatee County, directed the study of mumps epidemiology in the families described above, participated in a study of the neurologic and psychologic sequelae following a mumps viral central nervous system (CNS) infection, and assisted in a large-scale mass field trial of aerosol influenza vaccination. The latter was conducted in conjunction with the University of Florida, College of Medicine, Gainesville; the State Board of Health in Jacksonville; the Hillsborough County Health Department; and the Hillsborough County T. B. and Health Association.

Other special studies conducted during the year included continuation of the immunization and cross challenge experiments with dengue-2 and SLE viruses, the development of an immunodiffusion technique for identification of EE, and a cooperative study with St. Joseph's Hospital and USF in characterization of arboviruses under the electromicroscope.

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY THE UNIVERSITY OF TEXAS MEDICAL SCHOOL AT SAN ANTONIO

The Department of Microbiology at the new University of Texas Medical School at San Antonio has been recently established. As part of its overall program, arbovirus studies of both epidemiologic and fundamental nature will be initiated. Some of the viruses important to the Southwestern United States will be studied and other virological interests relevant to the projects conducted in Panama and in Bethesda will continue to be pursued. With the addition of Dr. Dennis Trent of Brigham Young University, biochemical studies of St. Louis encephalitis virus will also be initiated. Dr. K. O. Smith and his two associates currently at NIH will join the Department and will continue their electronmicroscopic virological studies.

(Alexis Shelokov)

REPORT FROM THE STATE DEPARTMENT OF PUBLIC HEALTH VIRUS LABORATORY, AUSTIN, TEXAS

Arboviral encephalitis as in 1967 remained at a low level of incidence in 1968. Laboratory confirmed or highly presumptive cases in the past 3 years are shown as follows:

Year	WE	SLE	CEV
1966	22	253	1
1967	4	2	
1968	5	1	

Since the clinical differentiation of arboviral encephalitis and enteroviral "meningo-encephalitis" or "encephalitis" remains a problem in children or young adults enterovirus "associated encephalitis" continued to receive attention. Coxsackievirus B2 and Echovirus 9 and 14 were incriminated most often in 1968.

Arbovirus surveillance activities were limited mostly to tests on pools of culicine mosquitoes and tests of bird sera obtained near Dallas and San Antonio. The SLE virus was found in two pools of mosquitoes from Dallas County in late 1968.

Further comparative serological studies on several members of the CEV complex suggest that the Houston virus should be considered as a new member of the group.

(T. Guedea, M. Guerra, and J. V. Irons)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY
THE UNIVERSITY OF TEXAS AT AUSTIN

Biological Functions Associated with Nucleocapsid and Envelope Components of Sindbis Virus

The biological functions associated with the nucleocapsid and envelope components of the Sindbis virion were studies. Sindbis virus preparations labeled with amino acids, uridine or P^{32} were treated with desoxycholate (DOC) and the major structural components of the virion were separated by zonal centrifugation in sucrose gradients. The nucleocapsid component of the Sindbis virion was not infectious in the conventional infectivity assay. Attachment studies conducted with labeled nucleocapsid and virus preparations indicated that the viral envelope was necessary for host cell attachment. The ability of intact Sindbis virus to agglutinate goose erythrocytes was destroyed by DOC treatment. Hemagglutinin activity was not detected in either the nucleocapsid or envelope fractions. Nucleocapsid preparations obtained from homogenates of infected cells also lacked hemagglutinin activity, indicating that the viral hemagglutinin is associated with the envelope of the virion.

Immunological studies were conducted to determine which component of the virus particle is involved in the complement fixation and viral neutralization reactions. Immune serum was prepared in rabbits and adsorbed against chicken embryo cells. Complement fixing antigens were detected in the nucleocapsid and envelope components of the Sindbis virion. To define the role of each of these structural components in viral neutralization reactions, immune serum was adsorbed with envelope and capsid preparations, adsorption of the antiserum with capsid protein did not alter the ability of the immune serum to neutralize Sindbis virus. Adsorption against the envelope preparation reduced the serum's ability to neutralize virus by 90%. Further adsorption of the serum with nucleocapsid or envelope preparations failed to alter these results. This suggests that neutralizing antibodies are directed against antigens in the viral envelope and that the antigens in the nucleocapsid and envelope are not identical.

(H. R. Bose, B. P. Sagik)

Separation of Sindbis Variants by Calcium Phosphate Chromatography

Two Sindbis virus variants have been isolated which differ in plaque-type antigenicity and neuroinvasiveness in mice. These biological differences in the variants are believed to reside in the viral structural protein. Therefore, procedures were sought to purify large quantities of Sindbis variants for subsequent amino acid analysis. One of the methods investigated was calcium phosphate chromatography. When these two Sindbis virus variants were subjected to chromatography on calcium phosphate they eluted from the column at different phosphate molarities. The virus was eluted from the column with a linear gradient of 0.005 to 0.5M phosphate buffer pH 7.4. Virus elution was initially monitored using the microhemagglutination technique. The large plaque variant began to elute from the column at a phosphate molarity of approximately 0.04M. The small plaque variant began to elute a 0.38M phosphate. To determine whether these plaque variants could be separated on a calcium phosphate column, an artificial mixture was prepared and chromatographed. Plaque assays were used to distinguish between the two variants. The large plaque variant predominated in a ratio of 10 to 1 in the zone of low ionic strength. In the range of high ionic strength small plaques predominated at approximately the same ratio.

(H. R. Bose, B. P. Sagik)

Purification of Sindbis Virus

A procedure was established for preparing Sindbis virus in quantities sufficient for chemical analysis. The procedures and degree of purity achieved are outlined in Table 1. The purity of the virus preparation was monitored by following removal of added C^{14}-labeled host protein, and host antigenic material. In addition the ratio of infectivity to total protein was monitored at each step in the procedure.

As shown in Table 1, fluids (1850 ml) were removed from 300 chick embryo (CE) monolayers 12 hours after infection with Sindbis virus. This virus suspension (5×10^9 PFU/ml) was centrifuged at 16,000 xg for 20 minutes to remove cell debris. The supernatant fluids were placed in ten 92-ml cellulose nitrate tubes. A cushion (5 ml) of 20% sucrose was added to the bottom of each tube with a needle and syringe, and the material

TABLE 1

PURIFICATION OF SINDBIS VIRUS FROM CE MONOLAYERS

	Total PFU	Total C^{14}-CPM	Protein (mg)	HA Units	CF Units: Viral	CF Units: CE
300 CE monolayers						
CE cell supernatant (1850 ml)	5.5×10^{12}	---	2,360.	6,400	9,500	9,250
C^{14}-grown cells; sonicated (65 ml)	---	1.3×10^{8}	26.	---	---	800
Centrifugation 59,000xg (1915 ml)						
Precipitate (45 ml)	2.7×10^{12}	1.5×10^{5}	98.	3,600	4,800	3,600
Supernatant (1900 ml)	1.0×10^{3}	1.0×10^{8}	140.	NR	NR	9,250
1) Centrifuge 63,500xg 2) Dialysis (20 ml)	5.2×10^{11}	3.3×10^{5}	22.8	6,400	6,400	800
Virus (4 ml)	1.0×10^{11}	6.6×10^{4}	4.5	1,280	1,280	160
Virus (16 ml)	4.2×10^{11}	2.5×10^{5}	18.3	5,120	5,120	640
1) NRS immunoadsorbent column 2) Ultrafiltration						
Concentrate (12 ml)	1.9×10^{11}	1.3×10^{4}	3.0	1,500	2,500	120
1) Anti-host immunoadsorbent column 2) Ultrafiltration						
Concentrate (25 ml)	3.5×10^{11}	5.0×10^{2}	8.2	6,400	6,000	NR
Density Gradient centrifugation						
Density gradient centrifugation						
Centrifugation 63,000xg (30 ml)	1.2×10^{11}	3.3×10^{3}	2.5	400	2,000	150
Centrifugation 63,000xg (30 ml)	7.1×10^{11}	7.5×10^{1}	7.4	130,000	10,240	NR
Pellet (1 ml)	1.0×10^{11}	1.0×10^{3}	2.4	2,400	1,000	100
Pellet (1 ml) (purified virus)	6.0×10^{11}	6.0×10^{1}	7.1	12,800	4,000	NR

was centrifuged at 59,000 xg for 3 hours in the Spinco 21 rotor at 4 C. The supernatant was discarded and the pellets were overlaid with 4 ml of a saline solution, pH 8.6, containing 0.2 M tris-(hydroxymethyl)-aminomethane, 0.02M disodium ethylaminediaminetetraacetic acid, and 0.005M 2-mercaptoethanol. After gentle agitation for eight hours at 4 C, the resuspended virus (45 ml) was layered over discontinuous gradients formed by layering 6 ml of 20% sucrose solution over 4 ml of 40% sucrose solution in each of two 32-ml cellulose nitrate tubes, and was then centrifuged at 63,500 xg for 2.5 hours. The viral material at the 40-20% sucrose interface was collected and dialyzed against the Tris-saline-EDTA buffer (no mercaptoethanol present).

Virus was then passed through an immunoadsorbent column prepared by treating anti-host serum with ethyl chloroformate (Avrameas and Ternynck, 1967). The virus was not adsorbed by the immunoadsorbent and passed through the column. The eluant was centrifuged into three 20-45% sucrose gradients (volume 25 ml) at 63,500 xg in the SW 25.1 rotor. The band containing the infectious virus was collected, diluted with buffer and the virus pelleted by centrifugation for two hours at 63,500 xg.

The level of purification obtained was 330-fold based on the change in PFU/mg total protein and at least 2000-fold based on the removal of added labeled host material. The overall recovery of infectivity was approximately 11 percent.

(G. Z. Carl, B. P. Sagik)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY THE UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL SCHOOL, DALLAS, TEXAS

In a previous Information Exchange (No. 15, March, 1967) we presented preliminary data obtained in field studies in Japan designed to investigate the role of bats in the ecology of JBE virus and in south Texas to determine if these animals are involved in the persistence of SLE virus in that area of the United States. These field studies have now been completed except for a few viral isolates which have not as yet been fully characterized.

Results obtained in the Japan field studies have been assembled for publica- in the form of two reports which will appear in the November issue of the American Journal of Tropical Medicine and Hygiene. We are now in the process of analyzing data obtained in the survey of bat populations in south Texas for evidence of SLE virus infection during and subsequent to epidemics of SLE in Houston, Texas in 1964 and in Corpus Christi, Texas in 1966 and it is with these data that the present report will be concerned.

Although considerable knowledge has accumulated concerning the interrelationships of SLE virus, its vectors, and natural hosts since the original isolation of the agent in 1933 there are still many missing links in our understanding of the biological life cycle of SLE virus particularly with regard to the survival of the agent in temperate zones during the winter months. The epidemic of SLE in Houston, Texas in the summer of 1964 provided an opportunity to determine if bat populations in that area were serving as virus carriers in a manner that our experimental studies had indicated they were capable of doing (Information Exchange No. 10, October, 1964). Although we were unsuccessful in finding colonies of bats in the Houston metropolitan area where the center of the epidemic was located, a large colony of Mexican free-tailed bats (Tadarida briziliensis) was found in the attic of a house in Angleton, Brazoria County, approximately 40 miles south of Houston, a distance within the nocturnal feeding flight range of this species. The first collection of bats was made on 26 August 1964, which subsequently proved to be during the week when the epidemic reached a peak with regard to number of cases. In order to determine if SLE virus could be detected in the bat population subsequent to the epidemic period, additional groups of bats were netted in September, October, November, and December of 1964. Collections were resumed in April of 1965 and continued at approximately monthly intervals through January 1966 to determine if SLE virus was circulating in the bat population in what proved to be a nonepidemic year in the Houston area. A total of 1016 bats were collected with significant numbers being taken from nature during each of the four seasons of the year. Certain groups of bats were tested only for neutralizing antibodies against SLE virus and we failed to obtain blood specimens from a few animals so that only 851 bats were tested for viremia by the assay of either whole blood or spleen tissue. Two strains of SLE virus were isolated from bats obtained in the initial collection on 26 August 1964 and 11 additional strains were obtained from bats netted at intervals throughout the following 18 month period. The isolation of SLE virus from bats collected during the epidemic period in Houston was of interest since it represented the first reported isolation of this agent from a naturally infected mammal other than man but it showed only that natural SLE virus infection could be demonstrated in bat populations during the time when the agent is readily demonstrable in nature in mosquitoes and

avian hosts whose role in the epidemiology of SLE has long been recognized. However, the subsequent isolations of strains of SLE virus from bats netted after the epidemic period and during the following nonepidemic year indicated that populations of Mexican free-tailed bats in the Houston area are persistently infected with this agent.

In the summer of 1966 an outbreak of SLE occurred in Dallas, Texas but we were unable to find sufficient numbers of bats in Dallas or environs at that time to enable us to determine if these animals were harboring SLE virus. However, when a second outbreak of SLE occurred in Texas during the summer of 1966 in Corpus Christi we were fortunate in locating colonies of Tadarida braziliensis roosting along beams in the ceilings of warehouses in the dock area of the city. The first collection was obtained 11 September 1966 and continued at approximately 14 day intervals through November, then monthly until June 1967 when the colonies were disrupted by officials of the city-council health department. In an extensive study of the life history and ecology of Mexican free-tailed bats, little evidence was found that this species overwinters in buildings in Texas but it was suggested that this habit is more prevalent than observed, particularly along the Gulf coast. Our collections of bats from Angleton during the winters of 1965 and 1966 and from Corpus Christi throughout the winter months in 1966 and 1967 are evidence that at least a percentage of the free-tailed population which roosts in buildings in these locations in Texas remains throughout the winter. During the collection period in Corpus Christi 870 bats were obtained for study and 13 strains of SLE virus were isolated from the bloods of 798 of these animals. Although collection of bats was initiated late in the epidemic period in Corpus Christi when the attack rate was declining rapidly, 4 strains of SLE virus were isolated from bats obtained in the first collection on 11 September and additional strains were obtained from virtually all of the groups of bats collected subsequently. Although disruption of the bat colonies in Corpus Christi prevented our collecting bats from this site after May 1967 these colonies had been adequately sampled during the fall, winter, and spring months, the seasons in which we were most interested in detecting SLE virus infection in the bats in this area. The isolation of SLE virus from bats netted in November and December of 1966 and in February, April and May of 1967 demonstrates the persistence of SLE virus infection in the Mexican free-tailed bat population in the Corpus Christi area throughout the year. Serological evidence of SLE virus infection in Tadarida populations in south Texas has also been obtained. Neutralizing antibodies against this agent have been demonstrated in 10 to 20 per cent of the more than 500 plasma samples tested to date. In addition to the

strains of SLE virus isolated from the bats collected in south Texas, several strains of Rio Bravo virus and several as yet uncharacterized viral agents were recovered from the bloods of these bats.

(S. E. Sulkin and R. Allen)

REPORT FROM THE DEPARTMENT OF PATHOBIOLOGY AND COMPARATIVE MEDICINE, UNIVERSITY OF TEXAS, SCHOOL OF PUBLIC HEALTH, HOUSTON, TEXAS

One of the first research programs initiated in this new School of Public Health was a study of arboviruses in the metropolitan area of Houston, Texas. This will be a long term study involving continuous field observations and supported by an efficient laboratory. Although, emphasis will be directed to St. Louis encephalitis virus previous studies resulted in the isolation of Cache Valley and Hart Park viruses. The study will be designed to provide quantitative data useful to the Harris County Mosquito Abatement District and local health agencies.

One of the most important components of this research program is the selection of sites suitable for long term field study. As a result of field observations and examination of maps of several types, it was apparent that metropolitan Houston has a complicated anatomy.

A number of hydrologic features appear to favor an abundant mosquito population during certain periods of the year. The storm drainage in Houston flows into several bayous which form a criss-cross pattern. All of these lead into the Houston ship channel and this, in turn, into Galveston Bay. Due to the low contour and rather high soil moisture content, a small amount of precipitation results in filling dependent areas such as ditches and bayous.

Scattered throughout the city are inhabitants representing low socio-economic groups. In some of these areas, the dwellings are elevated several feet above ground level, and thus provide harborage for small domestic

creatures as well as resting sites for mosquitoes. In these situations sewage disposal is often not adequate.

In older established residential areas, large trees and shrubs are present. Along the margins of bayous and larger ditches, there is dense growth of luxuriant vegetation.

Most areas in which a detailed reconnaissance was made conformed to the distribution of residents in Houston who had a laboratory confirmed diagnosis of St. Louis encephalitis infection during the 1964 epidemic.

These observations as well as limited mosquito collections indicate that productive results should be forthcoming and accordingly, will be the subject of future reports from this unit.

REPORT FROM THE MEDICAL MICROBIOLOGY DIVISION GULF SOUTH RESEARCH INSTITUTE NEW ORLEANS, LOUISIANA

1. Responses of African Green Monkeys to Unadapted and Mouse-adapted Type 1 Dengue Viruses

Juvenile African Green monkeys (Cercopithicus aethiops) were infected by the subcutaneous and oral routes with Unadapted Type 1 virus in the form of acute phase human serum. This virus (designated as the Vandeever strain) characteristically produced encephalitis and death in suckling mice when inoculated intracerebrally; $10^{4.8}$ $SMLD_{50}$ was used subcutaneously and $10^{5.2}$ $SMLD_{50}$ administered orally. Animals were followed for clinical reactions, changes in blood chemistry, and hematology, viremia, and antibody responses over a 90-day period.

No significant clinical features including rash or fever were observed over a 14-day period. Several of the animals developed palpable inguinal and axillary lymph nodes, but these reactions were not marked. There were no significant changes in blood chemistries for total protein and γ globulin, glucose, or blood urea nitrogen. No significant changes in the hematological picture were noted.

Despite the fact that the virus used for infection produced definite encephalitis and death in suckling mice, attempts at demonstrating the viremia by inoculation of daily blood samples into baby mice (with subsequent challenge with adapted virus) failed to indicate definite evidence of viremia. Throat swabs taken from animals during the first week after inoculation also failed in most cases to yield virus. We were able to recover virus in one or two instances from the throat swabs of animals which had been inoculated orally. Even in these latter cases, only 1 or 2 animals per litter developed definite indications of infection.

Tests for the CF antibody responses have been completed. The findings representing 1 set of animals are presented in Figures 1 and 2. The response of the other monkeys was essentially the same. CF antibodies appeared between day 10 and 14. Titers peaked at 21 days, and ranged from 1:16 to 1:64. CF titers appeared to persist at low levels (1:8) in 4/6 animals at 91 days. The responses of the monkeys given virus orally were as good or better than those infected by the subcutaneous route.

In carrying out the CF tests, three antigens were employed - derived respectively from Hawaiian Dengue MP125, TH-Sman MP25, and Vandeever MP5. (Unadapted strain used for inoculation and passaged in suckling mice.)

The responses to Vandeever and MP125 Dengue were essentially identical in all animals, whereas the responses to TH-Sman were at least 2 and sometimes 4 to 8 fold lower. This difference was especially marked in the HAI tests. HAI antibodies (80-320) appeared by 14-21 days for MP125 Hawaiian and MP5 Vandeever hemagglutinins. Only 3/6 animals infected demonstrated HAI antibodies for the TH-Sman strain; these appeared later (21 days or later) and never reached titers greater than 1:80.

By day 59, HAI antibodies had receded to low levels (20 or < 20). The responses of animals infected orally were no different for those infected with virus by the subcutaneous route.

On day 91, animals were challenged with either MP125 Hawaiian Dengue, MP25 TH-Sman or MP5 Vandeever by the subcutaneous route.

Control animals, which had never been immunized, were inoculated with the mouse-adapted strains and their CF responses also measured.

As shown in Figure 1, animals previously infected with Unadapted virus and then challenged with either $10^{5.5}$ LD_{50} of MP125 Hawaiian or MP25 Sman, developed a very rapid anamestic response, which could be detected

CF Response Of African Green Monkeys (Cercopithicus Aethiops) Following Primary SQ Immunization With Unadapted Vandeever Type I Dengue Virus Followed By Secondary Immunization With Mouse-Adapted Dengue Virus Type I Hawaiian Strain Or. TH Sman

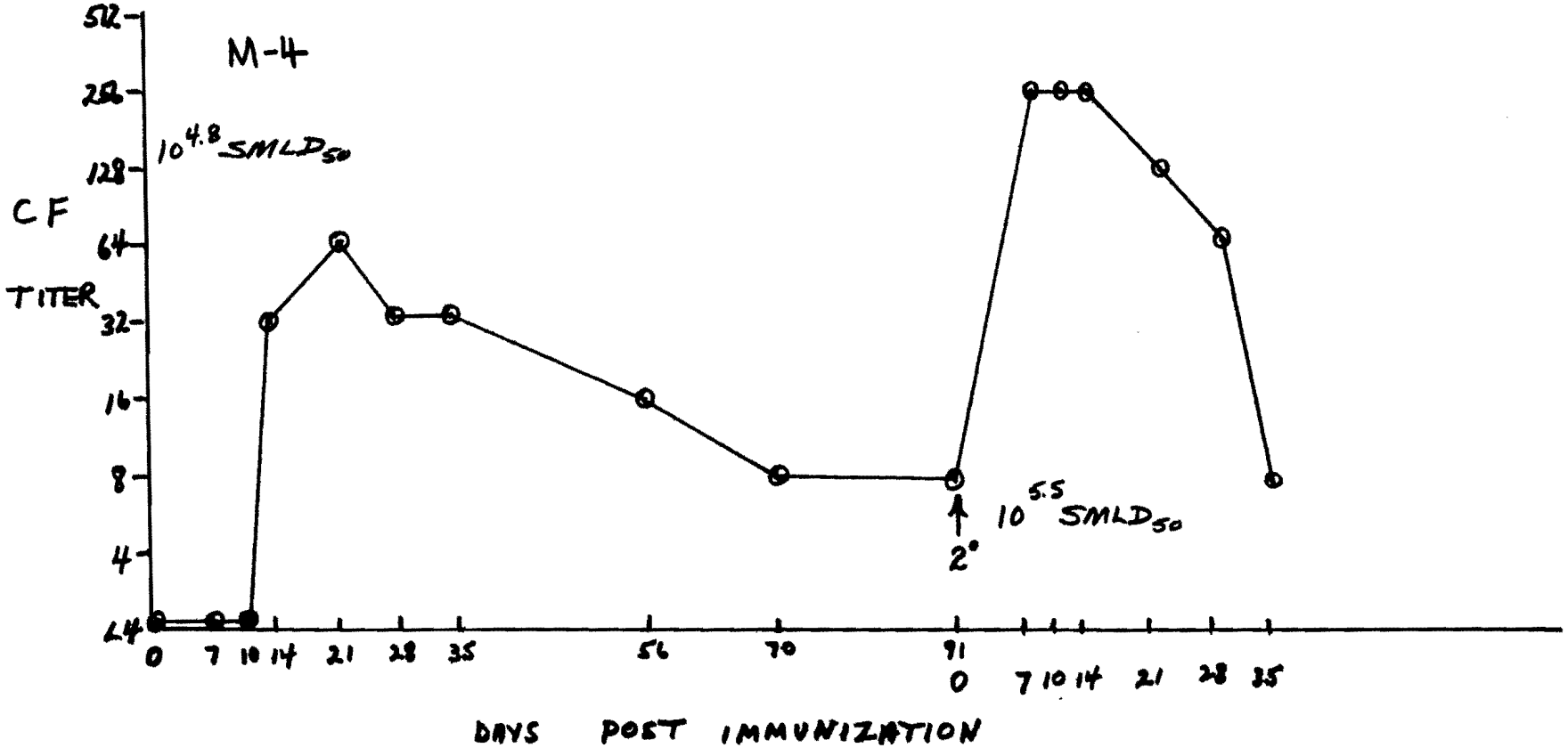

FIGURE 1

CF Response Of African Green Monkeys (Cercopithicus Aethiops) Following Oral Infection With Unadapted Vandeever Type I Dengue Virus Followed By Secondary Immunization With Mouse Passage 5 Vandeever Virus

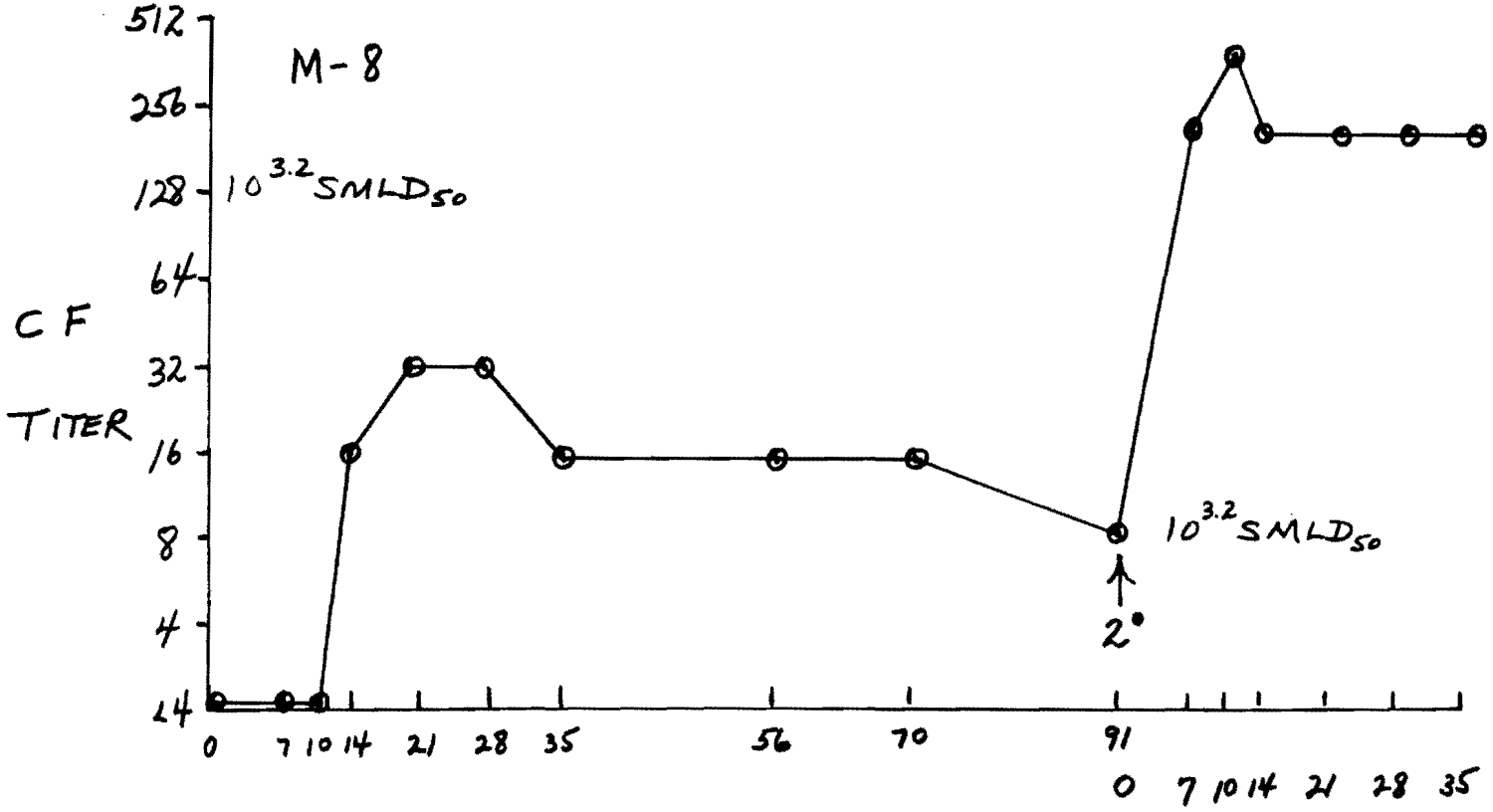

FIGURE 2

as early as 4 days post challenge, reached a peak by 7 days, and rapidly fell to the initial level by day 35. On the other hand, the monkeys initially infected with Unadapted virus and challenged with only $10^{3.2}$ LD_{50} of MP5 (Vandeever) not only developed a rapid CF response, but it persisted at high levels for at least 35 days.

The primary responses of monkeys inoculated with the mouse-adapted strains are shown in Figure 3. Despite the fact that the infecting dose of virus was larger, there was a noticeable suppressed primary response compared to those which had received Unadapted virus. One of the two monkeys inoculated with Vandeever MP5 seemed to behave like its Unadapted parent, while the other behaved more like the mouse-adapted virus.

These latter animals were subsequently challenged with the homologous virus on day 42. In general the CF response following booster inoculation was poor with animals who had been immunized with MP125 Hawaiian, moderate in one instance with TH-Sman, and very good with the Vandeever 5th mouse passage. The difference in primary and secondary responses of monkeys given Unadapted or Adapted viruses appears to be quite marked.

The serological responses of these animals to the heterotypic dengue virus will be the subject of a subsequent report.

2. Cross-immunity Pattern of Dengue Viruses in Mice as Demonstrated by Direct Challenge Experiments

A study was set up to determine the cross-immunity patterns in mice among the dengue types and other selected Group B arboviruses. In the first phase (reported here), groups of young mice were inoculated intracerebrally with 320 ID_{50} of Unadapted Type 1 virus and later challenged intracerebrally at 3 and 6 months with 100-320 LD_{50} of fully mouse-adapted strains. The initial results are shown in Table 1. Previous infection at 3 months with Type 1 virus protected against challenge with all dengue types as well as Zika. Unlike animals challenged with the Hawaiian or TH-Sman strains, a significant number of animals challenged with the Type 2, Type 4, and Zika viruses showed clinical signs, but did not die. This resistance pattern was also evident at 3 months despite the fact that a greater number of animals developed clinical signs. This may indicate a breakdown in the immunity patterns; 12/15 sera tested at 1 month for CF antibody to dengue Type 1 were positive. Although not shown - young animals infected intracerebrally 1 month previously with Sindbis virus did not resist challenge with the mouse-adapted Hawaiian strain, nor did the Vandeever strain afford protection against challenge with Nakayama, JBE or Powassan. Further extensions of this study utilizing 6 months challenge

PRIMARY AND SECONDARY CF RESPONSES OF AFRICAN GREEN MONKEYS TO MOUSE-ADAPTED DENGUE VIRUSES

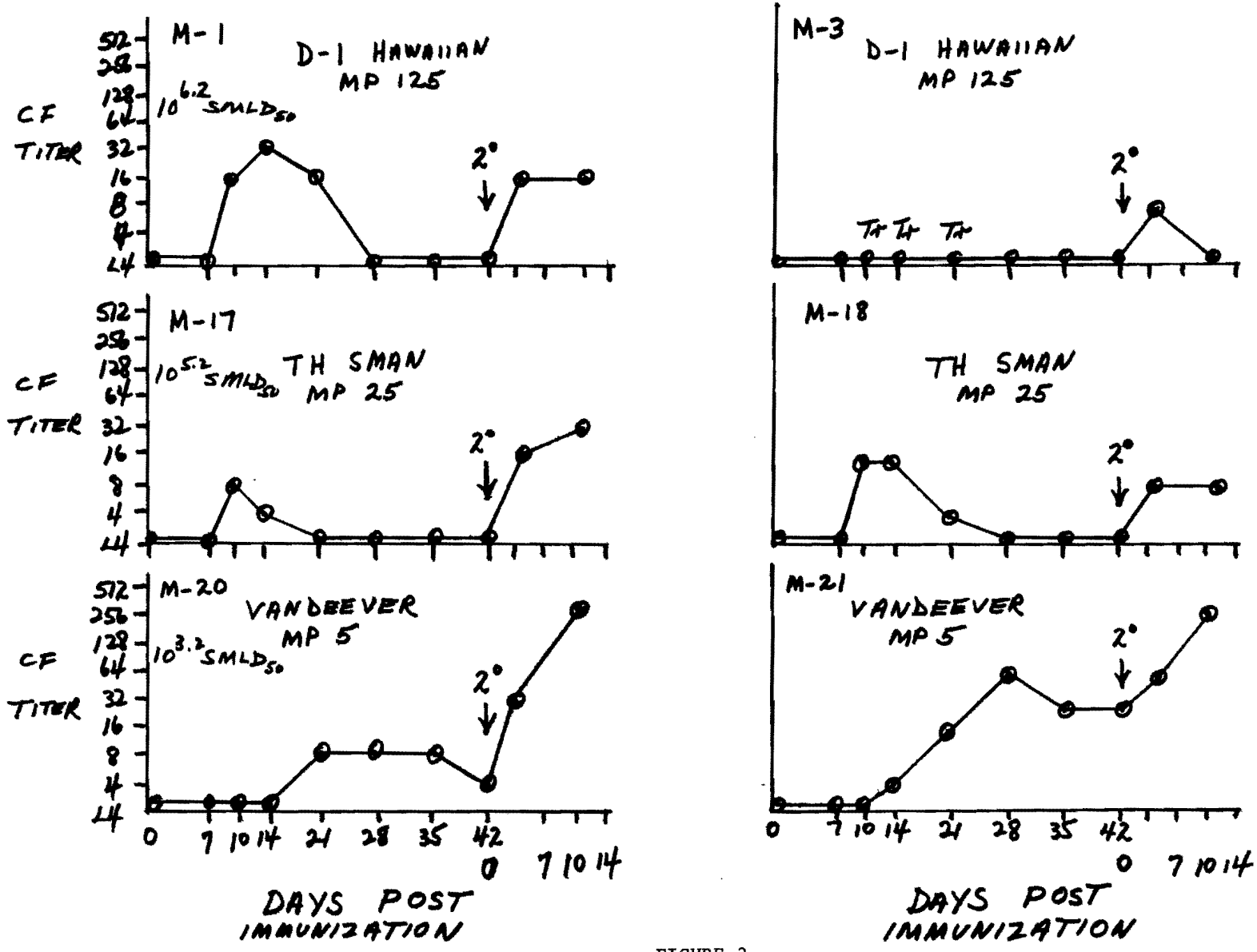

FIGURE 3

RESULTS OF EXPERIMENTS IN MICE INOCULATED WITH UNADAPTED TYPE 1 DENGUE VIRUS

AND CHALLENGED WITH MOUSE-ADAPTED DENGUES AND OTHER GROUP B VIRUSES

Original Inoculum: 320 suckling mice ID_{50} Acute Phase Human Serum, Type 1 (VanDeever Strain) Intracerebrally

Time of Challenge	Group	Clinical	Results of I.Cer. Challenge with Indicated Virus and LD_{50} Used								
			Type 1 Dengue		Type 2 Dengue			Type 4	Zika	Pow.	JBE
			Haw. (32-100)	Sman (100-320)	NGC (100-320)	TR-1751 (100-320)	TH-36 (320)	(100)	(320)	(320)	(100)
1 Month	Immuniz.	Signs*	1/8	1/8	4/8	5/8	2/8	3/8	6/8	8/8	-
		Mort.	1/8	0/8	0/8	0/8	0/8	0/8	1/8	8/8	-
	Control	Mort.	8/8	8/8	8/8	8/8	8/8	7/8	7/8	8/8	-
3 Months	Immuniz.	Signs	0/8	0/8	0/8	6/8	6/8	3/8	8/8	-	8/8
		Mort.	1/8	0/8	0/8	2/8	0/8	0/8	4/8	-	8/8
	Control	Mort.	5/5	5/5	5/5	5/5	4/4	4/5	5/5	-	5/5

* Weakness, encephalitis, or paralysis

Table 1

time are in progress. In addition, animals inoculated with Unadapted Type 2 and Type 3 dengue as well as low passaged Zika are on test and will be challenged with the heterotypic dengues and heterologous viruses of Group B.

3. Tissue Culture Studies with Dengue Viruses: Susceptibility of Y15 Line of Porcine Kidney Cells to Dengue

Utilizing Y15 cells incubated at 34° C, definite cytopathology ranging from 50-100% destruction of the cell sheets were observed for all mouse-adapted dengue types. Onset of cytopathology was 8-11 days in the case of Type 1 strains; 5-6 days in the case of Type 2 and 4 strains, and 7 days in the case of Type 3. Cytopathology end point titers/ml ranged from $10^{-3.5}$ to $10^{-5.5}$ for Type 1 strains and greater than 10^{-6}/ml for Type 2 and 4 strains.

By continued passage of the viruses in Y15 cells, the cytopathology was found to be reproducible. 5-6 passages of all types have been carried out. The incubation period of cytopathology has been reduced to 4-7 days in the case of Type 1 and Type 3 strains, and 4-6 days in the case of Type 2 strain. In general, only the Type 2 strains produce complete destruction of the cell sheets following inoculation with undiluted of 10^{-1} dilution of virus. The other 3 viral type strains produce from 50-75% destruction.

All virus types plaque well in Y15 system using either tissue culture passaged or mouse brain material. PFU titers for Dengue 1 are in the range of $5x10^{-5}$ to $5x10^{-6}$/ml depending upon the strain. PFU titers for Type 2 strains are never below $1x10^{-6}$/ml. Using a 10^{-3} or 10^{-4} inocula, onset of plaques are 6-8 days in the case of Type 1 viruses and 4-6 days in the case of Type 2 strains.

4. Mosquito Tissue Culture

Two cell lines derived from either newly emerged adult C. inornata or late pupal stage of A. vexans have been established. Both of these cell lines are similar in growth characteristics and cellular morphology, to the A. aegypti and Antheria lines of Grace. Both are grown in Grace's media supplemented with fetal bovine serum and cannot be grown on the media employed by Singh for his lines. By gel diffusion techniques, all the above 4 lines share common antigens, and thus far cannot be distinguished from one another.

Tests for viral susceptibility of C. inornata and A. vexans were compared to the Grace's line of A. aegypti. SLE and JBE viruses were shown to mul-

tiply at low levels in all three lines over a 6 week period at 28° C. Although the virus input was in the range of 10^5 to 10^6 $TCID_{50}$, the best virus titers achieved with SLE and JBE were in the range of 10^{-3} to 10^{-4} $TCID_{50}$/0.1 ml. EEE, California, and Cache Valley viruses did not multiply in any of the three cell lines.

The problem of proving the identity or lack of identity of the A. vexans and C. inornata with each other or with Grace's A. aegypti and moth lines will be difficult. Chromosome analysis and cloning of the cultures with further immunological studies will be necessary.

In studies carried out with the Singh line of A. aegypti and A. albopictus, we could not distinguish between the two cell lines by immunodiffusion tests; they appeared identical. Interestingly enough, there was no immunological crossing by immunodiffusion between the Singh's A. aegypti and the Grace's A. aegypti cell line. Singh's lines can be adjusted to grow in Grace's media. They still maintain the characteristic monolayer formation but the morphology of the cells appears different.

(B. H. Sweet)

REPORT FROM THE DIVISION OF ARTHROPOD MICROBIOLOGY, DEPARTMENT OF MICROBIOLOGY, NAVAL MEDICAL RESEARCH INSTITUTE, BETHESDA, MARYLAND

This laboratory has as its main goal the analysis in vitro of relationships between arthropod-borne microorganisms and their arthropod hosts. Continuously passaged arthropod cell lines are used in these studies. At present, these lines number ten; seven from mosquitoes, two from moths, and one from a leafhopper. Two examples of recent developments using these cells follow:

Plaque production in mosquito cell line. Singh's Aedes albopictus cell line, which forms complete monolayers, was used. After preliminary infectivity tests with the parent line, the smaller cell type present in the original mixed cell population was cloned, since this appeared to demonstrate the highest degree of sensitivity, in terms of cytopathogenicity, of

those present. Cloning was done using the coverslip method. The initial virus tested for plaquing capacity in the clonal line was Japanese encephalitis virus, OCT-541 (35-24) strain. The virus was adsorbed for 2 hours at 28 C on cells and then overlayed with Agarose (in culture medium). After 7 days incubation at 28 C, a second overlay containing neutral red was added. Plaques, about 4 mm in diameter with irregular edges, were visible within the next 24 hours. Their number was directly proportional to the virus concentration, and could be reduced significantly when the virus was neutralized by mouse JEV-immune ascitic fluid prior to adsorption.

Virus-like particles in mosquito cell line. Virus-like particles have been found by electron microscopy in normal Aedes albopictus cells (parent line, Singh). They measure approximately 30-35 mμ in diameter, have two distinct membranes, and either empty or filled cores around 27 mμ in diameter. They occur in clusters, imbedded in an electron-dense matrix, and are most commonly associated with the nucleolonema region of the nucleolus. Their presence does not appear to disturb the cells, and we assume they originated from the mosquitoes from whence the cells were derived.

REPORT FROM THE LABORATORY OF VIROLOGY AND RICKETTSIOLOGY, DIVISION OF BIOLOGICS STANDARDS NATIONAL INSTITUTES OF HEALTH BETHESDA, MARYLAND

Administrative Changes

On 31 October 1968 Dr. Alexis Shelokov, Chief of our Laboratory, resigned from the U. S. Public Health Service to become Professor and Chairman of the Department of Microbiology, The University of Texas, Medical School at San Antonio, Texas. On 21 January 1969 Dr. Nicola M. Tauraso became Acting Chief of this Laboratory, which has since been reorganized to consist of 2 Sections--the Virology and Rickettsiology and the Cell Biology Sections. The former Section consists of 7 Units (Immunoserology, Avian Leukosis, Immunofluorescence, Respirovirus, Arbovirus, Rickettsial and Viral Pathogenesis) and the latter of 2 Units (Cell Production and Cell Research). As you can see the Laboratory is involved in various research activities.

Yellow Fever Vaccine Studies

In our previous report to the Information Exchange we stated that we had developed an avian leukosis virus (ALV) - free primary yellow fever virus vaccine seed by ridding the 17D virus vaccine of its ALV contaminant. By differential filtration through Millipore membrane filters of different pore sizes, yellow fever virus was separated from its contaminant by physical means. Avian leukosis viruses could not be detected in the new primary and secondary vaccine seeds by RIF, COFAL and FAB tests.

Additional studies revealed that the ALV - free candidate primary and secondary seed lots were indistinguishable from corresponding ALV - contaminated lots with respect to: (1) potency as measured by titration in newborn and suckling mice and in the MA-104 plaque system; (2) degree of viscerotropism as measured by viremia in monkeys; (3) neurotropism as determined by the monkey neurovirulence test; and (4) potency as determined by antibody response in monkeys inoculated by the intracerebral route.

Studies on immunogenicity were conducted on a total of 265 rhesus monkeys and on 180 volunteers at the Lewisburg Federal Penitentiary. The ALV - free and -contaminated vaccine lots were indistinguishable with respect to reactions and to antibody response (Table 1).

As part of our Yellow Fever Vaccine studies, we have developed a convenient plaque neutralization (PN) test using the MA-104 embryonic rhesus monkey kidney cell line and a single agar-overlay procedure. When compared to mouse neutralization (MN) tests using suckling and weanling mice inoculated by the intracerebral route, the PN test was: the most sensitive for measuring neutralizing antibody, less variable, less costly, and quicker (5-6 days vs. 21 days for the PN and MN tests respectively) in achieving results.

Hemorrhagic Fever Studies

Simian hemorrhagic fever. Well known by this time were the two epizootics of simian hemorrhagic fever which occurred at the Sukhumi Institute of Experimental Pathology and Therapy, USSR (July 1964) and at the NIH Primate Quarantine Unit (Fall 1964). Another epizootic has since occurred at the National Center for Primate Biology, University of California at Davis in September 1967. The disease seen in all three epizootics has been clinically indistinguishable. We reported earlier that the viruses causing the Sukhumi and NIH epizootics were serologically identical. Sera obtained from monkeys involved in the Davis outbreak were inoculated by

Table 1

Results of Serological Tests Performed on Monkeys and Humans Inoculated with ALV Virus-Contaminated and -Free Yellow Fever (YF) Vaccine

Host	Group	ALV Status of Vaccines: Contaminated				Free			
		No. in Group	HI[a] %	PNT[b] LNI[c]	PNT[b] %[d]	No. in Group	HI %	PNT LNI	PNT %
Rhesus monkeys	1	42 (38)[g]	76 (76)[h]	2.3[e]	94	40 (36)[g]	73 (78)[h]	2.5	97
	2	17 (17)	77 (77)	ND[f]		25 (25)	72 (72)	ND	
	3	49 (47)	78 (77)	2.1	100	48 (48)	46 (46)	1.4	95
	4	21 (20)	91 (95)	2.4	100	23 (23)	91 (91)	2.5	100
	Total	129 (122)				136 (132)			
	average		81 (81)	2.3	98		71 (72)	2.1	97
Humans	1	89 (65)	62 (71)	2.2	99	91 (68)	69 (78)	2.2	98

[a] Percent of individuals developing a 4-fold or greater rise in HI antibody titer.
[b] PNT = plaque neutralization test.
[c] LNI = log neutralization index.
[d] Percent of individuals having an LNI rise of ≥ 0.7.
[e] Average value.
[f] Not done yet.
[g] No. having no YF HI Ab in preinoculation serum.
[h] Percent of individuals not having YF HI Ab in preinoculation serum who developed 4-fold or greater rise in HI Ab.

the intramuscular route into rhesus monkeys housed in a specially constructed negative pressure isolator. The inoculated monkeys developed a disease indistinguishable from that seen during the previous two epizootics. We were unable to isolate a virus from the sera from these monkeys, even after repeated attempts. However, serum obtained from monkeys dying late in the course of the disease contained antibodies to viruses which had been isolated several years previously from both the NIH and Sukhumi epizootics. We feel that this serologic evidence demonstrates that the Davis outbreak was caused by a closely related (if not similar) virus.

American hemorrhagic fever. Studies were performed on 2 members of the Tacaribe group of American hemorrhagic fever viruses. Adult guinea pigs immunized with live Amapari virus (not known to be pathogenic for the adult guinea pig) did not resist challenge with Junin virus (Argentinian hemorrhagic fever virus) to which they are normally quite susceptible. This was quite unexpected because, as reported previously (initially by Dr. A. Parodi and subsequently by our Laboratory), immunization with Tacaribe virus, another member of the same virus group, did protect guinea pigs from subsequent challenge with Junin virus.

(N. M. Tauraso)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE BALTIMORE, MARYLAND

A Plaque Assay for Dengue Viruses (Eylar)

Continued efforts have been directed towards the development of a satisfactory tissue culture assay system for the dengue viruses, especially type 1 dengue virus. Although previous work had indicated that several strains of type 1 dengue virus would form plaques in chick embryo cell cultures, HeLa cell cultures and BS-C-1 cell cultures, plaque size was often small, plaques often hazy and difficult to count, and it was difficult to maintain the cultures long enough to obtain maximum plaque counts.

Another cell culture system, the BHK-21/clone 15 line of baby hamster kidney cells, was selected for study. This line has the added advantage of maintaining uniform monolayer integrity under a methyl cellulose medium for at least two weeks. The growth medium consisted of 10% bovine serum, double strength Eagles BME vitamins and amino acids with glutamine, in Hanks' BSS. In all cases the pH of the medium was adjusted to pH 7.1-7.3 with sodium bicarbonate and incubation was carried out in a humidified atmosphere of 5% carbon dioxide, 95% air.

The virus diluent was 1/15 M phosphate buffer supplemented with 0.1% bovine serum albumin at pH 7.0. Adsorption of virus was carried out at room temperature (usually 26-27 C) for 120 minutes and the infected monolayer overlayed with a medium consisting of 10% bovine serum, 2XBME vitamins and amino acids with glutamine, 15 mM bicarbonate, in Hanks' BSS with 1 1/4% methyl cellulose as a solidifying agent. Sterile NaOH was added to adjust the medium to pH 7.2. Incubation was at 37 C in 5% CO_2 - 95% air. After 11-14 days incubation, the methyl cellulose overlay was removed by repeated washing with GKN (a physiologic solution containing NaCl, KCl and glucose). The cell sheets were then stained with crystal violet, excess stain removed with tap water and the bottles dried overnight. Virus plaques appeared as unstained holes in a blue cell sheet.

The results of typical titrations are presented in Table 1. All four strains of type 1 dengue virus produced plaques in the BHK-21/c 15 monolayer cultures. In all instances the titer of the mouse brain pool, determined by the plaque count method, was equal to or better than the titers obtained when the same virus seed material was titered in suckling mice. Representative members of dengue virus types 2, 3, 4, 5 and 6 were assayed by the same method and in all instances the BHK-21/c 15 plaque assay method was as sensitive if not more sensitive than the standard suckling mouse titration technique.

This technique has the additional advantage that once the cell sheets are stained by crystal violet, plaques may be counted whenever time permits. The neutral red staining technique does not have this advantage for once the cell sheets are exposed to direct light, plaques must be counted before photoinactivation of cells occurs.

Table 1

Plaque Assay of Dengue Viruses in Monolayer Cultures of BHK-21/ c15 Cells

Dengue serotype	Strain	Mouse passage	Plaque development Time (days)	Plaque development Diameter (mm)	Titer $\log_{10}$ (PFU/gm of brain)
1	Hawaiian	122	10-14	2-3	9.1
1	Duff	3	11-14	1-2	7.5
1	MD-1	34	11-14	1-2	7.7
1	Mochizuki	93	11-13	1-1½	7.9
2	NGB	20	7-10	2-4	9.9
3	H87	41	10-14	2-3	8.9
4	H241	42	7-10	1-2	8.5
5	TH-36	15	7-10	2-3	>9.5
6	TH Sman	15	7-10	2-4	7.9

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

Use of Hamsters for Potency Assay of Eastern and Western Equine Encephalitis (EEE, WEE) Vaccines

An antigen extinction-type test employing hamsters has been extensively evaluated. The animals are given two 0.5 ml doses of 5-fold dilutions of vaccine intraperitoneally (IP) with a 7 day interval between doses. Twenty-one (21) days after the last vaccine dose the hamsters are challenged IP with 10^3 - 10^4 LD_{50} of virulent EEE or WEE virus.

Comparative potency assays with guinea pigs and hamsters (Table 1) showed the latter to be a suitable replacement animal for the guinea pig. Use of the hamster offers several advantages including savings in animal costs, space, and manhours. More significant is the higher susceptibility of the hamster to peripheral challenge; the guinea pig, in contrast, requires intracerebral challenge, a more unnatural route which bypasses most circulating antibody and natural body defenses.

(F. E. Cole, Jr. and R. W. McKinney)

Production and Testing of an Experimental, Inactivated EEE Vaccine Propagated in Chick Embryo Cell (CEC) Cultures

A formalin-inactivated EEE vaccine was prepared from the PE-6 strain virus, propagated in primary CEC cultures maintained with Medium 199 containing 0.25% human serum albumin. To effect an increase in antigenic mass per unit volume of fluid harvest, the pooled, virus containing fluids from the first tissue culture passage were used as maintenance medium for two additional passages of the virus in fresh CEC cultures.

After centrifugation and filtration, (Millipore, 45μ), the virus suspension was inactivated with 1:2000 (0.05%) formalin at 37° C for 24 hours. Safety tests in tissue culture, suckling and adult mice, 8-hour-old chicks and guinea pigs documented the absence of residual live virus in the "in process" and final vaccine; suitable, standard tests showed the product to be sterile.

TABLE 1

WEE and EEE Vaccine Potency Assays

In Hamsters and Guinea Pigs

	ED_{50} (ml)	
Vaccine[a]	Hamster[b]	Guinea pig[c]
WEE #6	0.0008	$\leq$0.0008
WEE-TC	$\leq$0.0008	0.012
WEE-7	0.045	<0.5
EEE #4	0.0031	0.0049
EEE-TC	0.11	0.35
EEE-1-1966	0.009	0.012

[a] All animals given two 0.5 ml doses: i.p. route in hamsters; s.c. route in guinea pigs.

[b] Intraperitoneal challenge with 10^3-10^4 LD_{50} contained in 0.5 ml.

[c] Intracerebral challenge with 10^3-10^4 LD_{50} contained in 0.15 ml.

Potency tests on the final lyophilized product were performed in hamsters and guinea pigs, yielding ED_{50} values of 0.009 ml and 0.012 ml, respectively. After storage of the final product at -20° C for 17 months, no change in potency had occurred.

(L. F. Maire, R. W. McKinney and F. E. Cole, Jr.)

Evaluation of an Experimental EEE Vaccine in Man

A formalin-inactivated EEE vaccine prepared in CEC cultures has been evaluated in man. Studies with 16 volunteers given 3 doses of 0.5 ml subcutaneously, revealed only mild local or systemic reactions in some individuals. No meaningful changes occurred in clinical laboratory values such as those for electrocardiograms, WBC, platelet and differential count, hematocrit, urinalysis and liver function studies during a 14-day observation period. These results indicated that the vaccine was safe for use in man and could be further evaluated in personnel "at-risk" to infection.

The serological responses of the volunteers suggested that a two-dose series with a 28-day interval between doses would result in significant and persistant titers. This schedule was employed for immunization of 92 "at-risk" personnel who were virgin to EEE. As shown in Table 1, 28 days after the second dose of vaccine 93% (86/92) had an LNI $\geq$ 1.7, with all but 2 individuals having a titer $\geq$ 2.0 logs.

The vaccine has also been tested as a booster in 117 EEE-experienced persons, the majority of which had previously received a basic series of 3 doses and numerous yearly boosters with another EEE vaccine. As indicated in Table 2, 81% of the individuals had an LNI of $\geq$ 1.6 at the time of booster administration (Day 0), whereas only 8.6% remained at this level by 28 days after the booster. This represents a serological conversion of 89% of the persons with low initial titers and is indicative of the high immunogenicity of the vaccine given intradermally in a 0.1 ml dose.

(P. J. Bartelloni, R. W. McKinney, J. P. Duffy and F. E. Cole, Jr.)

TABLE 1

Neutralizing antibody responses in EEE non-experienced, "at risk" personnel administered two doses of EEE vaccine

	Number responding after vaccine dose*		
LNI+	Pre-	1	2
3.0 - 3.9		1	15
2.0 - 2.9		26	69
1.7 - 1.9		17	2
1.0 - 1.6		38	6
<1.0	92	10	0

*Dose was 0.5 ml, subcutaneously; 28 day interval between doses.

+Log_{10} serum neutralization index of serum obtained 28 days after each vaccine dose.

TABLE 2

Neutralizing antibody responses in EEE-experienced, "at risk" personnel administered 0.1 ml booster dose of EEE vaccine

	% Responders (No./Total) on:			
LNI*	Day 0	(Pre-booster)	Day 28	(Post-booster)
≥4.0	0	(0/117)	0.85	(1/117)
3.0 - 3.9	0	(0/117)	19.7	(23/117)
2.0 - 2.9	11.1	(13/117)	59.8	(70/117)
1.7 - 1.9	7.7	(9/117)	11.1	(13/117)
1.0 - 1.6	29.0	(34/117)	7.7	(9/117)
<1.0	52.2	(61/117)	0.85	(1/117)

*Log_{10} serum neutralization index.

REPORT FROM THE
WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
PHILADELPHIA, PENNSYLVANIA

Interferon-Mediated Natural Resistance of Mice to ArboB Virus Infection

Cells of congenic mouse strains C3H and C3HRv, which are known to differ only at the genetic locus which determines resistance to infection with arboB viruses, produce equal amounts of interferon after arboB virus infection. However, the virus-resistant C3HRv cultures were much more susceptible to the inhibitory effect of the interferon, when tested with arboB viruses, than the C3H cultures. The mouse interferons used in these experiments were obtained from various sources, and their enchanced effect in C3HRv cultures was specific for arboB viruses. In vivo, C3HRv mice did not produce more interferon after West Nile virus infection than C3H mice but, again, C3HRv mice were more readily protected by interferon from lethal West Nile virus infection than were C3H mice.

(B. Hanson and H. Koprowski)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY
RUTGERS MEDICAL SCHOOL, NEW BRUNSWICK, NEW JERSEY

During the past year the attention of this laboratory has been directed to a study of the structural proteins of type 2 dengue virus (New Guinea B strain). Previous investigations had shown that dengue-2 virus produced by infected KB cells consisted of two types of particles separable by sucrose gradient centrifugation. The more rapidly sedimenting particles (complete virus) were infectious, had HA activity and contained an intact 45S RNA molecule. The slowly sedimenting particles (incomplete virus) also had HA activity, but they were not infectious and probably contained no RNA.

Dengue-2 virus was produced from KB cells in the presence of C^{14} amino acids. The virus was concentrated and then purified by sucrose gradient

centrifugation. The complete virus peak was dissociated with SDS mercaptoethanol and examined by acrylamide gel electrophoresis. There were 3 peaks of radioactivity, 2 small rapidly moving peaks (VSP-1 and VSP-2) and a large slowly moving peak (VSP-3). Sindbis virus from infected BHK21 cells, labeled with C^{14} amino acids and treated in the same way as dengue virus, showed only two peaks on gel electrophoresis. This is in accord with the results of Strauss et al. (Proc. Natl. Acad. Sci. U.S. 59, 533, 1968) who examined Sindbis virus from infected chick embryo cells.

Incomplete dengue-2 virus contained VSP-1 and VSP-3, but VSP-2 was either much diminished or absent. When dengue virus was labeled with C^{14} or S^{35} methionine, the electrophoretic pattern of complete virus was quite similar to that obtained when labeling was with a mixture of C^{14} amino acids. While C^{14} histidine was incorporated into VSP-1 and VSP-3, there was little or no incorporation into VSP-2. C^{14} lysine, in contrast, was incorporated very well into VSP-2 but poorly into VSP-1. Incorporation of glucosamine 1-C^{14} into VSP-3 was demonstrated suggesting that this protein may be a glycoprotein. Nonidet 40, a nonionic detergent, was used to degrade dengue-2 virus into an RNA-containing core and into small fragments of the outer viral components. Acrylamide gel electrophoresis of these subviral structures showed that VSP-2 is associated with the RNA-containing core structure, while VSP-1 and VSP-3 are associated with the outer viral components.

Thus, dengue-2 virus contains 3 structural polypeptides one of which is an inner protein associated with the RNA-containing core. This protein is rich in lysine but deficient in histidine.

The other two proteins are associated with the outer structures or the envelope of the virus. One of these, VSP-3, is probably a glycoprotein.

(V. Stollar, R. W. Schlesinger)

REPORT FROM THE NEW JERSEY DEPARTMENT OF HEALTH

Eastern Encephalitis in New Jersey during 1968

The spring of 1968 was marked by excessive rainfall in New Jersey leading to severe flooding in several areas of the State. Later, most localities in the southern part of the State experienced excessive rainfall during the month of June averaging about 1-1/2 inches more than the monthly norm. Since excessive rainfall in the preceding spring had been experienced prior to the 1959 outbreak of EE in New Jersey, these events led to some increased sensitization concerning the possibility of local recurrence of EE epidemic activity. As has been the case every year during the past decade, surveillance of EE epizootic activity was continued by virologic studies of wild birds netted, banded and bled and of mosquitoes collected and speciated at certain fixed sites in the State. Surveillance of equine disease was reinstituted, as usual, in late spring and all practising veterinarians in the State were contacted by mail. Similarly, all physicians were alerted by letter, and special instructions were forwarded to all hospital pathologists.

Epizootic surveillance activities during the latter part of the month of July began to reveal very disquieting information indeed. Mosquitoes collected began to yield large numbers of isolates of EE virus, eventually amounting to 32 strains from mosquitoes trapped during that month. By way of comparison, studies between 1961 and 1968 had never before yielded more than 8 isolations of EE from mosquitoes so early in the season. Moreover, no prior year had ever revealed EE-infected C. melanura mosquitoes as early as July, and this mosquito species had yielded the bulk of EE isolates in July of 1968. The sheer number of strains of EE recovered was anxiety provoking since it represented more than had ever before been detected in mosquitoes during a whole summer season in prior years. When seven EE viremic wild birds were also detected during the last week in July, the evidence seemed clear that New Jersey was experiencing unprecedently early and heavy EE epizootic activity. The threat of an impending outbreak was communicated to various agencies, periodic phone surveillance of all of southern New Jersey's hospitals begun and emergency mosquito adulticiding activities were instituted. Equine cases of EE were detected during the last week in July and were rapidly confirmed by fluorescent antibody diagnostic techniques developed in this laboratory and applied for the past four years. On August 9th, information was obtained about a boy who had been hospitalized in Philadelphia with a diagnosis of encephalitis that had its onset on July 17th. Studies over that week-end revealed that the boy had

indeed been infected with the virus of EE, providing confirmation that earlier anxiety had been justified. Thus the 1968 outbreak of EE in New Jersey represents the first outbreak due to this virus in which epizootic surveillance provided forewarning and permitted the institution of emergency mosquito control activities at the very onset of an epidemic.

A total of 12 human cases of EE were detected. As seen in Table 1, 6 succumbed to the disease. In contrast to previous experience in New Jersey of equal numbers of cases in males and females, eleven of the twelve cases in 1968 were males. As noted in prior years, the cases tended to occur in those under 15 and over 55 years of age. Table 2 reveals that the human cases were distributed in 6 southern counties of the State, namely; Atlantic, Burlington, Cape May, Cumberland, Ocean and Salem.

The stars on Figure 1 reveal the probable sites of exposure of all 12 human cases. With one exception, all had been exposed within 10 miles of the salt marsh-fresh water swamp boundary. The single exception, and the first inland case ever detected in New Jersey, was that of a young child exceedingly heavily bitten by mosquitoes at his home on a river opposite a sewage treatment plant.

As seen in Table 2, eight cultivated flocks of pheasants distributed in 5 counties were discovered to have incurred epizootics of EE during the 1968 summer season. Their locations are indicated on Figure 2 by stars. The relatively small number of pheasant flock outbreaks during 1968 is undoubtedly attributable both to the efficacy of commercially available EE and WE vaccine and to its widespread use by those who cultivate sizable flocks of pheasants.

Equine cases of EE were documented as early as the week ending July 26th (Table 3), 2 weeks earlier than experienced in any year during the last decade. As seen in this tabulation of the temporal distribution of cases, the occurrence of equine EE peaked during August, a couple of weeks prior to the epidemic peak of human cases. A total of 126 equine cases of EE were recognized during a 12 week period. Only 14 survived, yielding a case fatality rate of 89%. One hundred and fifteen of the equine cases were documented by various combinations of virologic, serologic and characteristic pathologic findings. Thus the New Jersey equine epizootic of EE during 1968 bears the rather dubious distinction of having been the largest accumulation of documented cases heretofore described anywhere in the world, to the authors' knowledge.

The distribution of equine cases of EE by county can be seen in Table 2. Eleven of New Jersey's 21 counties yielded cases, including all of South

Table 1

DISTRIBUTION OF HUMAN EE CASES

NEW JERSEY

1968

Age (Yrs)	Cases			Deaths		
	Male	Female	All	Male	Female	All
0-4	1	1	2	0	0	0
5-14	4	0	4	2	0	2
15-24	1	0	1	0	0	0
25-34	0	0	0			
35-44	0	0	0			
45-54	1	0	1	0	0	0
55-64	0	0	0	0	0	0
65+	4	0	4	4	0	4
All Ages	11	1	12	6	0	6

Table 2

DISTRIBUTION OF HUMAN, EQUINE AND PHEASANT

EE IN NEW JERSEY BY COUNTY

1968

County	Human Cases	Equine Cases	Pheasant Flocks
Atlantic	3	17	2
Burlington	1	36	2
Camden		20	
Cape May	2[1]	4	
Cumberland	3[2]	10	
Gloucester		10	
Mercer		2	1
Middlesex		1	
Monmouth		9	1
Ocean	2	10	2
Salem	1	7	
All	12	126	8

[1]one of these human cases was also exposed in Salem County

[2]one of these human cases was also exposed in Ocean County

FIGURE 1

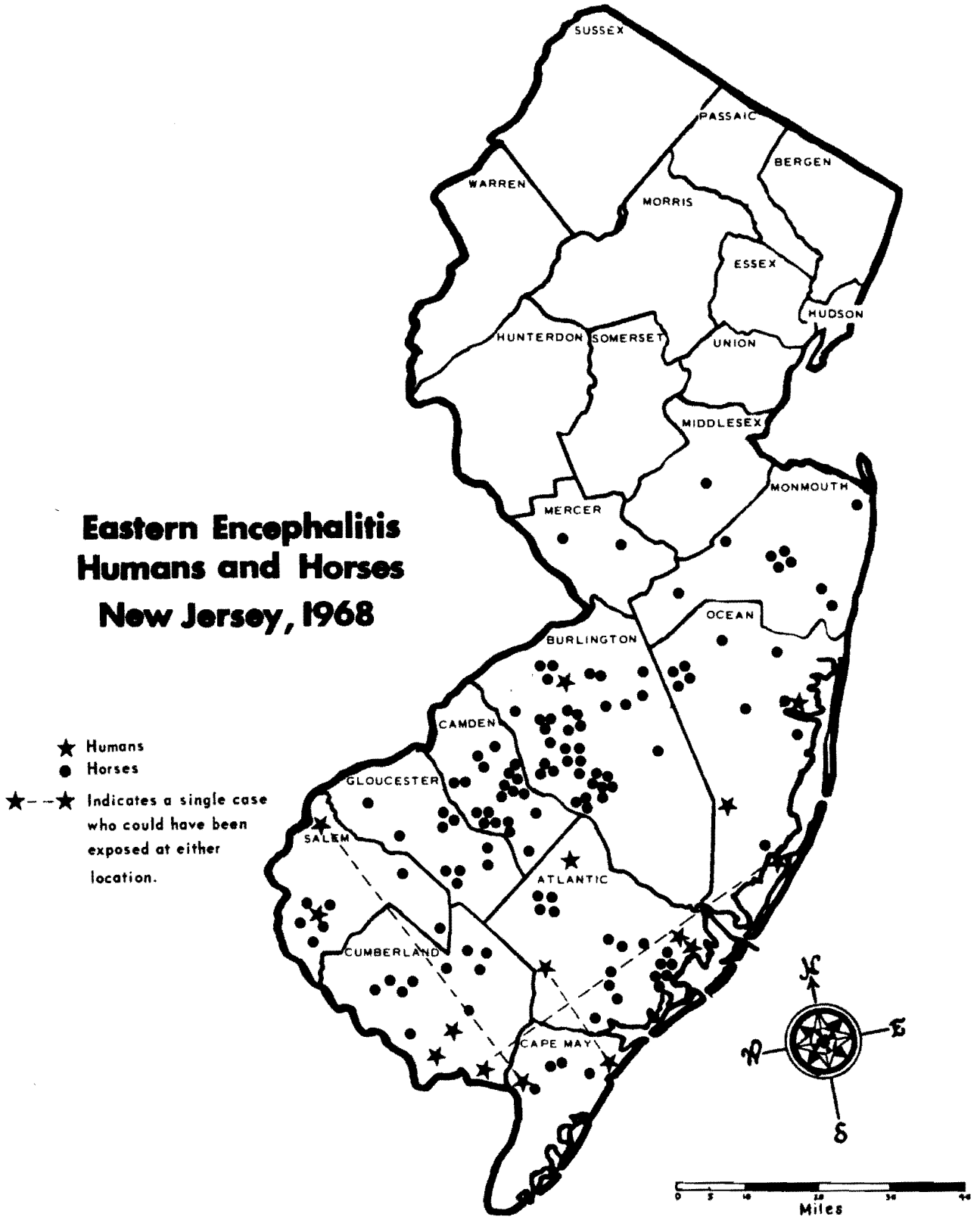
Eastern Encephalitis
Humans and Horses
New Jersey, 1968
Humans
Horses
Indicates a single case who could have been exposed at either location.
SUSSEX
PASSAIC
BERGEN
WARREN
MORRIS
ESSEX
HUDSON
HUNTERDON
SOMERSET
UNION
MIDDLESEX
MONMOUTH
MERCER
OCEAN
BURLINGTON
CAMDEN
GLOUCESTER
SALEM
ATLANTIC
CUMBERLAND
CAPE MAY
Miles
M6389

Figure 2

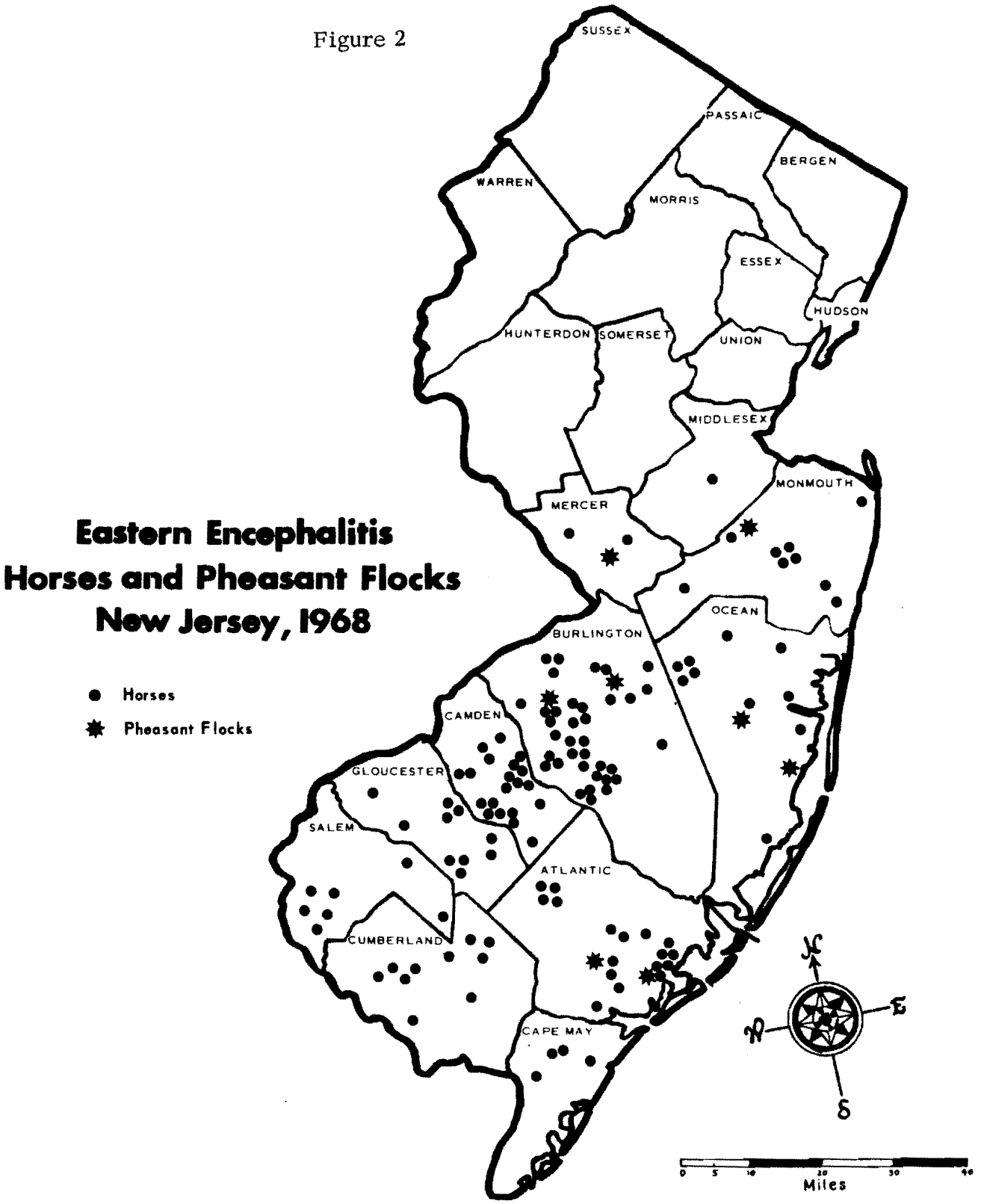
Eastern Encephalitis
Horses and Pheasant Flocks
New Jersey, 1968
Horses
Pheasant Flocks
SUSSEX
PASSAIC
BERGEN
WARREN
MORRIS
ESSEX
HUDSON
HUNTERDON
SOMERSET
UNION
MIDDLESEX
MONMOUTH
MERCER
OCEAN
BURLINGTON
CAMDEN
GLOUCESTER
SALEM
ATLANTIC
CUMBERLAND
CAPE MAY
0 5 10 20 30 40
Miles
M6389

Table 3

TEMPORAL DISTRIBUTION OF EASTERN ENCEPHALITIS IN HUMANS AND HORSES

NEW JERSEY

1968

Week Ending	Man	Horses
7/19	1	0
7/26	0	4
8/2	0	4
8/9	1	18
8/16	1	32
8/23	1	19
8/30	2	25
9/6	4	9
9/13	0	6
9/20	1	6
9/27	0	2
10/4	1	0
10/11	0	1
Total	12	126

Jersey and extending solidly as far north as Middlesex County. Easily discernible in Figure 1 is the fact that the heaviest accumulation of equine cases occurred in an inland zone extending through Burlington, Camden and Gloucester Counties. The extension of equine cases relatively deep inland and the limitation of human cases to the margins of salt marsh and fresh water swamp have been consistent findings in prior years in New Jersey and elsewhere. New Jersey's experience in 1968 was no exception.

A particular effort was made during the course of the equine epizootic to obtain blood samples from horses early in the course of the disease. Acute phase bloods were obtained from 91 equine cases of EE. Three of the 91 cases revealed detectable EE viremia at the time of the first blood specimen, all 3 of which revealed absence of detectable EE, CF, HI and neutralizing activity. The serum specimens from the three viremic horses were each titrated on two separate occasions in wet chicks, recently weaned mice and suckling mice. EE log titers varied from 3.6 to 4.4 in recently weaned mice, from 4.1 to 4.9 in wet chicks and from 4.3 to 5.6 in suckling mice. This appears to provide solid evidence, for the first time, that horses naturally infected with EE can develop high enough titers of virus in circulating blood to act as potential amplifying hosts.

Studies of mosquitoes and wild birds were continued throughout 1968. The extent of epizootic activity detected was truly astounding. One hundred and sixty-six isolations of EE and 46 of WE were obtained from 151,314 mosquitoes, representing larger numbers of isolates of both of these agents than were obtained in all of the preceding seven years combined. Isolations of EE were obtained from A. sollicitans mosquitoes, as they had been in 1959, 1965 and 1967, when human cases of EE were also detected. A. sollicitans mosquitoes have yielded EE in all years when human cases occurred and have failed to yield EE in all other years since 1959 when no human involvement was detected. Non-avian vertebrates yielded 13 additional strains of EE and 2 of WE.

EE was isolated from none of 1,682 wild birds netted between January 1 and July 22nd, 1968, and from 84 of 2,859 netted between July 23rd and the end of October, a rate of 2.9%. Assuming an average duration of EE viremia in species netted of 3 1/2 days, the average EE viremia prevalence rate of 2.9% over the 14 week period can be converted to an estimate of incidence. If this is done, it can be estimated that of the order of magnitude of 81% of wild birds (of the species netted) experienced an EE infection during the period. This figure would be valid, however, only if the avian population had remained static through the summer and fall. Obviously it did not, particularly during peak migratory activity in the fall. It is likely, there-

fore, that immunity rates in birds netted toward the end of the season will be found to have been much lower than 81%.

An avian EE viremia rate of 1.8% was observed at a fixed site in northern New Jersey at a distance of 40 miles from the northernmost equine case of EE and 70 miles north of the closest human case, as compared with average viremia rates of 3.1% at three fixed sites adjoining New Jersey shoreline and in the vicinity of equine, human and pheasant cases.

In view of the overwhelming evidence of unprecedented EE epizootic activity in New Jersey during the summer of 1968, it is appropriate for us to address ourselves to the question "Why didn't a larger number of human cases of EE occur?" It is possible that emergency mosquito control measures instituted very early in the season played a significant role in reducing the risk of human infection. The diffuse occurrence of human cases all along the salt marsh-fresh water swamp boundary adjoining both the Delaware Bay and the Atlantic Ocean, however, made it impossible to consider use of intensive area-wide blanketing as has been recently applied to the control of urban outbreaks of Saint Louis Encephalitis. It is likely, therefore, that the vagaries of the weather played an even more important role in saving us from a more tragic experience. After a wet June, South Jersey experienced an average deficit of 2 1/2 inches of rainfall in July, 2 inches in August and a record-shattering deficit of 3 inches in September. It is most tempting to speculate that the drought last summer played a significant role in diminishing populations of mosquitoes, such as A. solicitans, capable of transmitting EE from infected birds to man.

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

Study on the Role of California Encephalitis Virus in Selected Areas of New York State

Indicator Rabbit Study

The Partridge Run site in the Helderbergs was used for indicator rabbits in 1968 as it was in 1967. Six laboratory-bred rabbits were set out May 21, 1968 and were bled bi-weekly until September 10, 1968. One rabbit devel-

oped a reproducible complement-fixation (CF) titer of 4 with Cache Valley (CV) antigen of the Bunyamwera (BUN) arbovirus group in serum collected July 23. This same titer was demonstrated also in sera collected through August 9. Neutralizing antibodies for CV, however, were never demonstrated, and no infectious agent was isolated from blood clots collected prior to July 23. Sera collected prior to July 23, 2 samples taken during the period of July 23 to August 9, and another at the end of the experiment, were shipped to Doctor Reeves of Berkeley, California, for neutralization (NEUT) tests with some of the new BUN group isolates.

Year-long Antibody Study of a Naturally CEV Infected Rabbit

A year-long follow-up study was carried out on rabbit No. 372 which showed seroconversion on July 14, 1967 to California encephalitis virus (CEV) (New York strain 65-8569). Neutralizing antibody reached a peak 9 weeks later and CF antibody 10 weeks later; the titers than dropped slightly and persisted at this lower level until the experiment was terminated on August 20, 1968. At no time were CF antibodies detectable against the prototype strain BFS 283.

Antibody Studies in Rabbits and Woodchucks

Laboratory-bred rabbits were inoculated subcutaneously with varying multiplicities of virus (New York strain 65-8569). The animals were examined daily for viremia, neutralizing, CF, and HI antibodies. Viremia was noted on day 3, NEUT antibodies on day 7, CF and HI antibodies on day 9. The maximum NEUT titer usually occurred 4 weeks after inoculation; the maximum HI titer 10 days post-inoculation and the maximum CF titer from 2 to 4 weeks post-inoculation. The least dose of CEV to elicit antibody response was 63 LD_{50}.

Eighteen woodchucks were live-trapped in Albany County during the spring and summer of 1968. All animals were bled from the heart; no infectious agent was isolated from blood samples. Hemagglutination-inhibition tests showed no group A, BUN, or CEV reactions or NEUT antibodies; however, 72.2% had POW HI titers ranging from 10 to 320 and 50% cross-reacted with St. Louis encephalitis virus (SLE) with titers of 20 to 80. Powassan neutralizing antibodies were present in all but one animal, while SLE antibodies were noted in 3 (16%). In our original arbovirus survey of human sera, we found 1 serum from an 11-year-old boy who had been an all-time resident of Albany County to have POW, HI, and NEUT antibodies.

Similar studies with the same strain of CEV were undertaken with 11 live-trapped woodchucks. Neutralizing antibodies were detected on day 4 in 2

animals in which the immunizing dose was 1 x 10^8 and 10^7. With lesser doses the antibody did not develop until 6 to 8 days. One animal which received 1 x 10^5 infectious doses of CEV failed to show a serologic response in 50 days. However, on reinoculation with 1 x 10^7 infectious doses of CEV, NEUT antibodies were found on day 7. Viremia was detected in only 1 woodchuck in a blood sample collected 3 days after inoculation; this was the only woodchuck in which HI antibodies were present.

Arbovirus Survey of Suspected Encephalitis Cases during the Months of June - October, 1968

One hundred and fifty-two sera from 87 patients were examined by HI and/or NEUT tests with 6 virus strains representing groups A, B, BUN, and CEV. All sera were examined in NEUT tests with CV and the New York strain of CEV 65-8569. Neutralization tests with the other agents were performed only if HI titers were found. Sera of 9 patients from 8 widely scattered counties had CEV neutralizing antibodies, and sera of 2 patients demonstrated CV neutralizing activity. None of these samples reacted in the HI test.

Sera from 4 patients demonstrated group A or group B antibodies. See Table 1. The first blood sample from patient No. 3 was collected 6 days after onset, and the results unfortunately were indeterminate. The antibodies detected in patients Nos. 1, 2, and 4 undoubtedly reflect a past experience with these agents.

No reactions were obtained with sera from 72 patients.

Arbovirus Survey of Deer Sera in New York State

In previous years, 262 sera from deer herds in Albany, Delaware, Erie, Seneca, St. Lawrence, and Dutchess Counties were examined by HI and/or suckling mice NEUT tests against 6 test viruses: Eastern and western encephalomyelitis (EE, WE) of group A, Powassan (POW) and SLE of group B, Maguari (MA) in HI and CV in NEUT for BUN group, BFS-283 strain of CEV in HI and the New York isolate 65-8569 in NEUT for the CEV complex. Four sera had detectable group A and B antibodies, 3 had detectable WE NEUT activity, and 1 had a reproducible HI titer with SLE antigen. However, BUN and CEV antibodies were detected frequently by both technics.

Ninety-three deer sera have been collected so far this year from 3 upstate areas - Howland Island, Seneca County, and Moose River Plains - and Suffolk County, Long Island. The results of the HI tests on the 59 samples from upstate were similar to those obtained in previous years. Using the

Table 1

Patient	County	Date Collected	Results											
			A				B				BUN		CEV	
			EE		WE		POW		SLE		MA	CV	BFS-283	N.Y.S.
			HI	NEUT	HI	NEUT	HI	NEUT	HI	NEUT	HI	NEUT	HI	NEUT
1	Jefferson	5/18/68	< 10	-	< 10	-	< 10	-	40	-	< 10	-	< 10	-
		6/4/68	< 10	-	< 10	-	< 10	-	40	-	< 10	-	< 10	-
2	Suffolk	9/24/68	20		40	7/8*	< 10		< 10		< 10	-	< 10	-
3	Westchester	7/19/68	< 10		< 10		20		160	4/8	< 10	-	< 10	-
		7/29/68	< 10		< 10		20		320	7/8	< 10	-		-
4	Oneida	8/10/68	< 10		< 10		20	8/8	< 10	-	< 10	-	< 10	-
		9/1/68	< 10		< 10		20	8/8	< 10	-	< 10	-	< 10	-

* Number of mice lived/total number of mice inoculated.

Table 2

Arbovirus Survey of Deer Sera from Ten Areas in New York State

County	Year	No. Sera	Group A				Group B				BUN		CEV	
			EE		WE		POW		SLE		MA	CV	BFS-283	65-8569
			% + HI	NEUT	% + HI	NEUT	% + HI	NEUT	% + HI	NEUT	% + HI	% + NEUT	% + HI	% + NEUT
Seneca	1959-60	67	0	0	0	3.0	0	0	0	0				
Albany	1960-61	11	0	0	0	9.0	0	0	9.0	0				
St. Lawrence	1964	15	0		0		0		0		0			33.3
Delaware	1965	30	0		0		0		0		3.3	3.3	0	30.0
Erie	1965	25	0		0		0		0		36.0	62.5	0	25.0
Dutchess	1967	63	0		0		0		0		16.0	35.0	6.3	20.6
Seneca	1968	50	0		0		0		0		52.0	60.0	14.0	<12.0
Howlands Island (Seneca)	Nov. 1968	16	0		0		0		0		31.2	in test	12.5	in test
Seneca	1969	28	0		0		0		0		60.7	"	21.4	"
Moose River Plains (Hamilton)	1969	15	0		0		0		0		6.6	"	13.3	"
Suffolk County	1969													
Shelter Island		23	0		0		56.9		21.7		0	"	0	"
Middle Island		8	0		0		0		0		12.5	"	0	"
Quogue		3	0		0		0		0		0	"	0	"

HI method for the first time we have found a focus of group B arbovirus activity among deer in 1 area of Long Island. The titers were higher against the POW antigen than against the SLE antigen. Neutralization tests demonstrated POW antibodies confirming the HI reactions.

(Elinor Whitney)

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

Isolation and Characterization of Virus from Fatal Disease Among Nigerian Mission Nurses

A virus has been isolated from serum of two fatal cases and from serum and pleural fluid of a third non-fatal case of hemorrhagic-febrile illness of Nigerian mission nurses. The clinical disease included fever, albuminuria, leukopenia, azotemia, rash, petechiae, pharyngeal ulcers, pneumonitis, pleural effusion, and in one case terminal gastrointestinal hemorrhage.

Patient W. became ill January 20 while working in the Biu-Mubi region of Nigeria, returned to the mission hospital at Jos where she died January 26, 1969. Virus was isolated from her post-mortem serum. Patient S. who cared for patient W. became ill on February 3. Virus was isolated from her serum on day 3 and at the time of her death on day 10. Patient P. became ill February 20 after attending both patients W. and S.. She was flown to New York City while acutely ill. Virus was recovered from serum specimens taken days 5, 12, 13, and from pleural fluid day 13. No virus was found in serum of days 27 or 36 nor in throat and fecal specimens day 36 at which time she was gradually convalescing.

The virus from serum of patient P. had an incubation period of 6-8 days in VERO cell cultures shortening to 4 days on passage. Basophilic cytoplasmic aggregates were seen in infected but not in control VERO cultures stained with Giemsa. The virus was sensitive to sodium deoxycholate, passed a 220 mμ filter but not 100 mμ, did not hemadsorb goose or guinea

pig erythrocytes at pH 7.2 or hemagglutinate goose red blood cells after acetone extraction of infected culture fluid. A CF antigen from VERO cell fluid reacted $\geq$ 1:64 with convalescent serum from patient P. and was negative with acute. Convalescent serum neutralized 2.0 log TCD_{50} of virus. Patient P.'s acute serum did not kill baby mice on intracerbral inoculation although the mice developed CF antibody.

Infectivity of the virus was unchanged when titrated in the presence of BUDR. The development of vaccinia, used as a control, was inhibited whereas that of Mayaro (a RNA virus) remained unaffected. The virus was inactivated by 0.1% BPL. It did not multiply in Aedes albopictus cell culture.

CF tests of patient P.'s convalescent serum with rabies, LCM, herpes, poxvirus, NDV, EMC, Marburg, simian hemorrhagic fever, and 104 different arboviral antigens - among which were included Omsk hemorrhagic fever, yellow fever, Congo and all known Tacaribe group agents - were negative. Negative were also the reactions between antisera for epizootic hemorrhagic disease of deer (New Jersey), group Tacaribe, Rift Valley fever, Nairobi sheep disease, simian hemorrhagic fever and Marburg viruses and a tissue culture antigen for the P. virus. Further studies are in progress.

(S. Buckley, J. Casals, and J. Frame)

Antigenic Similarity Between the Virus of Crimean Hemorrhagic Fever and Congo Virus

In serological tests the Drosdov virus strain, isolated in 1967 by Chumakov and associates from acute phase blood of a patient with Crimean hemorrhagic fever, has been found to be very closely related to Congo virus.

By CF test Drosdov virus reacted reciprocally to titer with two strains of Congo virus, JD 206 and K 2/61. The results are shown in Table 1. Convalescent sera from 6 patients with a clinical diagnosis of Crimean hemorrhagic fever reacted equally by CF with Drosdov and Congo virus antigens.

TABLE I. CF Test with the Drosdov Strain, Isolated from a Case of Crimean Hemorrhagic Fever, and Strains JD 206 and K2/61 of Congo Virus.

Antigen	Mouse serum or ascitic fluid		
	Drosdov	JD 206	K2/61
Drosdov	256/1024*	256/1024*	16/1024
Congo, JD 206	256/256	256/256	32/128
Congo, K2/61	256/256	256/256	32/256
Normal	0	0	0

* Reciprocal of serum titer / reciprocal of antigen titer.
0 = no fixation at dilution 1:8 of serum and antigen, lowest used.

By neutralization test, a Drosdov mouse serum, a Congo mouse serum, and a poos of convalescent human Crimean hemorrhagic fever sera neutralized both Drosdov and Congo viruses (Table 2).

A close similarity between the Drosdov strain and Congo strain 3010 was also noted by agar gel precipitin test. Complete fusion of the precipitation lines occurred when a human convalescent serum was placed in the center wells of the slide and the antigens, either undiluted or diluted 1:4, were placed in the peripheral wells.

(Jordi Casals)

REPORT FROM THE ANIMAL HEALTH DIVISION NATIONAL ANIMAL DISEASE LABORATORY AMES, IOWA

The southeastern United States from South Carolina to Texas, New Mexico, and Colorado have been the enzootic areas of vesicular stomatitis (VS) in the United States. In the southeastern states the annual appearance of the New Jersey type of VS infection of cattle, horses, and swine has been expected; in Texas, Colorado, New Mexico, and Oklahoma either the New Jersey or the Indiana type has appeared at unpredictable intervals. Both types spread northward and in 1949 spread into Manitoba, Canada. Single cases are occasionally noted as early as January in Louisiana or along the Rio Grande River in Texas. Many outbreaks appear to start during May or at least spread rapidly from May throughout the summer and disappear during the fall months. Very little is known about the epidemiology of VS. It is believed to be endemic in tropical Mexico throughout the year.

New Jersey VS was last reported in Georgia during 1964. It occurred in the western end of the southern states area during 1965, 1966, and 1967. During 1967 the only case diagnosed in the United States was in Louisiana, and during 1968 a dozen New Jersey VS positive herds were diagnosed near-by in close association with a game management area of central Louisiana.

Several surveys were attempted in an effort to determine if subclinical VS has been occurring in enzootic areas and in an effort to detect which wild

TABLE 2. N Tests with the Drosdov Strain and Congo Virus.

Serum	Virus and Dilutions (log_{10})											
	Drosdov						Congo, 3010					
	-2	-3	-4	-5	LD_{50}*	NI*	-2	-3	-4	-5	LD_{50}	NI
Drosdov, mouse	2†	0	0	0	≤1.7	≥1.9	0	0	0	0	≤1.5	≥2.5
Congo, K2/61, mouse	6	1	0	0	2.3	1.3	2	0	0	0	≤1.7	≥2.3
CHF, pool,man	0	0	0	0	≤1.5	≥2.1	0	0	0	0	≤1.5	≥2.5
Normal, mouse	8	8	1	0	3.6		8	8	4	0	4.0	

* LD_{50} and neutralization index (NI) expressed in dex as reciprocals of log_{10}.

† Number of mice dead of 8 inoculated; i.c. test in 2-day-old mice.

mammals become infected and to determine if nidi of infection occurs among wild mammals.

Bovine Serum Survey

Alabama, Georgia, Louisiana, Texas, and New Mexico were selected as infected states; Indiana and New Jersey were selected as control states, and Puerto Rico was added as an interesting subtropical area. Two hundred serums were to be submitted from each survey state at the rate of five serums per county, selected from animals born since the last known outbreak. This survey is less than one half completed. Table 1 shows the samples which have been tested by the neutralization test using 8 day old chicken eggs.

Serums are being selected by the brucellosis labs. With the size of the sampling there is a 50 percent chance of detecting a positive animal if the infection is around 1 percent. We hope to get some indication if subclinical VS is occurring. To date, one positive and one suspicious case have been detected among 300 serums from Alabama and Texas, and no antibodies were detected in 200 serums from Puerto Rico.

Deer Survey

A total of 540 serums collected from white-tailed deer (Odocoileus virginianus) during the period 1964 to 1968 have been tested for neutralizing antibodies to VS. These serums have been received through the courtesy of Dr. Frank Hayes of the Southeastern Wildlife Disease Survey and from the Louisiana Wildlife and Fisheries Commission. Serology is summarized in Table 2. Generally, antibodies were not found. The most significant find was the presence of a significant level of New Jersey type VS neutralizing antibodies in deer of the 1967 fall kill at the Saline Game Management Area in Louisiana; 8 of 10 deer mostly 1 1/2 years of age had antibody titers to 2.4 (1:256). A lower level of antibodies was found on 2 of 14 deer from Ossabaw Island along the east coast of Georgia. Five suspicious reactions, serums positive at .6 (the 1:8 serum dilution), were omitted from the chart; there were 4 from Mississippi and 1 from Florida, all bled during 1965.

Mammal Survey

ANH personnel, and in Louisiana personnel of the Louisiana Wildlife and Fisheries Commission, collected 112 rodents, and in Louisiana serums from 2 swamp rabbits and 6 barred owls. One juvenile cotton rat, Sigmodon hispidus, and one Peromyscus had New Jersey VS neutralizing antibodies

Table 1. Summary of Vesicular Stomatitis Bovine Serum Survey.

	Maximum Age	Bovine Serums Tested to Date	Positive New Jersey		Positive Indiana
			No.	Titer	No.
Alabama	4	220	1	1.2(1:16)	0
Texas	2	80	1	.9(1:8)	0
Indiana	None	160	0		0
Puerto Rico	None	200	0		0

Table 2. Summary of Vesicular Stomatitis Serology on White Tailed Deer.

	Dates Collected	Serums Tested	Positive New Jersey	Positive Indiana
Alabama	1965, 67, 68	45	0	0
Arkansas	1965, 67	13	0	0
Florida	1965, 67, 68	50	0	0
Georgia	1965, 67, 68	42	2*	0
	1967		1**	
Louisiana	1964, 68, 69 rec'd 1966	269	3†	0
	1967		8‡	0
	1969		1§	0
Mississippi	1965	34	0	0
North Carolina	1967, 68	20	0	0
South Carolina	1965, 67, 68	36	0	0
Virginia	1965, 67, 68	31	0	0

* Ossabaw Island, 2/14 collected during February 1965; titers were 1.5 (1:32) and 2.1 (1:128).

** Ft. Stewart, 1/5 collected May 23, 1967; titer 1.2 (1:16).

† West Bay GMA, Allen Parish, 3/5 collected June 30, 1966; titers were >2.8 >(1:512), 2.4 (1:256), and 1.5.

‡ Saline GMA, LaSalle Catahoula Parish, 8/10 collected fall 1967; titers 1.2 (1:16) through 2.4 (1:256).

§ Pass-a-Loutre, 1/117 collected February 5, 1969; titer 1.2 (1:16).

Endpoints were figured by the Karber method with the titer expressed as the logarithm of the serum dilution neutralized.

Table 3. Summary of Vesicular Stomatitis Serology on Wild Mammals.

	Total No.	New Jersey VS	Indiana VS
Georgia (16) Heard, Carroll and Liberty counties		Coll. July 1968	
Sigmodon hispidus	8	0	0
Mus musculus	6	0	0
Peromyscus	2	0	0
Louisiana (18) (Saline Game Management Area)		La Salle Parish	Coll. May 1968
Sigmodon hispidus	3	1 pos. at 1:20	0
Peromyscus	6	1 pos. at 1:20	0
Reithrodontomys	1	0	0
Sylvilagus aquaticus	2	0	0
Barred owl	6	0	0
Texas (86)			
Laredo (45), Webb Co.	Coll. June 1968		
Sigmodon hispidus	41	0	0
Neotoma micropus	4	0	0
LaJoya (12), Hidalgo Co.	Coll. June 1968		
Sigmodon hispidus	6	0	0
Neotoma micropus	4	0	0
Peromyscus leucopus	2	0	0
Mission (14), Hidalgo Co.	Coll. October 1968		
Sigmodon hispidus	11	0	0
Peromyscus leucopus	1	0	0
Liomys irroratus	2	0	0
Rio Grande City (15), Starr Co.	Coll. January 1969		
Sigmodon hispidus	7	0	0
Neotoma micropus	2	0	0
Onychomys leucogaster	3	0	0
Peromyscus	3	0	0

at a 1:20 serum dilution; all other serums were negative to both the New Jersey and Indiana types of VS virus and are summarized in Table 3.

Ectoparasites have been combed from the rodents. Several pools have been made for virus isolation and others preserved for taxonomic studies. No Gigantolaelaps mites have been collected. Steptolaelaps were combed from the Liomys irroratus. No virus was isolated from organ pools of spleen-liver-heart and kidney-bladder from the positive Sigmodon and Peromyscus.

The positive Sigmodon and Peromyscus were collected in Louisiana's Saline Game Management Area (SGMA) during May 1968; the rodents were collected at the same time clinical VS was appearing in horses located in the SGMA. The first of the twelve herds found infected in Louisiana were near the SGMA and in the only state in which VS was diagnosed during 1968. The number of mammals sampled was too small to be significant, particularly in a year when clinical VS was not diagnosed outside of Louisiana. From an epidemiological viewpoint it is encouraging that the two positive rodents were collected in the area in which VS virus was active and where antibodies are found in deer.

(E. W. Jenney)

REPORT FROM THE DEPARTMENT OF VETERINARY SCIENCE UNIVERSITY OF WISCONSIN MADISON, WISCONSIN

Sixteen agents lethal for the suckling mouse and sensitive to ether were recovered from arthropods in 1968. Identifications have not been completed.

Over the past 16 years evidence has been obtained for the presence of at least 12 kinds of arboviruses in Wisconsin. Some appear to persist year after year and others to be detected at infrequent intervals. Eastern encephalitis was isolated from a horse in 1952, from a pheasant in 1958 and from black flies in 1960. Neutralizing antibodies were demonstrated in horses and a variety of wild birds before 1960 but not in the past 10 years. Western encephalitis was isolated from a horse in 1953. Although the

virus has not been isolated since then, clinical disease in horses associated with a rise in neutralizing antibody titer is observed in a few animals annually and there is evidence for two cases in man. Virus of St. Louis encephalitis has not been isolated. Sera which neutralize the virus have been obtained from wild rodents, domestic rabbits and sheep. Although no isolation has been made of Powassan virus, neutralizing sera have been obtained from woodchucks.

Many isolations have been made of the viruses of the California group. LaCrosse virus was first isolated from a child who died in 1960. Starting in 1964, it has been recovered every year from arthropods and/or sentinel rabbits. Five isolations of Jamestown Canyon virus were made from arthropods in 1965 and only in that year. Snowshoe hare virus was isolated twice in 1966. Trivittatus virus was first recovered from arthropods in 1964 and has been isolated every year since.

Eleven isolates of the Bunyamwera virus group were closely related or possibly identical to Cache Valley were recovered from arthropods in 1964, 1966 and 1967. Antibodies are commonly found in horses and possibly occur rarely in man.

Eleven viruses similar to Flanders virus have been isolated (one) in 1966 and (ten) in 1967. Silverwater virus was recovered from a tick in 1965.

No isolations have been made of Buttonwillow but Dr. W. Reeves found neutralizing substances in sera of cottontails from south central Wisconsin.

Six of the viruses studied in gel-diffusion have not shown reactions with any of the locally recognized California group viruses, or Bunyamwera group viruses and although serology has not been performed on all of them to exclude eastern or western encephalitis neither titers nor death patterns in mice or chicks have made it likely that they are closely related to either agent.

REPORT FROM THE DEPARTMENT OF ENTOMOLOGY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

During the years 1964-1967 attempts were made in Wisconsin to isolate arboviruses from mosquitoes (Culicidae), horseflies and deerflies (Tabanidae) and blackflies (Simuliidae).

From mosquitoes there were 46 isolations from 96,914 specimens of genus Aedes, 4 isolations from 2,004 specimens of Culex, and 2 isolations from 8,763 Mansonia. Viruses isolated from Aedes included one of the Bunyamwera group, one presently unnamed virus similar to Flanders and 4 members of the California complex: Jamestown Canyon, LaCrosse, Snowshoe hare and Trivittatus.

From horseflies and deerflies, there were 7 isolations from 20,487 specimens of genus Hybomitra, one from 3,429 Chrysops and none from 4,623 Tabanus. The viruses isolated were Jamestown Canyon the virus similar to Flanders, and, tentatively, LaCrosse. We consider LaCrosse virus to be closely though not exclusively associated with Aedes triseriatus in Wisconsin since at least one of every 142 triseriatus tested was infected.

From blackflies, there was one isolation from 15,588 specimens of 3 bird-feeding species and no isolations from 27,745 mammal-feeding species. Identity of this isolate has not yet been established.

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

Comparison of interferon induction and infectivity of various dengue strains cultivated in two mammalian tissue culture cells

Dengue virus 1-4 strains cultivated in hamster embyronic (HEM) and human embyronic (HET 35) diploid cells were titrated intracranically in suckling mice. Dengue 1 and 3 strains reached a titer 1.5 logs higher in HET 35 cells than the same inoculum of these strains in HEM cells. Dengue 2 and 4 strains reached approximately the same titers in both cell types. These observations suggested a difference in susceptibility, growth capacity, or interferon induction in different mammalian cells to infection by different dengue strains which could be used to distinguish these strains. In particular, the HEM cell seemed to group dengue 1 and 3 and dengue 2 and 4 together, whereas no distinction was observed in HET cells.

A chikungunya interferon inhibition test was used to determine the interferon forming units (IFU) induced by the four dengue strains. HEM cells produced negligible quantities of interferon induced by dengue 1 and 3 strains, but 3.7 and 6.5 Log_{10} IFU/0.1 ml of interferon was induced by dengue 2 and 4, respectively.

In HET cells 4.5, 2.0, 4.0 and 6.5 Log_{10} IFU/0.1 ml of interferon was induced by dengue strains 1, 2, 3 and 4, respectively. HET cells seemed to be more readily induced to produce interferon by all dengue strains and this induction was variable with the strain although the same starting inoculum was used.

Induction of interferon in HEM cells by dengue followed a surprisingly inverse pattern than one would have expected from the infectivity levels that were observed. Dengue 4 strain induced the same level of interferon in both cell types which was a higher level than dengue 1, 2 and 3. Mechanisms to explain these observations are under further investigation.

(H. M. Jenkin and S. Yu)

Biological and antigenic variations of Japanese encephalitis viruses

Two Japanese encephalitis viruses isolated from a mosquito pool and a human were serially cultivated in cell culture of weanling mice. Each isolate shows characteristic changes in their mouse pathogenicity after serial passage through primary hamster kidney (PHK) cells.

The strains and origins were designated as follows:

> G1 strain - human origin, about 250 passages in mice - virulence for mice maintained with 100 further passages in PHK cells.
>
> MH 30 strain - mosquito origin, PHK isolation and passage - mouse attenuation after 20 passages which was stable through 50 further passages in PHK.

Two biological parameters, hemagglutination (HA) and plaque formation (PFU), were used to distinguish the strains after DEAE column chromatography in a sodium chloride-phosphate buffer gradient. Strain MH 30, passaged in PHK cells, was eluted in a single early peak whereas strain G1, passaged in mice, showed the same peak plus an additional peak in a later fraction. HA and PFU activities were higher in the early fraction than in the last fraction.

After passage in PHK cells, the G1 strain pattern was similar to that of MH30, however, after mouse brain passage, MH30 showed no chromatographic changes. The data suggested possible phenotypic changes had occurred when virus-host changes were made.

Plaques produced by isolate MH30 in BHK-21 cell culture were found and measured 2.0 - 3.0 mm by day 6 but plaques of the G1 strain were small, 1.0 - 1.5 mm, irregular and tended to be crescent-shaped. This observation was explained by a difference in a one-step growth curve in the same cell culture system.

Viral antisera were prepared by hyperimmunization of rabbits. Cross neutralization tests and neutralization kinetics of MH30 and G1 were performed in a plaque reduction test in BHK-21 cells. Percentage of surviving virus (plotted as a probit value) were proportional to serum dilution (reciprocal of $\log_2$). The results of the cross-neutralization tests showed the same slope for each of the neutralizations. Relative potency of anti-MH30 serum against G1 virus was, however, about twice that of anti-G1 serum against MH30 virus.

Results of kinetics study showed anti-G1 serum of high homologous titer had low neutralizing capacity against MH30 strain, whereas homologous and heterologous titers of anti-MH30 strain were almost identical.

The data suggests that there are some genetic differences among JE virus strains other than phenotypic expression for example as demonstrated in pathogenicity tests.

Further investigation of these observations from a biochemical standpoint will be pursued.

(S. Makino)

REPORT FROM ONTARIO VETERINARY COLLEGE UNIVERSITY OF GUELPH, GUELPH, ONTARIO, CANADA

Electron Microscopic Study of the Virus of Epizootic Hemorrhagic Disease (EHD) of Deer

The 8th intracerebral suckling mouse passage of the Alberta strain (New Jersey serotype) of EHD virus was used in this study. The suckling mouse intracerebral infectivity titer was approximately 10^6 LD 50/ml. Seed virus was prepared as a 10% infected mouse brain suspension and stored at -70 C until used. Infected BHK-21 cell cultures and brain tissues from newborn mice were examined by ultrathin section and negative contrast electron microscopy.

Viral sequestration: At 48 hours post-inoculation the virus particles could be seen sequestered within dense phagocytic vacuoles of BHK-21 cells. Frequently these dense vacuoles were bounded by multilaminar membranes or myelin figures. Viral particles lacking distinctive protective coats were more numerous inside the phagocytic bodies and sometimes the central nucleoids were tightly packed.

Sites of virus formation: Infected BHK-21 cells as well as neurons of infected mouse brains contained foci of dense materials, consisting of granular and filamentous elements, scattered throughout the cytoplasm. These appeared to be viral matrices. The dense aggregates were associated with the

appearance of recognizable progeny particles of 40 mμ in diameter within the viral matrix. The progression of infection was marked by the appearance of increasing numbers of viral particles at the periphery of granular matrices. These particles were surrounded by an electron lucid layer. In many areas the mature particles were observed to migrate from the viral matrix to the adjacent areas of cytoplasm as they were formed.

Structure of EHD virus: The fully developed particles were 65-70 mμ in diameter. The intact virus consisted of three principal morphological components: 1) the outer capsid, 2) the inner structure layer and 3) the core. The capsid consisted of hollow and distinctly separate capsomeres. The inner layer structure, measuring 45 A wide, was present subjacent to the capsomeres. This structure was particularly prominent in damaged particles. It will be noted that this description of EHD virus differs from earlier published descriptions. We are unable to explain these apparent discrepancies.

Both in negative contrast preparations and in ultrathin sections, EHD virus resembles the reoviruses. Bluetongue virus also has been described as reovirus-like. EHD of deer has many similarities in clinical and pathologic signs to bluetongue in deer. Biochemical and serological comparisons should be made of their causative viruses.

(K. S. Tsai and L. Karstad)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY WESTERN COLLEGE OF VETERINARY MEDICINE AND CANADA AGRICULTURE RESEARCH STATION SASKATOON, SASKATCHEWAN

Western equine encephalitis surveillance in Saskatchewan during 1967 and 1968 indicated a decreasing level of virus activity and contraction in the incidence of active WEE infections to the endemic southeast corner of the Province.

In 1967 one human case (fatal) and nine unconfirmed horse cases were reported. From the bloods of 553 nestling birds representing 33 species collected between April 12 and August 30, two isolations of WEE virus were

made, one from a house sparrow and the other from a rock dove. WEE virus was not isolated from the bloods of 164 rodents representing four species collected between April 13 and August 18, but the virus was obtained from the liver of a Peromyscus sp. collected on May 5. No WEE infections were acquired in six sentinel chicken flocks of 24 birds each and the virus was not isolated from 20, 288 mosquitoes representing 18 species in 1144 species pools.

In 1968, no human cases of WEE were reported in the Province and of 10 reported horse cases, three were confirmed. No WEE virus isolations were made from the bloods of 851 wild birds including 300 molting Pintail ducks, from the bloods and other tissues of 124 Richardson's ground squirrels and 21 wild mice. No infections were acquired in the six sentinel flocks and the virus was not isolated from 14, 836 mosquitoes representing 14 species in 897 species pools. The three confirmed cases in horses were the only evidence obtained that the virus was still present in the Province.

Two of the three virus isolations made in 1967 were from specimens taken in the endemic southeast corner of the Province and two of the three confirmed horse cases in 1968 were also in that area. The 1968 season was the third in succession in which no epidemics of WEE had occurred in Saskatchewan.

(A. N. Burton and J. McLintock)

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

Field studies, summer 1968

Sera from 411 of 1243 small forest rodents collected in south-eastern British Columbia (116° W, 49° 30' N) between May and August 1968 inhibited hemagglutination by one or more group B arboviruses. In intracerebral mouse neutralization tests, conducted simultaneously on HI-positive sera against Powassan and St. Louis encephalitis viruses, 26 of 409 neutralized Powassan virus and 61 of 399 neutralized St. Louis encephalitis virus, including 18 sera which neutralized both agents. Powassan neutralizing

antibodies were found in an additional 9 of 522 HI-negative sera. Field investigations for 1969 will be initiated on 1st April, one month earlier than in previous years, in attempts to determine the initial stages in the cycle of arbovirus transfer between tick vectors (powassan virus), mosquito vectors (St. Louis encephalitis virus) and their respective vertebrate reservoirs in mountainous portions of south-eastern British Columbia.

(D. M. McLean)

Powassan virus replication in ticks

Larvae of Dermacentor andersoni ticks become infected by ingestion of $10^{4.5}$ mouse LD_{50} of Powassan virus contained in blood from a rabbit rendered viremic by intravenous injection. At 17 days, nymphs which emerged from infected larvae contained no virus, but at 3 weeks $10^{6.0}$ LD_{50} were detected in nymphs, and they transmitted virus by biting hamsters at 4 weeks. At 8 weeks when virus titers had declined to $10^{3.0}$ LD_{50}, nymphs transmitted virus by biting guinea pigs. Following the second molt, emergent adults contained no infectivity at 9 weeks, but maximum titers of $10^{6.0}$ LD_{50} were attained by 18 weeks, at which time they transmitted virus by biting guinea pigs. Furthermore, immunofluorescent foci were demonstrated in the cytoplasm of salivary glands of adult ticks at 18 weeks by direct or indirect fluorescent antibody techniques.

When adult D. andersoni ticks were fed on viremic rabbits, infectivity was detected exclusively in the gut washings shortly after the intravenous injection of the hosts. No infectivity was detected in any organ at 5 days, minimal amounts of virus were found only in the gut wall at 9 days, but by 17 days minimal infectivity was found in the salivary glands which also showed virus-specific immunofluorescence. At 24 days, infectivity was detected additionally in the Malpighian tubules and gland of Gené's organs. At 32 days immunofluorescent foci were observed in gut with an infectivity titer of $10^{3.0}$ LD_{50} glands of Gené's organ ($10^{2.0}$ LD_{50}) and salivary glands ($10^{5.0}$ LD_{50}). Virus titers remained almost unchanged in salivary glands, and glands of Gené's organ, and immunofluorescence was again detected at 61 days.

These results show that Powassan virus replicates in D. andersoni tick tissues, especially the salivary glands and glands of Gené's organ, that it

is transferred trans-stadially, and that experimentally infected ticks transmit virus by biting vertebrates.

(M. A. Chernesky)

REPORT FROM SOUTH DAKOTA STATE UNIVERSITY BROOKINGS, SOUTH DAKOTA

Isolation and Identification of EEE Virus from 1967 Epizootic on South Dakota Pheasant Farm

There has been no known EEE virus isolation in South Dakota until 1967. The virus epizootic occurred on Southeast South Dakota pheasant farm located in Lincoln County near the Iowa border on Big Sioux River at Long 96° 30' and Lat 43° 20'. The epizootic which began in July 1967 involved 10, 862, sixteen to eighteen weeks old pheasants in which the mortality rate was 89.8%. Thorough examination of (1) site where first outbreak occurred, (2) the physical setting of the farm, (3) aerial photography of the country and (4) environmental factors suggested possible mosquito the involvement in the arbovirus outbreak in pheasants. Commercial pheasant farmer said that there was unusually high mosquito population. The species of mosquitoes identified in the area were Aedes sollicitans, Aedes triseriatus, Culex restuans, Culex salinarius, and Mansonia perterbans. Which of these or other mosquito species played a role in virus transmission to pheasants is as yet not clear. Detailed work is under investigation.

The gross symptoms, unusually high mortality rates, and serological evidences were similar to the EEE outbreaks reported in pheasants of Eastern United States. The symptomatology of the disease syndrome was sequentially followed in 300 birds by photography. 16mm color movie of the diseased birds showing first signs of illness to death was also produced.

The problem of virus transmission from pheasant to pheasant still remains obscured. The once reported method of debeaking and controlling the number of diseased birds during an epizootic was not successful. More than 900 debeaked birds had the same percent mortality (89.8%) as non-debeaked birds. There is a possibility that some pheasant ectoparasite serves as a vector for quick transmission on the farm birds from a pheasant to another.

The South Dakota EEE virus was isolated from a pool of 20 diseased and paralysed pheasant brains using embryonated and new born mice. The virus infected embryo suspension showed 1:16 CF titer and 1:128 and HA titer. The nonpurified infected mouse brain suspension showed 1:8 CF and 1:256 HA titer. The South Dakota EEE virus suspension of 105 mice LD50 was completely neutralized by EEE guinea pig antisera obtained from NCDC. The virus HA was inhibited by EEE-HAI goat serum (also obtained from NCDC), even at 1:64 dilution. Thus the virus isolate was identified to be EEE. Further comparison of this agent with other EEE strains is anticipated.

Nine weeks after the outbreak, the sera from 130 epizootic survivors showed (a) 20.8% positive for EEE CF antibodies (at least 1:4 to 1:32, majority 1:8) and (b) 22% HAI antibody (titer rate 1:32 to 1:512, majority 1:128). The remainder of the birds had probably escaped infection.

Development of Serological Tests for Epizootic Hemorrhagic Disease (EHD)

In order to perform EHD epidemiological work, laboratory serological tools of complement fixation and precipitation test were investigated. EHD antisera were produced in (a) horses and (b) rabbits. The horses and rabbits were inoculated respectively with differentially centrifuged and genetron extracted infected deer spleen and 10% homogenized and purified (35,000 rpm) infected mouse brain as antigen. These animals received at least 10 injections and then were bled. From three deer inoculated with clarified (15,000 rpm) SD 10 EHD 10% spleen suspensions, two hundred cc of deer immune sera were obtained. These antisera were then tested against a specific EHD antigen. They showed at the least, a CF titre of 1:16 and precipitin titer of 1:4. When examined by Ouchterlony immuno-diffusion tests, different animal (horse, rabbit and deer) antisera showed one precipitating line with complete identity, when tested against above virus antigenic material prepared from deer spleen; or mouse brain. Various controls and checkerboard testing were also carried out. Results showed that there was adequate specificity and our method of preparation of antigenic material was found to be satisfactory. Precipitin titers in wild deer sear were low (L1:2) and at times more animals (approx. markedly 10%) showed positive CF titre than precipitaton reaction. These antigens were then utilized to detect EHD complement fixing antibodies in wild deer, cow and pig sera. We surveyed over 2300 cow and pig sera for

EHD CF antibody and found that 8.8% of these animals had the antibody. Thus, there is a possibility of EHD or EHD related virus in livestock.

(G. C. Parikh)

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

Colorado: During 1968 there was a total of 56 clinical encephalitis cases reported; two were serologically confirmed as western encephalitis (WE). Only Turlock virus isolations were obtained from mosquitoes during 1968; whereas, Turlock, WE and St. Louis have been obtained each of the past several years. Markedly lower C. tarsalis population indices at the collecting sites were obtained during the 1968 season in comparison to those obtained the previous few years. The disappearance of WE and SLE viruses and the persistence of Turlock virus during periods of C. tarsalis population ebb is similar to the pattern being observed in Kern County, California. Evidence of California encephalitis (CE) virus infections among humans was detected among suspect human encephalitis cases in 1967 and 1968. Serums from nine of 48 (19%) of the 1967 cases and seven of 41 (17%) of the 1968 cases contained neutralizing antibodies against CE virus (LaCrosse strain). However, no rises in antibody titer was demonstrable between acute and convalescent serum pairs from any of the 16 persons with CE virus antibodies. Thus, no evidence was found that CE virus infections had any eitologic significance in this group of patients.

Texas: No confirmed WE or SLE case was found among the 29 clinical encephalitis cases in Hale County during 1968. Arboviral disease occurrence in Texas was very low during the year, as throughout the U.S.A., and only four cases were confirmed in the state. Culex tarsalis population levels also were lower than during recent years, but isolations of WE, SLE and Turlock viruses were obtained from C. tarsalis. To date, only WE virus isolations have been identified from nestling house sparrows (Passer domesticus), and two isolates from the nestlings are not yet identified. The first isolation of SLE from Culiseta inornata was obtained this year.

from the Hale County area. Arbovirus isolations also were obtained from pools of Psorophora signipennis and Aedes nigromaculis. These included CE virus from both species.

The first known breeding colonies of P. domesticus were established at the Plainview field station for experimental studies with WE virus. One colony consisted of WE virus immune adult birds (HI 1:80) and the second of WE virus non-immunes (HI 1:10). Nestlings were inoculated with 95-275 pfu of a second DECC passage C. tarsalis WE isolate or diluent. The average age at time of inoculation of nestlings from non-immune parent birds was 10.3 days and from immune parents 9.3 days. All nestlings were bled until they died or fledged.

Twenty of 22 inoculated nestlings from WE virus non-immune parents developed viremia; 18 of 21 from WE immune parents had demonstrable viremias. No virus was recovered from 40 uninoculated nestlings which were, for the most part, nestmates of WE virus inoculated nestlings.

Peak viremia levels usually occurred 24-48 hours post-inoculation and the highest level of viremia occurring in a nestling was $10^{10.4}$ DECC pfu/ml. The longest period of detectable viremia was nine days post-inoculation, however, only a few nestlings survived that long or longer. Generally, viremia levels in nestlings from immune parents appeared to be somewhat lower, but persisted longer than in nestlings from non-immune parents.

REPORT FROM THE ANIMAL DISEASE AND PARASITE RESEARCH DIVISION, ANIMAL DISEASE RESEARCH LABORATORY DENVER, COLORADO

An infection rate of about 30% for Culicoides variipennis flies infected with bluetongue by blood meals given through a membrane was equivalent for 3 groups of flies in spite of the fact that 1 group received 2 infected meals, another a normal blood meal followed by an infected one, and the 3rd group only a single infected meal. Because the threshold to infection seems definite and limited, it is likely that the genetic inheritance of the transmissibility of bluetongue virus can be used so that both transmitting and nontransmitting colonies of flies can be developed for research on the mechanism of insect transmission of an arbovirus from an infected to a susceptible host and for research on the control of this vector fly.

Biological serial transmission of bluetongue from sheep to sheep by the bites of Culicoides variipennis has been accomplished over a period of 13 months. Fifteen serial transmissions were conducted during this period in which the infection rate of the Culicoides was 2.5% at the first passage level. The infection rate then varied from 16.6% to 65.1% over the next 14 serial passage levels. In general, the peak infection rates occurred during the spring and summer months while the infection rate during the winter months varied from 26% to 40%.

A survey of 97 antelope sera collected during the 1968 pronghorn antelope hunt in Colorado revealed 34 (35%) positive micro-agar gel precipitin tests (Jochim and Chow, 1969) for bluetongue. Only 2 of 51 (4%) of the samples collected in western and northeastern Colorado were positive, whereas 32 of 46 (70%) collected in southeastern and east central Colorado were positive.

Bluetongue virus has been determined to have a complex micromorphology. The capsid containing the nucleoid has reovirus-like morphology and is 55-60 Nm in diameter. The interesting fact concerns the progressively complex coats that may be added to the virus particle which increases its diameters from 60 to 80 Nm. The virus also has been found on occasions to possess an envelope (unit membrane), presumably of host cell origin. These extra coatings found on the virus particle may account for the fact that virulent virus may circulate freely with specific neutralizing antibodies in cattle infected with bluetongue disease.