ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

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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 S.I.R.A.C.A. OF THE AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

The immunological inter-relationships among all catalogued members of the California encephalitis (CE) group of arboviruses have been studied in detail by S.I.R.A.C.A. This information has been drawn from published reports together with additional unpublished data derived by S.I.R.A.C.A. members and other investigators from laboratory studies on arboviruses within this group. Although the conclusions are provisional, they represent the best possible synthesis of information available at the time of formulation of the report on 19th March, 1969. The conclusions are subject to modification in the light of additional information. S.I.R.A.C.A. welcomes the submission of any experimental information on intra-group antigenic relationships by interested investigators.

The conclusions of S.I.R.A.C.A.'s deliberations are as follows:

1. Examination of available results of neutralization tests, including tests with one- and multiple-injection sera, from mice and rabbits leads to the conclusion that there are hardly any major differences among the viruses now included in the California group.

2. Differences between individual members of the group have been observed, when apparent, mainly by CF, HI and double diffusion gel precipitation tests.

3. Tahyna and Lumbo viruses are as close as they can to being indistinguishable from each other, by N, CF, HI and gel diffusion tests. Slight differences have been reported in serum-dilution neutralization tests between the two viruses, which fact is of undoubted epidemiological value. However, it seemed to this Subcommittee that greater differences exist among strains of other given arboviruses.

4. Jamestown Canyon and Jerry Slough offer a similar situation; all available evidence indicates that they are nearly indistinguishable.

5. In the absence of major differences by N test; of hardly any experimental inclusive cross-challenge tests; of little serological surveying that includes all antigens or viruses as well as sera from different areas, simultaneously done; and from the fact that

differences between serotypes detected by CF or HI tests are, on the whole, not systematic and at times even contradictory: S. I. R. A. C. A. is of the opinion that the current members of the California group are closer to each other than are some of the closer viruses in complexes of Groups A (Chikungunya-O'nyong-nyong) or B (MVE-Kunjin). Therefore, it seems advisable to consider the present California group, no longer a group but one virus with subtypes, as follows: California encephalitis (1943, year reported or first isolated), Trivittatus (1948), Melao (1955), Tahyna-Lumbo (1958-1959), San Angelo (1958), Snowshoe hare (1959), Jamestown Canyon-Jerry Slough (1961-1961), La Crosse (1964), and Keystone (1964).

6. The 9 distinguishable subtypes listed in 5, can be grouped in the following units or cluster, on the basis of their greater or lesser closeness:

   i. California encephalitis, Tahyna-Lumbo, San Angelo, Snow-show hare, Jamestown Canyon-Jerry Slough, La Crosse, Keystone.

   ii. Trivittatus

   iii. Melao

7. S. I. R. A. C. A. is against withdrawing, or suggesting withdrawal of any of the current cards of the California group. There may be and, in fact, there are good reasons for keeping them. We, however, advise that cross-referencing on the cards be done that explains the situation as we see it.

A few final comments may serve to clarify S. I. R. A. C. A.'s position.

There is no doubt that in some instances when two of the virus prototypes now listed in the California group are serologically compared, differences large enough may be detected to justify considering these viruses as distinct. However, if a third prototype is brought into the comparative study, it may differ from either of the first two by such small values that it no longer is justifiable to consider number 3 as different from either number 1 or 2, therefore 1 and 2 are brought together through the agency of 3.

The conclusion to consider the now registered viruses of the California group as serologic subtypes (or antigenic variants) of one virus is tentative, subject to change and undoubtedly colored by S.I.R.A.C.A.'s leaning at this time towards lumping rather than splitting; it is to be stressed again that this conclusion is based on presently available sources.

The following must be clearly understood: in no way must this report by S.I.R.A.C.A. be interpreted as saying that there are no antigenic differences among the subtypes (or viruses) of the California virus (or group); and that any subtype can be substituted for any other in, for example, serological surveys or diagnostic work.

(E.L. Buesher, C.H. Calisher, J. Casals - Chairman, W.F. Scherer, and G.E. Sather)

REPORT FROM THE YALE ARBOVIRUS RESEARCH UNIT DEPARTMENT OF EPIDEMIOLOGY AND PUBLIC HEALTH YALE UNIVERSITY SCHOOL OF MEDICINE NEW HAVEN, CONNECTICUT

Lassa Virus

As reported in the previous issue (No. 19, July 1969) of the Information Exchange, a virus was isolated from serum of two fatal cases and from serum and pleural fluid of a third, non-fatal case of a hemorrhagic febrile illness of American nurses in Nigeria working for the Sudan Interior Mission.

A fourth case has now been observed in a laboratory investigator who, while working with this agent, particularly conducting work with mice, became infected in the middle of June, 1969, and recovered after a serious illness that required hospitalization for one month. The main symptoms in this case were severe pain in the muscular mass of the lower thighs, fever and chills. Clinical and clinical laboratory studies indicated pronounced myositis and abnormalities in the electrocardiogram. On the 4th day of hospitalization, the patient was given 500 ml of plasma from the nurse who had recovered from the infection, as it seemed apparent that the same

agent was responsible for the current case. During the 24 hours after administration of the plasma the temperature dropped to normal and there was clinical improvement. At the time of release from the hospital, the patient had fully recovered.

A virus identified as Lassa by CF was isolated on repeated occasions from the fourth patient; from serum, urine and throat washings. It is to be noted that while virus was no longer isolated from the blood after administration of immune plasma, it remained present in the throat for the next 8 or 10 days and in the urine until the 20-22nd day from onset.

Additional properties of Lassa virus have been determined. Inoculation of material containing virus by the intracerebral route into 1- or 2-day old mice failed to cause visible signs of illness - only one mouse of about 35 or 40 inoculated was found dead on the 9th day; the rest survived for over 3 months. On the other hand, when the same materials were inoculated to 3- to 4-week-old mice, after a symptomless period of 5 to 6 days, 10 mice of 15 inoculated were found dead; the surviving ones appeared sick, hunched up, and with ruffled fur; when stimulated they went into convulsions with stretched out, rigid hind legs; in this position some would die, with respiratory paralysis. When holding these mice by the tail, a characteristic fine tremor could be felt. These signs immediately brought to mind the picture observed in mice infected with LCM, even though it was known then that the serum from the 3rd patient had failed to react with LCM antigen at the time when the patient had high titered CF antibodies against Lassa virus. Furthermore, electronmicroscope examination (Owen Wood, personal communication) showed an image similar to that reported for LCM virus, also for some Tacaribe group viruses: spheric or spheroidal, with a membrane. Electron dense particles were prominent.

Serologic studies were undertaken employing sera from the mice which survived inoculation of Lassa virus when they were 1-day-old, reinoculated 60 to 70 days later with a suspension of infected newborn mouse brain tissue (Lassa virus). Three different pools of 2-inoculation sera having a high homologous titer, against Lassa antigen, by complement-fixation test (1:256) were prepared; all 3 reacted with LCM antigen, with titers 1:8 or 1:16.

There appeared also a low titer cross-reaction between these samples of Lassa virus immune sera and some of the Tacaribe group antigens, particularly Amapari.

The current evidence indicates therefore, that a distant but reproducible cross-reaction exists by complement-fixation test between Lassa virus and LCM antigen; also with some of group Tacaribe antigens.

Brain tissue from newborn mice inoculated with Lassa virus is now on hand with which it is planned to prepare a sucrose-acetone antigen, inactivated. If this has a high homologous titer it is then planned to test against it hyper-immune sera for LCM and Tacaribe group agents.

(Yale Arbovirus Research Unit)

REPORT FROM THE MASSACHUSETTS VIRUS LABORATORY BOSTON, MASSACHUSETTS

In 1969 Eastern encephalitis virus (EEV) was isolated from arthropods for the first time since 1956, the last epidemic year in Massachusetts. Eastern encephalitis virus activity has been monitored each of the intervening years. Evidence of activity, often neutralizing antibody conversion in recaptured wild birds has been found each year. When Eastern encephalitis virus has been isolated from wild birds it has been late in the year, as was also the overt disease in pheasants in 1967, suggesting that the amount of virus transmission was too little and too late to cause human disease. There was, however, one human death in 1968 which was probably attributable to Eastern encephalitis (low titer neutralizing antibody in post mortem serum, four day illness).

Our data supports the hypothesis that Eastern encephalitis overwinters here. Its epidemiological behavior as a "place disease" is also compatible with this concept. If arthropods are sufficiently seeded with virus to be detected in a small sampling in 1969, we may expect an earlier build up in 1970 if favored by other factors. It will be important to continue both arthropod and wild bird surveillance.

Techniques: Mammalian cell lines susceptible to wild arboviruses have been sought with special emphasis on California virus (CEV). EE, WE, SLE, and Powassan are isolated in chick embryo cell cultures. In 1969 two strains belonging to the California group were isolated from Aedes

in December.

1957 2/51 sentinel pheasants

1958 None

1959 4 pheasants (1 flock)

1960 None

1961 None

1962 None

1963 None

1964 Viremia swamp sparrow Sept. 28, Raynham

1965 Viremia chickadee Oct. 14, Raynham

1966 Fatal horse case mid August, Middleboro

1967 3 pheasant flocks Oct., 3 towns in SE Mass.

1968 None**

1969 Viremia immature yellow throat Sept. 10, Easton
Culiseta melanura pool Sept. 17, Easton

None

None

Western encephalitis (WEV) sick pheasant, 27 mosquito pools (MP)

WEV MP

WEV MP

WEV MP

WEV MP

WEV MP

WEV MP

Very few MP completed-freezer failure

WEV MP: WE viremia swamp sparrow 10/4. Powassan Oct. skunk and ticks thereon. *Flanders in Culiseta melanura and other MP.

WEV MP: CEV group from Aedes canadensis

no WEV: CEV group from Aedes canadensis

canadensis directly in Vero cells. One of these strains (Hockomock) will be used as a complement fixing antigen in the search for human infections. The BFS^{283} strain has been so used since 1966. Paired sera from aseptic meningitis, encephalitis, and paralytic diseases will be tested.

Indirect fluorescent antibody using a "master conjugate" directed against mouse globulin has been successfully used to identify new isolates of EE and CEV and reagents standardized for the other viruses of interest in Massachusetts WE, SLE, Pow., Flanders, MHV (Mouse hepatitis virus - used only on mouse passage material). This can be applied rapidly to any virus-cell culture system to give a clue and often a definitive answer. Confirmation is by reisolation and neutralization in the cell culture.

The usual series of mouse immune ascites fluid applied to a new unknown is: normal, broad group A, broad group B, CEV (BFS), Bunyamwera group, Flanders, MHV, CEV (Lacrosse), EE, WE, Powassan.

(Joan B. Daniels)

REPORT FROM THE DEPARTMENT OF TROPICAL HEALTH, HARVARD UNIVERSITY SCHOOL OF PUBLIC HEALTH, BOSTON, MASSACHUSETTS

Vector Potential of Aedes albonotatus

Aedes albonotatus, a common, domestic mosquito of the Bahamas, has been collected from human bait, but nothing is known of its potential as an arbovirus vector. This report compares the survival and transmission in the laboratory of chikungunya and dengue 2 virus in A. albonotatus and in A. aegypti.

Chikungunya virus was assayed in agar-overlaid cultures of embryonic chick cells while dengue 2 virus was assayed in a similar system including a challenge-virus-resistance-test (with poliovirus type II). Mosquitoes

were infected with chikungunya by feeding on viremic infant mice and with dengue by inoculation. Similarly, transmissibility was assessed by feeding on infant mice.

The incubation period of chikungunya in infant mice was 4 days and in A. aegypti, 10 days. A. albonotatus did not infect mice and contained no detectable virus after 5 days. Dengue virus was present in all A. aegypti sampled but was absent from A. albonotatus 15 days after inoculation.

These observations indicate that A. albonotatus may be a poor vector in nature for these viruses. Studies, currently in progress, indicate that A. albonotatus displaces or excludes A. aegypti. Introduction of A. albonotatus into areas beyond its normal range would be permissible only if this species appears to be less dangerous as an arbovirus vector than is A. aegypti.

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

During the past year, research included investigations of arthropod-borne viruses isolated from the Atlantic lowlands of British Honduras, Honduras and Guatemala, and the Pacific lowlands of Guatemala. By use of sentinel hamsters, Venezuelan encephalitis, a group C and Patois group viruses were recovered from each of these geographic regions during 1967 and/or 1968. Some of these endemic areas are closely associated with human populations. Nepuyo virus was found to produce cytopathic effects in HeLa human epithelial cells and plaques in primary hamster embryonic cell cultures. Of the 96 strains of Patois group virus recovered from Mexico and Central America during 1963 through 1967, five have so far been identified as Zegla rather than Patois viruses. Zegla virus was recovered from a cotton rat caught at Puerto Cortez, Honduras, during August 1967. Patois viruses produced plaques in BHK 21 hamster kidney and in primary hamster embryonic cell cultures and CPE in HeLa cell cultures.

Further studies of Mexican and Hawaiian cattle and other domestic animal sera by chicken embryonic cell microculture neutralization tests for anti-

bodies to western and Venezuelan encephalitis viruses have indicated that cattle sera frequently contain nonspecific viral neutralizing substances detectable by the cultural neutralization test, whereas pig, goat and sheep sera seldom contain nonspecific inhibitors of these viruses.

A comparison of the biologic and immunologic properties of VE, Mucambo and Pixuna viruses revealed Mucambo virus to be less virulent for hamsters inoculated subcutaneously than VE virus, and Pixuna virus to be essentially nonpathogenic, though infectious, for hamsters. Cross protection tests utilizing hamsters immunized either with the TC83 vaccine strain of VE virus or with Pixuna virus have shown solid cross protection to subcutaneous challenge with virulent VE or Mucambo viruses.

Studies of the effect of pH on dengue virus yields and plaque formation in LLCMK2 rhesus monkey kidney cell cultures were carried out with dengue type 2, New Guinea C strain, and type 1, Hawaii strain. Although it was possible to raise the pH of medium to 7.6 or 7.7 and maintain it in the 7.0-7.5 range, this did not significantly increase plaque counts or yields of these dengue virus strains when compared with medium which began at 7.4 and maintained 6.9-7.2. The failure of dengue 4, strain H241, to produce plaques in LLCMK2 cells maintained under agar medium was found not to be related to the line of cells employed, but was influenced by the type of agar medium. The vital constituent has not yet been identified, but seems to be an ingredient of Eagle's solution. However, a different strain of dengue 4, 4328S, produced good plaques in our ordinary agar medium which works successfully for type 1, Hawaii strain, type 2, New Guinea C strain and type 3, H87 and AP20 strains. Thus, whatever the phenomenon is with dengue 4, strain H241, it could from a practical viewpoint be circumvented by use of 4328S strain.

Cross protection experiments among dengue viruses, types 1-4, in terms of viremia in New World monkeys have indicated lack of cross protection among type 3 and type 2 viruses, strains New Guinea C and AP20 respectively. Small numbers of marmosets, squirrel monkeys and night monkeys have been infected by intranasal inoculation of large doses of dengue type 1, Hawaii strain, type 2, New Guinea C strain, and by relatively small doses of dengue type 3, AP20 strain.

Nodamura virus was found to be unrelated to type 10, bluetongue virus, but the development of Nodamura virus neutralizing antibodies in a sheep undergoing immunization to this bluetongue virus in South Africa suggested that Nodamura or a closely related virus may exist there. Results of filtration

experiments with Nodamura virus varied with the technique employed, but in some cases fitted with the 20 millimicron virions visualized during the past year by Dr. F. Murphy at NCDC in muscle and liver cells of infected mice.

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

Arboviruses and Singh's Mosquito Cell Lines

We have been investigating the usefulness of two mosquito cell lines (Singh, 1967) for our continuing studies of arbovirus-host cell relationships. Both of these invertebrate cell lines have great potential for large scale use due to their lack of requirement of insect hemolymph for growth. The cell line derived from Aedes albopictus larvae has proved to be more versatile in our laboratory, as in others, than that from A. aegypti larvae, in terms of susceptibility to and maximum titers produced by infection with Sindbis and dengue-2 viruses.

We have established the A. albopictus line in stirred suspended ("spinner") culture. In M-M medium (Mitsuhashi and Maramorosch 1964) these cells in suspension have a doubling time of 24 to 30 hours at 22 or 28° C. They do not survive at 37° C. We have also adapted the A. albopictus cells in both monolayer and spinner culture to Eagle's medium enriched with 1% tryptose phosphate and 10% fetal calf serum (heat inactivated at 56° C for 30').

Chromosome counts have disclosed approximately 75% of figures having six chromosomes and 25% having 12. All are mesocentric, two pairs being longer than the third. Since mosquitoes are known to have six chromosomes, and such low chromosome numbers are unknown in vertebrates, our counts would be quite reassuring evidence that the cell line is really mosquito in origin and uncontaminated by cells of higher forms.

Monolayers of mosquito cells have been infected with dengue-2 (New Guinea B, grown in KB and assayed on Vero cells) or Sindbis virus (grown and assayed in BHK21 cells) at multiplicities of 1-2 PFU/cell. The yields of

infectious dengue-2 virus have been low, while those of Sindbis are of the order of 100 PFU/cell in A. albopictus, only 1 PFU/cell in A. aegypti cells. However, the rates of virus production in all these systems are equivalent to those in the mammalian reference cells, i.e., dengue-2 has a latent period of about 12 hrs. and reaches a peak titer at 24-30 hrs., while the latent period for Sindbis is about 3 hours in both mosquito cell lines.

Six weeks after infection with Sindbis virus, monolayer cultures were put into spinners, and virus yields and cell counts were followed for a week. Under these conditions the cells were found to release virus at a rate of about 1 PFU/cell/day. It is unknown at this point whether this reflects the response of a few or of many cells in the culture. No cytopathic effect (CPE) was evident and the growth rate of the cells was unaffected.

Sindbis virus grown in A. albopictus cells sediments in sucrose gradients with the same velocity as BHK21-grown virus. The viral RNA has the same sedimentation coefficient (42S) regardless of the kind of cell from which it is derived. Comparative protein (and especially glycoprotein) analyses of purified virus from both sources are in progress.

The mosquito cells are similar to mammalian cells in response to exposure to actinomycin D. A concentration of 1 μg actinomycin/ml in the medium causes extensive CPE and concomitant reduction of Sindbis virus yield in a 24 hour period of exposure. Lower concentrations do not. H^3-uridine labeling shows that more than 90% of cell RNA synthesis is turned off in the presence of higher concentrations (5 μg/ml) of actinomycin with four hour exposures. Residual cell RNA is concentrated in a 12S peak under these circumstances. The pattern of virus specific RNA's is similar to that found for Group A arboviruses in vertebrate cells.

(T.M. Stevens, V. Stollar, R. W. Schlesinger)

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

A series of tissue culture and cell culture systems were tested for their capacity to support the growth of a highly attenuated strain of type 1 dengue virus, the MD-1 strain. Primary cultures of chick embryo cells and baby hamster kidney cells produced only limited amounts of virus, $10^{3.7}$ PFU/ml and $10^{4.8}$ PFU/ml, respectively. Primary and secondary cultures of rhesus monkey cells varied significantly in their capacity to replicate the MD-1 virus. Primary monkey kidney cultures produced $10^{3.7}$ PFU/ml while secondary monkey cultures produced $10^{5.2}$ PFU/ml. When poly-L-ornithine was included in the adsorption and growth medium, primary and secondary monkey cultures produced $10^{5.0}$ PFU/ml and $10^{5.7}$ PFU/ml respectively. The WI-38 human diploid cell line produced $10^{5.9}$ PFU/ml of the MD-1 virus while $10^{6.1}$ PFU/ml were produced when poly-L-ornithine was incorporated in the adsorption and growth medium. Thus it appears that the highly attenuated MD-1 strain of type 1 dengue virus replicates reasonably well in the WI-38 cell line and that it may be possible to produce this candidate vaccine strain in a potentially acceptable cell culture system.

REPORT FROM THE EPIDEMIOLOGY BRANCH AND LABORATORY OF SLOW, LATENT, AND TEMPERATE VIRUS INFECTIONS, COLLABORATIVE AND FIELD RESEARCH, NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND STROKE, NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND

The NINDS, through its Epidemiology Branch in Bethesda, its Research Center in Guam, its Laboratory of Slow, Latent, and Temperate Virus Infections, is conducting studies of Japanese encephalitis infection on Guam.

An epidemic of Japanese encephalitis occurred on Guam in 1948, which was well documented by Hammon, Reeves and others. Their evidence indicated

Year of Birth	1900 09	1910 19	1920 29
No. with Ab/No. Bled	3/3	0/1	24/53
% with Ab	100	0	45

1930	1940	1945	1950	1955	1960	1965
39	44	49	54	59	64	67
43/140	9/28	1/150	6/54	2/57	1/7	0/5
31	11	1	11	4	14	

that the virus completely disappeared from the area following the epidemic. Recently an entomologic survey of the island has revealed that the prevalence of Culex tritaeniorhynchus is increasing. Although this mosquito was reported to be uninvolved in the 1948 epidemic it has been reported as the major vector of JEV in Japan, and as a vector in other areas. A recent serologic survey of 100 pigs turned up only one pig with a titer of 1:20. Pigs did participate in the 1948 epidemic.

In order to determine the pattern of Japanese encephalitis infection on Guam, we have bled 552 individuals born since 1910 and measured their antibody level to JEV using the hemagglutination inhibition test. The prevalence of HI antibody to JEV is shown in the enclosed table by decade for the years preceding 1940 and by 5 year intervals following 1940.

Sera from 40 of these individuals born since 1950 were run against Russian Spring-Summer Encephalitis virus, Dengue I virus, and St. Louis Encephalitis virus in addition to JEV. None of the 40 individuals had HI antibody to either RSSE or Dengue I viruses. One had a HI titer of 1:80 and three of 1:20 against SLE only. Two had a titer of 1:40 against SLE; one with a titer of 1:40 and the other with a titer of 1:20 to JEV. One individual had a titer of 1:80 against JEV only.

These findings confirm that the epidemic of 1948 involved less than 20 percent of the susceptibles and emphasize that the epidemiology of JEV on Guam differs from that of Japan, Korea and Taiwan. The findings suggest the possibility that there is some continuing arbovirus activity on Guam even though no epidemics have been reported. In order to determine the cause of the arbovirus activity on Guam we plan to subject all the sera with HI titer to JEV and a randomly selected aliquot of negative sera to neutralization tests.

REPORT OF THE LABORATORY OF SLOW, LATENT, AND TEMPERATE VIRUS INFECTIONS AND THE STUDY OF CHILD GROWTH AND DEVELOPMENT AND DISEASE PATTERNS IN PRIMITIVE CULTURES, NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND STROKE, NATIONAL INSTITUTES OF HEALTH BETHESDA, MARYLAND

Our studies on slow infections of the nervous system have established infection as the etiology of two degenerative diseases of the human brain: kuru and Creutzfeldt-Jakob diseases. The successful transmissions of these diseases to chimpanzees with spongiform encephalopathy of gray matter as a major characteristic suggest that they be included with the virus infections which may be designated the "subacute spongiform viral encephalopathies: kuru, Creutzfeldt-Jakob, scrapie and mink encephalopathy".

Concurrent with these transmission studies isolation and related characterization studies are being conducted on the isolation and identification of viruses from chimpanzee tissues grown as explant cultures in vitro. We have previously reported over 47 isolations in primary human embryo kidney cell cultures inoculated with supernatant fluid from explant cultures of chimpanzee tissues. Since that earlier report additional chimpanzees affected with neurological disease have been killed and from their tissues additional virus isolations have been successfully accomplished. They now number over 100 isolations.

Among the isolates are two serologically distinct viruses designated Pan 1 and Pan 2 isolated from brain, spinal cord, sympathetic ganglia, spleen, thymus, kidney, lymph node and salivary gland tissues from 11 chimpanzees. The viruses produce a vacuolated, foamy, multinucleated syncytia without inclusion bodies in HEK and MA-111 rabbit kidney cell cultures. Both viruses are ether, chloroform, and pH-sensitive and are inactivated by exposure to 56°C/30 mins. They pass through 200nm millipore filters but not through 100nm millipore filters. Homologous antibody occurs in the serum of each chimpanzee from whose tissue the virus has been recovered. They do not cross serologically with previously described Simian Foamy Viruses Types 1, 2, 3 or 4. Full characterization of Pan 1 and Pan 2 viruses will shortly be reported as a Ph.D. Thesis of Mr. John Hooks of this laboratory.

In a similar study we have isolated cytomegalovirus (CMV) from the urines of 9 healthy young rhesus monkeys among 11 surveyed. Viruria has been found to persist for over 4 months. The virus was isolated in WI-38 cell cultures in which it produces a CPE similar to that caused by CMV strains isolated from African green monkeys. Rhesus CMV strains produce CPE in cell cultures of newborn human foreskin (MA-184), of rhesus monkey fetal lung (MA-101) and skin-muscle (MA-105) and of African green monkey fetal lung (MA-192) and liver (MA-121) origin. A serological survey is now in progress to determine the prevalence of antibody to rhesus monkey CMV in monkeys of several species as well as the relationship of rhesus monkey CMV strains to those from African green monkeys. These studies have been primarily conducted by Dr. David Asher and are being continued by him as part of a special fellowship in the laboratory of Dr. A. A. Smorodintsev, Leningrad, U. S. S. R.

REPORT FROM THE DIVISION OF VETERINARY MEDICINE, WALTER REED ARMY INSTITUTE OF RESEARCH, WASHINGTON, D. C.

Preparation of a Tween-ether extracted vaccine for chikungunya virus

Tween-ether (TE) extraction of arboviruses has been used in the past for preparation of hemagglutinins for use in HI tests (Saturno, A. 1967. Wld. Hlth. Org. Bull. 36: 347-349). After suitable treatment of a lipid-containing virus with Tween 80 and ether, infectivity in most cases is completely lost and hemagglutinin activity is enhanced. The application of this procedure for preparation of an inactivated vaccine for chikungunya (CHIK) virus has been investigated and vaccines prepared by formalin-inactivation (FI) and TE extraction have been compared.

Chikungunya virus grown in African green monkey kidney cell cultures was harvested between 48 and 96 hr and the culture fluids clarified by centrifugation. Tween 80 was added to the virus fluids at a concentration of 5 mg/ml and the mixture shaken 15 min at room temperature. An equal volume of diethyl ether was added and shaking repeated for 15 min at room temperature. Ether was separated from the aqueous phase by centrifugation and

residual ether was removed by aeration with nitrogen gas. The extracted fluids were filtered through a 0.45μ Nalgene filter unit and bacterial sterility and safety tests were performed with satisfactory results.

Formalin-inactivated vaccine was prepared by the method of Harrison, Binn, and Randall (1967. Am. J. Trop. Med. Hyg. 16: 786-791) from the same CHIK virus seed used for the preparation of TE vaccine.

Potency and antibody assays of FI and TE vaccines were performed in adult male mice using a single dose or a two dose vaccination schedule, seven days between doses. Challenge with live virus and bleedings were done 14 days after the final vaccine dose.

Serological testing of post-vaccination sera was performed using CF, HI, and neutralization tests. The bead neutralization test of Porterfield (1960. Wld. Hlth. Org. Bull. 22: 373-380) was used and microtiter CF and HI tests were performed using antigens prepared by sucrose-acetone extraction of CHIK-infected suckling mouse brain. Several strains of CHIK virus were included in the neutralization tests to determine the degree of specificity of antibodies produced in response to the vaccines. Table 1 lists the antibody response in mice to either FI or TE vaccine. Antibody response was quite similar in the TE and FI vaccines and potency after one dose of vaccine was adequate in all four vaccines. The ED_{50} value given in Table 1 is the volume of vaccine that protects fifty per cent of animals against a live virus challenge.

Another lot of FI and TE vaccine was prepared with the purpose in mind to determine stability of the vaccines stored in the fluid or freeze-dried state over an extended period of time. Table 2 compares the results of potency assays and serological tests in mice after three months storage of the vaccines. The dried vaccines were held at -20C and the fluid vaccines at 4C. The immunogenicity of the vaccines remained stable over this period of time with further tests planned to reveal more about the stability of the vaccines held for longer periods.

The hemagglutinin produced as a result of TE extraction of CHIK virus also remained stable over a period of three months with no significant reduction in HA titer. Future experiments will attempt to elucidate the relationship between the hemagglutinin and the antigenic and immunogenic components of CHIK virus.

(V. R. Harrison, K. H. Eckels, C. M. Hampton)

Table 1. Antibody response and potency in mice after vaccination with FI and TE CHIK vaccines.

Vaccine[a]	HI test	CF test	Bead neut tests[b]			ED_{50}
	CHIK 168	CHIK 168	CHIK 168	C-266	BAH-306	
Lot 1-FI	20	8	11	8	9	0.17
Lot 1-TE	20	4	14	8	11	0.17
Lot 2-FI	10	4	10	0	9	0.17
Lot 2-TE	20	16	14	9	11	0.24

[a]Vaccine given in one dose, 0.5 ml/IP.

[b]Neutralization measured as the diameter of the zone of plaque inhibition in mm.

Table 2. Comparison of fluid and freeze-dried FI and TE CHIK vaccines after one and three month storage intervals.

Vaccine[a]	HI test[b]		CF test[b]		Bead neut test[b]		ED_{50}	
	1 mo[c]	3 mo	1 mo	3 mo	1 mo	3 mo	1 mo	3 mo
Lot 3-FI fluid	20	10	4	8	10	12	0.03	0.04
Lot 3-FI dry	40	20	8	8	12	11	0.05	≤0.1
Lot 3-TE fluid	20	10	<4	<4	10	9	0.17	0.04
Lot 3-TE dry	10	20	<4	<4	10	12	0.13	0.06

[a]Vaccine given in two doses, 0.25 ml/IP, 7 days apart.

[b]CHIK 168 strain used.

[c]mo = month.

REPORT FROM THE DEPARTMENT OF ZOOLOGY, NORTH CAROLINA STATE UNIVERSITY RALEIGH, NORTH CAROLINA

This project will study wild birds in the winter and spring seasons in suburban habitats, to provide new ornithological data bearing on two aspects of disease: (1) the hypothesis that an arbovirus is brought in from the tropics by migrating birds and then transmitted to the local populations in the temperate regions, and (2) the possibility that avian malaria is maintained in nature by a relapse phenomenon in birds in early spring and then transmitted to other birds by mosquitoes in spring and summer.

Field research was initiated in the Fall of 1968 on a one-year bio-medical grant from North Carolina State University. The work will be advanced and expanded in 1969-1970 by a new research grant from the Department of Health, Education, and Welfare, NIAID, in which T. L. Quay is Principal Investigator and David E. Davis is Consultant.

(T. L. Quay)

REPORT FROM THE ENTOMOLOGICAL RESEARCH CENTER DIVISION OF HEALTH, FLORIDA DEPARTMENT OF HEALTH AND REHABILITATIVE SERVICES

The Florida mosquitoes of the Culex subgenera Melanoconion and Mochlostyrax could formerly be identified to species as larvae or adult males, but not as adult females. After Chamberlain, Sudia, Coleman, and Work (Science, July 17, 1964) had reported the recovery of Venezuelan equine encephalitis from mosquitoes of these subgenera caught in south Florida, it became desirable to have means of distinguishing all the adult females, especially for studies of the natural hosts and the vectoring potential of the various species. Examination of females taken in excellent condition from daytime resting boxes first suggested that useful diagnostic characters might possibly exist. In the course of our studies, some specific features

of the scales and their distribution proved too transitory for the reliable identification of worn specimens, but other characters, especially color patterns of the mesepimeron, were found distinctive even though requiring some experience to interpret. A working key to all seven species, which has now been prepared, is intended especially for the identification of frozen material received from the field. Some engorged specimens from Indian River County, tentatively identified, have been subjected to precipitin tests: Culex (Melanoconion) erraticus appears to be a general feeder on warm-blooded vertebrates; Culex (Melanoconion) opisthopus and Culex (Mochlostyrax) pilosus seem to feed mainly on mammals; and Culex (Melanoconion) peccator mainly on cold-blooded vertebrates. Despite the difficulty found in separating the female of C. pilosus from the others, its unique biology seems to justify its assignment to the separate subgenus Mochlostyrax. The eggs are laid separately, not in a raft as in 4 of the other species, and they hatch, not upon completion of embryonation, but when flooded, though not necessarily the first time this happens. Three of the six species of Melanoconion have been successfully colonized at this laboratory.

Other work with a direct bearing on virology includes several studies either underway or recently completed. (1) Mosquitoes engorged with blood are commonly supposed to move only short distances, but blood-fed females that react with bovine antisera have been found routinely on an island without cattle that is surrounded by marsh. The mosquitoes, which belong to several species including Culex nigripalpus, appear to have flown a total distance of at least one mile after taking their blood meal. (2) The nightly activity of C. nigripalpus, estimated by day-to-day differences in trap catches, has been shown to be closely associated with day-to-day differences in the relative humidity one hour after sunset. The impression obtained is that dry conditions actually suppress female activity while moist conditions favor it. (3) Information on the normal flight habits of different mosquitoes, obtained in part by using different types of collecting methods, is providing important new clues regarding blood-seeking behavior and the selection of hosts. Culiseta melanura, which feeds mainly on birds, may do so mostly because it tends to remain in dense vegetation; Anopheles crucians, which moves into the open, has been found to feed mainly on rabbits; Culex nigripalpus may have an annual pattern of flight behavior that brings it seasonally into habitats where mammals are more accessible than birds. Much more observation and experimentation will be needed to put these hypotheses related to flight behavior on a secure basis. In the field of insect migration, another area of much interest to this laboratory, it was found that when Aedes taeniorhynchus was reared at two different population densities, the adults reared under crowded conditions

had a main flight peak on the day of emergence while those from non-crowded conditions did not. The early flying, potential migrants were lighter in dry body weight and had lower percentages of lipid.

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

Dengue activity in Haiti, W. I.*

Between August and October 1969, 684 human sera were collected from the environs of the Haitian capital, Port-au-Prince, and from a coastal rural town, Gonaive. Hemagglutination-inhibition (HAI) tests with 146 of these sera have revealed HAI activity against dengue type-2, dengue type-3 and St. Louis encephalitis viral antigens. All the sera were negative to VEE antigen in HAI tests (Table 1).

Sera from 12 persons who had suffered clinical dengue-like infections in the recent past displayed high HAI antibody titers (Table 2).

Two dengue type-2 isolates have so far been recovered, one from a febrile 7 year old boy and the other from a febrile 40 year old woman.

These results together with clinical evidence suggest continuing dengue activity in Haiti for at least the past 12 months. This is the first documentation of arbovirus activity in Haiti.

Footnote:

* The HAI tests were conducted in Dr. W. F. Scherer's laboratory at Cornell, New York City, and the reisolation and identification of viruses were done in the Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, D.C.

(A. K. Ventura, N. J. Ehrenkranz and R. R. Cuadrado)

TABLE I

SUMMARY OF HAI TESTS CONDUCTED ON HAITIAN SERA

Age Group in years	Number of sera tested by HAI	% Positive* Dengue 2	% Positive Dengue 3	% Positive St. Louis
1-10	14	35%	37%	36%
11-20	13	70%	70%	70%
21-30	50	82%	80%	80%
31-40	28	86%	88%	88%
41-50	24	87%	90%	90%
≥ 51	17	77%	82%	70%
Totals	146**	73%	74%	72%

*Greater than or equal to 1:10 serum dilutions against 8-16 units of sucrose acetone extracted suckling mouse brain antigens.

**All sera were negative against 8 units of VEE antigens in HAI tests.

TABLE 2

HAI RESULTS OF SERA FROM DENGUE PATIENTS FROM PORT-AU-PRINCE

Laboratory number	Approximate time between bleeding and Dengue-like illness	Subject's Age in years	RECIPROCAL HAI TITERS*		
			Dengue 2	Dengue 3	SLE
H44	6 months	44	40	160	40
H50	6 months	53	640	>1280	640
H57	6 months	42	80	320	80
H58	6 months	30	<10	80	<10
H60	6 months	29	640	640	160
H62	6 months	45	640	160	80
H73	6 months	32	320	>1280	320
H61	4 months	34	640	160	40
H66	3 months	30	320	640	320
H72	3 months	27	320	320	40
H65	1 month	18	640	>1280	320
H68	2 weeks	18	320	10	<10

* As determined by antigen titers corrected to 4 units.

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

I. Responses of African Green Monkeys to Unadapted and Mouse-Adapted Type 1 Dengue Virus

The CF responses of animals inoculated orally or subcutaneously with Unadapted virus followed by a booster 90 days later with early, middle, or late passaged virus has been previously reported. The primary and secondary CF responses of the same species of animals to mouse-adapted strains of DEN-1 were also reported in a previous issue. The results show a difference in responses between mouse-passaged and Unadapted virus, especially in regard to primary and secondary responses and persistance of antibody.

The primary and secondary HI responses following Unadapted virus infection were essentially identical to those observed by CF. HI antibodies appeared by day 14, peaked at 21, and persisted for at least 91 days. The lower TH-Sman titers were again evident in the early phase of the primary response. In the secondary phase of infection, HI titers rapidly "tailed off" when the booster inoculum consisted of high or intermediate mouse passage virus. This was not found when MP5 material was used as a booster. Immunodiffusion antibodies were only found in sera collected after the booster inoculum. The responses obtained are apparently related to the mouse level passage and not due to the amount of virus inoculated. Immunodiffusion reactions were only found in those sera obtained after the booster inoculum and only in those monkeys infected with low passaged virus.

In Tables 1 and 2 are demonstrations of the plaque neutralizing antibodies in selected sera of those primates infected with Unadapted virus and boosted with low or high passaged viruses.

Moderate neutralizing antibody titers were present in monkeys initially infected by the subcutaneous or oral routes and a marked anamnestic response was detected following the "booster." The "tailing off pattern" is also shown. Monkeys initially infected with Unadapted virus and boosted with MP5 again showed elevated titers on day 35. In Table 2 are shown the neutralizing antibody responses following mouse-adapted virus. Low passaged virus elicited comparatively high neutralizing antibody titers,

TABLE 1

Inoculum, Route, and Dose		Primate	Time Interval in Days	50% Plaque Neutralization Titers vs 25 - 75 PFU's of Indicated Virus		
Primary	Secondary			Hawaiian MP 124 TC-P6	Vandeever MP 5 TC-P6	TH-Sman. MP 24 TC-P6
Unadapted Vandeever (Acute Phase Serum) SQ $10^{4.8}$ SMLD$_{50}$	MP 124 Hawaiian SQ $10^{7.5}$ SMLD$_{50}$	4	Pre	0 =>40	0	0
			21	200	320	[80]
			91	200	120	140
			2° 8	⩾2560	⩾2560	⩾2560
			35	1440	280	480
Unadapted Vandeever (Acute Phase Serum) Oral $10^{5.2}$ SMLD$_{50}$	MP 5 Vandeever SQ $10^{4.6}$ SMLD$_{50}$	8	Pre	0	0	0
			21	[0]	240	80
			91	110	220	480
			2° 8	1760	⩾2560	⩾2560
			35	⩾2560	1760	⩾2560

PLAQUE NEUTRALIZATION TITERS OF PRIMATES INOCULATED WITH THREE DIFFERENT PASSAGE LEVELS OF DENGUE TYPE 1 VIRUS

Inoculum, Route, and Dose		Primate	Time in Days	50% Plaque Neutralization Titers vs. 27 - 75 PFU's of Indicated Virus		
Primary	Secondary			Hawaiian MP 124 TC-P6	Vandeever MP 5 TC-P6	TH-Sman MP 24 TC-P6
MP 5 Vandeever SQ $10^{4.6}$SMLD$_{50}$	MP 5 Vandeever TC-P5 SQ $10^{5.3}$SMLD$_{50}$	20	Pre	- on test	-	0 =<40
			21	≥640	≥640	[0]
			2° 42	[≥640]	100	50
			11	≥640	480	580
		21	Pre	-	0	-
			21	≥2560	1600	[280]
			2° 42	≥2560	800	1040
			11	≥640	420	≥640
MP 24 TH-Sman SQ $10^{6.5}$SMLD$_{50}$	MP 24 TH-Sman TC-P5 SQ $10^{4.9}$SMLD$_{50}$	17	Pre	-	0	-
			21	0	0	0
			2° 42	40	0	0
			11	160	80	250
		18	Pre	-	0	-
			21	0	0	0
			2° 42	110	50	0
			11	≥640	200	140
MP 125 Hawaiian SQ $10^{7.5}$SMLD$_{50}$	MP 125 Hawaiian TC-P6 SQ $10^{5.0}$SMLD$_{50}$	1	Pre	-	0	-
			21	0	0	0
			2° 42	0	0	0
			11	0	0	0
		3	Pre	-	0	-
			21	0	0	0
			2° 42	0	0	0
			11	40	0	0

TABLE 2

whereas intermediate passaged virus elicited antibody of low titer during the late phase of the primary response and a moderate titer after the booster. The high passage line elicited no detectable antibody during the primary phase and little or no antibody after the booster.

These results, taken as a whole, would indicate that the intrinsic property or properties of the virus to initially sensitize and to elicit a sustained booster response appears to diminish upon increased serial passage in mice. The phenomenon appears to be independent of virus dose. The primary dose of Unadapted and low mouse-adapted virus was approximately 5.0 logs, whereas the intermediate and high passage inocula were 32 to 1000-fold higher. The booster doses used were essentially the same.

Further studies were carried out on the role mouse passage plays on oral infection. Attempts were made to infect monkeys utilizing MP5 material (which was derived from the Unadapted strain and which, as has been stated, infects monkeys by the oral route). Utilizing $10^{6.7}$ $SMLD_{50}$ we were unable to invoke infection in monkeys with such material. It is apparent that by adaption of Type 1 virus to suckling mouse brain, a loss of infectivity for the primate by the oral route seems to occur.

II. Cross Immunity Pattern of Dengue Virus in Mice as Demonstrated by Direct Challenge Experiments

As an extension of the findings previously reported, the intracerebral cross-resistance pattern to dengue types following primary immunization 6 months earlier with Unadapted or early mouse passage was studied. The results presented in Table 3 indicated that cross-resistance to TR-1751 and Zika was lost, whereas it remained for the other dengue viruses. No protection was found when Spondweni and Wesselsbron were used as challenge viruses at six months.

The role of the primary immunizing virus dose was also assessed. Graded dilutions of MP6 DEN-1 virus representing 10^5, 10^3, and 10^2 $SMLD_{50}$ were employed, and the animals were challenged six weeks later with fully mouse-adapted viruses as indicated in Table 4. The results show that as little as 10 $SMLD_{50}$ of DEN-1 protects only against challenge with the homotypic virus, whereas 1000 intracerebral $SMLD_{50}$ immunizing doses were necessary to protect against TH-Sman, NG"C", TH-36, TR-1751, and DEN-4. Partial protection (50% of the animals survived) was found against Zika; 100,000 $SMLD_{50}$ was necessary to show complete protection against all viruses.

I. Intercerebral immunizing dose: $\geq 10^{2.5}$ Suckling Mouse LD_{50}

Time of Challenge (months)	Group		Results of Intracerebral Challenge with Indicated Mouse-adapted Virus*										
			Type 1 Dengue		Type 2 Dengue			DEN-4					
			MP124	TH-Sman	NG-C	TH-36	TR-1751	H241	Zika	Powassan	JBE	Spondweni	WSL
1	Exper.	Signs**	1/8	0/8	3/8	5/8	1/8	0/8	4/8	7/7	-	-	-
		Deaths	1/8	0/8	1/8	0/8	0/8	0/8	1/8	7/7	-	-	-
		MST***	13.0		19.0				11.0	7.5			
	Control	Deaths	8/8†	8/8	8/8	7/8	8/8	7/8	7/8	8/8	-	-	-
		MST	13.8	14.0	10.5	10.6	11.4	13.7	12.2	8.2	-	-	-
3	Exper.	Signs	0/8	0/8	1/8	4/8	6/8	1/8	6/8	-	8/8	-	-
		Deaths	1/8	0/8	0/8	0/8	2/8	0/8	4/8	-	8/8	-	-
		MST	11.0				19.0		11.7		8.3		
	Control	Deaths	5/5	5/5	5/5	4/4	5/5	4/5	5/5	-	5/5		
		MST	17.8	12.2	12.0	12.2	9.5	14.2	9.8		7.9		
6	Exper.	Signs	3/7	0/7	2/7	2/8	8/8	1/8	6/8	-	-	4/4	4/4
		Deaths	3/7	0/7	1/7	1/8	7/8	0/8	5/8	-	-	4/4	4/4
		MST	17.0		12.0	17.0	12.2		12.0			11.5	10.0
	Control	Deaths	8/8†	5/5	5/5	5/5	4/4	5/5	4/4	-	-	5/5	5/5
		MST	15.5	12.6	13.4	12.2	10.8	11.8	10.0	-	-	9.4	10.0

TABLE 3

* 32-320 LD_{50} doses used in challenge unless indicated; number dead or with signs/number challenged

** Weakness or partial paralysis, but recovered.

TABLE 4

Immunizing Virus: Type 1 Hawaiian MP6
(Vandeever Strain)

Virus Dose Used for Infection	Results of Intracerebral Challenge with Indicated Mouse-adapted Virus*								
	Group		Type 1 Dengue		Type 2 Dengue			DEN-4	
			MP 124 Haw.	TH-Sman	NG "C"	TH-36	TR-1751	H241	Zika
10^5 $SMLD_{50}$	Exper.	Deaths MST**	1/8 15.0	0/8	0/4	0/3	0/7	1/3 12.0	0/4
10^3 $SMLD_{50}$	Exper.	Deaths MST	1/6 11.0	0/6	0/4	1/4 18.0	1/3 7.0	0/3	2/4 11.5
10 $SMLD_{50}$	Exper.	Deaths MST	2/6 11.5	4/5 14.0	4/4 11.0	... ...	4/4 9.0	... ...	... ...
None-Bov. Alb.Dil.	Control	Deaths MST	5/5 12.2	5/5 13.4	5/5 9.8	5/5 12.8	5/5 8.8	5/5 12.7	5/5 9/0

Mice inoculated six weeks previously with as little as 15 $SMLD_{50}$ of DEN-3 (Table 5) showed 50% or greater protection to Hawaiian, TH-Sman, New Guinea "C" and TH-36 viruses when challenged. No protection was afforded for TR-1751, DEN-4 or Zika viruses. When the immunizing dose was increased to 1500 $SMLD_{50}$, protection against the previous dengue types and DEN-4 was more pronounced. Protection against Zika was partial and against TR-1751 was poor. One had to employ approximately 150,000 $SMLD_{50}$ to fully protect against Zika and TR-1751 challenge. Using the largest immunizing dose (150,000 $SMLD_{50}$), cross protection was also seen against other Group B arboviruses (Table 6): namely, Spondweni (SPO), Usutu (USU), and Ntaya (NTA), but not with Uganda S (UGS), Yellow Fever (YF), St. Louis Encephalitis (SLE), West Nile (WN), Wesselsbron (WSL), Japanese B Encephalitis (JBE), and Banzi (BAN). Similar results were obtained when MP5 of DEN-1 was used.

To determine the role of antibody and the cross protection observed, representative animals in the aforementioned experiment were bled prior to challenge and the sera checked by CF for antibodies against the challenge viruses. The percentage of animals developing antibody was compared to percentage resisting challenge. These results with the dengue are shown in Table 7. As can be seen, the protection observed against the heterologous Group B viruses does not correlate well with resistance to challenge observed. The nature of this cross resistance is currently under investigation. Preliminary studies using Zika MP5 as a primary immunogen and then challenge six weeks later with the various dengues have indicated some unusual relationships which have to be confirmed. Thus, although no protection is offered to challenge with MP 124 DEN-1 (Hawaiian) or Sman, or DEN-2 (TR-1751), protection was noted for DEN-4 and perhaps NG "C" and TH-36. When heterologous Group B viruses were used as challenge inocula, protection was not only afforded to challenge with high passage Zika, but to SPO also. Negative or inconclusive results were obtained with NTA, USU, YF, WSL, WN, BAN, JBE, and UGS.

III. Studies with Singh's Line of Aedes albopictus Cells

A. Dengue

The findings with the dengue viruses in Singh's A. albopictus line are quite striking and indicate definite evidence of multiplication and cytopathology with certain viral types and/or strains (Table 8).

TABLE 5

EFFECT OF INITIAL DOSE OF INFECTIOUS VIRUS (DENGUE 3) ON RESISTANCE TO INTRACEREBRAL CHALLENGE SIX WEEKS LATER WITH MOUSE-ADAPTED DENGUE VIRUSES

Immunizing Virus: Dengue 3 H87
$MP_{24}TC_4MP_2$

Virus Dose Used for Infection	Results of Intracerebral Challenge with Indicated Mouse LD_{50} of Adapted Virus*								
	Group		Type 1 Dengue		Type 2 Dengue			DEN-4	Zika (160)
			MP 124 Haw. (10)	TH-Sman (25)	NG "C" (32)	TH-36 (320)	TR-1751 (320)	H 241 (63)	
$10^{5.2}$ $SMLD_{50}$	Exper.	Deaths	1/4		1/3	1/6	1/5	0/5	0/5
		MST**	9.0		12.0	15.0	9.0		
$10^{3.2}$ $SMLD_{50}$	Exper.	Deaths	1/6	1/6	2/6	1/6	4/5	0/6	3/6
		MST	12.0	2.0	12.5	16.0	13.0		9.7
$10^{1.2}$ $SMLD_{50}$	Exper.	Deaths	2/5	3/6	1/2	3/6	4/6	5/6	6/6
		MST	11.0	14.0	11.0	11.0	8.5	11.0	10.8
None Bovine Alb.Dil.	Control	Deaths	4/4	4/5	5/5	4/4	5/5	5/5	4/5
		MST	11.5	12.2	10.4	11.5	9.4	12.0	10.8

* Number Deaths/Number Challenged.

CROSS RESISTANCE PATTERNS IN MICE TO GROUP B ARBOVIRUSES

ONE MONTH FOLLOWING PRIMARY INFECTION WITH DENGUE 3 VIRUS

Immunizing Virus: DEN-3(H87) $MP_{24}TC_4MP_2$ - $10^{5.2}SMLD_{50}$

Group		Results of Intracerebral Challenge with Indicated Mouse-Adapted Virus*										
		Zika (250)*	SPO (32)	Usutu (20)	Ntaya (25)	UGS (100)	YF (10)	WSL (10)	WN (40)	SLE (250)	JBE (10)	Banzi (10)
Immunized	Deaths**	0/5	0/3	1/4	1/3	3/4	3/4	5/5	5/5	5/5	3/4	5/5
	MST***	-	-	14.0	10.0	8.0	13.2	9.8	7.0	4.6	7.3	7.0
Control	Deaths	4/5	5/5	5/5	5/5	5/5	4/4	4/5	5/5	5/5	5/5	5/5
	MST	10.8	8.0	9.0	10.2	7.0	12.5	9.3	6.8	5.8	8.0	8.2

TABLE 6

* Number of Mouse LD_{50} used in challenge.

** Mortality/Number of mice challenged.

*** Mean Survival Time (days) of animals that died.

RELATIONSHIP OF CF ANTIBODY TO RESISTANCE TO CHALLENGE TO GROUP B ARBOVIRUSES IN ANIMALS INFECTED WITH DENGUE VIRUSES

TABLE 7

Exper. No.	Infecting Virus and Time	% Animals with CF Antibody* Prior to Challenge/% Animals Resisting Challenge with Indicated Virus															
		DEN-1	NG"C"	TR1751	DEN-4	Ntaya	Usutu	SPO	Zika	YF	JBE	WN	Banzi	WSL	UGS	SLE	POW
1	D-1 30-40 days prior	78/88	48/88	26/100	20/100	9/50	0/100	4/100	4/88	9/0	4/0		0/NT**		NT/0		NT/0
2	D-1 90 days prior	67/88	47/100	33/75	47/100	20/[illegible]	20/NT	7/NT	0/50	0/NT	7/0		6/0				
3	D-3 30 days prior	39/86	33/100	11/90	28/83	22/66	11/75	11/100	0/75	11/25	6/25	NT/0	5/0	NT/0	NT/25	NT/0	

*CF Antibody of 1:10 or greater.

**Not Tested.

TABLE 8

EXPERIENCE TO DATE OF DENGUE, JBE, AND SLE VIRUSES
IN *A. albopictus* TISSUE CULTURE

Virus	Adaptation and Titer in Y15*			CPE, Incubation Period (days) and Titer* in *A. albopictus*		
Dengue 1	Unadapted (Haw)	HEK_1	≥5.0†	3+	7	...
	Serain VD	HEK_1^1 $Albo_4$		4+	2	OT**
	Unadapted (Haw)	HEK_1	4.1†	<1+	[illegible]	
	Serain Harr.	HEK_1^1 $Albo_4$		2+	2-3	OT
	Hawaiian	MP_5	2.5	3+	6	5.5
		MP_5 $Albo_4$		4+	2	OT
		MP_5 Y_5	3.5	1-2+	8	4.5
	Hawaiian	MP_{124}	4.5	±-+	14	<1.5
		MP_{124} $Albo_4$			Negative	
		MP_{124} Y_6	3.5	±	9	1.5
	TA-Sman	MP_{24}	3.0		Negative	
		MP_{24} Y_5	5.0		Negative	
Dengue 2		Human Serum	0.0	2+	5	3.5
	N.G. "C"	MP_{26}	5.0	3+	6-7	≥6.5
		MP_{26} $Albo_4$		4+	2-3	OT
		MP_{26} Y_5	5.0	3+	6	6.0
	TA-36	MP_{24}	4.5	3+	10	≥5.5
	TA-1751	MP_{62}	4.0	2+	10	6.0
Dengue 3	H-87	MP_{29}	4.0		Negative	
		MP_{29} Y_6	2.5		Negative	
Dengue 4	H-241	MP_{27}	5.0		Negative	
		MP_{29} Y_6	4.5		Negative	
JBE	Nakayama	MP_{44}	7.0	4+	3-5	≥9.5
SLE	Hubbard	MP_{124}	3.7		Negative	

* All titers/0.1 ml $\log_{10}$

† $SMLD_{50}$/0.1 ml

** On Test

Unadapted strains of Type 1 and Type 2 dengue viruses in the form of acute phase human serum produce marked cytopathology (multinucleation) with eventual destruction of the cell sheet. Subsequent passage appears to increase the virulence as indicated by a marked decrease in incubation period to time of onset of cytopathology and increased titer. There is evidence of auto interference if large doses of virus are used in passage. Early mouse-passaged Type 1 virus (MP5) also propagates well in the Albopictus cultures. Virus titers appear to be 10 to 100-fold higher than those found by titration in mice. On the other hand, Type 1 virus seed stocks derived from late-passaged mouse material (MP124) failed to produce marked cytopathology in Albopictus cells despite numerous attempts. Whether multiplication occurs has not been determined. TH-Sman, DEN-3, and DEN-4 viruses derived from infected mouse brain or tissue culture (Y15) sources also failed to produce cytopathology in this line. These results are in contrast to the behavior of these viruses in Y15 where cytopathology end point titers can be obtained.

Type 2 adapted strains (NG "C", TH-36, and TR-1751 - whether mouse or Y15 derived) all produce marked cytopathology and propagation in Albopictus cultures which can be subpassaged. Titers achieved are greater than those found in mice or in Y15 tissue cultures. Mouse-adapted Nakayama JBE virus is also markedly virulent for this cell line; on the other hand, the Hubbard strain of SLE which had been passaged in mice produces no evidence of cytopathology.

These results would indicate that the Albopictus line is exquisitely sensitive for Unadapted or early-passaged Type 1 viruses, but not for mouse-adapted or tissue culture-adapted strains of DEN-3, DEN-4, Hawaiian, and TH-Sman. Regardless of the source, Type 2 viruses apparently can propagate well. Examination is being made to determine if the differences observed with the Type 1 strains are due to biological changes after passage in mice.

B. Recovery of a "Group A" (Chikungunya-like virus) from "Normal" Cultures of Singh's Line of A. albopictus Obtained from Dr. Suitor's Laboratory

The late Earl Suitor et al had a note in a recent issue of Virology (38:482, 1969) concerning the use of the A. albopictus cell line for titration of dengue. In the article they made the point that the line should be carried on glass containers and when put on plastic containers the "morphology" of the cell line changes. They went so far as to suggest the presence of virus from EM data. We received this glass line from Suitor's laboratory in September 1969 at the 70th passage and confirmed their findings. How-

ever, we scraped off the cells which showed "cytopathology," prepared a cell pack, and passed it intracerebrally into suckling mice.

CNS signs were observed between 48 and 72 hours. A further passage was made in suckling mice which brought them down in 24 to 48 hours. A seed prepared from the 2nd passage was titrated in suckling mice and weanling mice by the intracerebral route. This material titered $10^{-8.0}/0.02$ ml in baby mice with incubation time at all dilutions of 5 days or less. The agent was only irregularly pathogenic for weanling mice at all dilutions, but there was some evidence of an "interference type" of phenomena at the most concentrated dilutions tested. A KCl-Borate antigen was prepared. In a "box" titration a CF antigen titer of 8 to 16 was obtained against 16 units of NCDC polyvalent Group A burro serum, and 16/16 vs. mouse IAF for Chikungunya prepared by YARU. An HA titer of 1:8 at pH 6.0 only and at 37° C was found. When tested by HI vs. the polyvalent Group A burro serum, polyvalent Group B monkey serum (NCDC), and YARU Chikungunya mouse IAF, a HI titer of 1:40 was found only with the polyvalent Group A antiserum.

Tests carried out on the line as it is carried on glass only revealed no evidence of cytopathology; however, cell packs are pathogenic for suckling mice to a titer of $10^{-3.6}/0.02$ ml. A simultaneous titration of first passage of this glass line onto plastic revealed a titer of $10^{-3.3}/0.02$ ml.

We wish to point out that our laboratory is not working with Chikungunya nor with other Group A virus. Whether all the Singh's Albopictus lines that are in use in the United States laboratories are contaminated is unknown. Suitor's laboratory has in the past worked with Chikungunya. They received their line from Dr. Buckley at YARU, who originally obtained it from Singh.

To further complicate the matter, we also have another line of Singh's Albopictus which we received from Dr. Suitor's laboratory in August 1968 at passage 63. This line has been adapted to grow in plastic containers and is currently in the 160th passage. Thus far tests for the presence of an agent pathogenic for suckling mice by the intracerebral route of this plastic line have been negative with cell pack material.

IV. Immunological and Serological Studies with Dengue and Sandfly Fever Viruses

A. Dengue

Attempts to prepare type or strain specific CF or HI antisera or mouse IAF by infection or hyperimmunization have failed when such reagents were

tested in the conventional manner. The failure of 1 or 2 injections may indicate that common (group) antigen(s) in dengue is likely to be the major and/or more immunologic antigen for mice so that the common antibody(s) appear first. It may be that two or more injections are necessary for the more specific and/or type specific antigens to stimulate the production of antibodies. Perhaps the specific antigens are either minor antigens, or less immunogenic, or both.

We have begun a careful examination of the value of immunodiffusion methods for typing of dengue. Sera of monkeys inoculated with single or multiple doses of Unadapted, Mouse, or Tissue Culture-adapted strains of Type 1, 2, 3, and 4 were tested by immunodiffusion (ID) methods using sucrose-acetone extracted infected mouse brains as antigens.

All monkeys failed to develop precipitin antibodies 21 to 42 days following a single inoculation of any dengue strain or type even though CF, HI, and neutralizing antibodies were present. Multiple inoculations of animals with Mouse-adapted strains of Type 1 Hawaiian (MP124), TH-Sman, DEN-3, or DEN-4 also failed to produce ID reactive antibody. On the other hand, animals which received at least two inoculations of MP5 derived Type 1 virus or were initially infected with Unadapted virus and boosted with any Mouse-adapted strain of dengue Type 1 did develop type specific precipitin antibody. This reaction could be observed only when Hawaiian MP124 or TH-Sman ID antigens were employed. We have not as yet been able to produce an ID antigen from early mouse-passaged Type 1 dengue virus despite the fact that the same material has both CF and HA activity.

Animals which received multiple inoculations of Mouse-adapted Type 2 strains all produced ID antibody. The sera from these animals inoculated with TR-1751 were type specific. In some cases of animals inoculated with New Guinea "C" or TH-36 viruses, the ID antibodies appeared to be strain specific as well.

Type specific ID immune ascitic fluid (IAF) or sera can be produced by hyperimmunization of mice for Type 1 dengues. In some instances, the sera or IAF is strain specific as well. The same is probably true for TH-36, TR-1751, and New Guinea "C" strains of Type 2 viruses. Type specific ID antisera for DEN-4 has also been produced. Thus far, we have not been able to produce reactive IAF or sera for DEN-3 in the mouse. As stated above, and in general, such sera or IAF produced in mice is not type specific or strain specific in HI, CF, or neutralization testing procedures. To obtain specificity one has to select very carefully the IAF or sera from individual animals.

B. Phlebotomus Fever Viruses

It was possible to produce mouse IAF against each of 11 different members of the Phlebotomus fever group. These reagents reacted monospecifically with their homologous antigens in immunodiffusion. Immunization of rabbits or hamsters with infected mouse brain of various members of the group resulted in antisera which reacted not only with the homologous virus preparation but also with all heterologous members of the group, as well as with EEE, WEE, WN, DEN-2, and CE. It was easy to identify the homologous reaction since it appeared as an additional strong precipitin line. By various experimental procedures it was found that the cross reaction observed was not due to normal mouse brain, Forsman, or heterophile antibody, or "C"-reactive protein-like substances. Precipitation of the antisera with 34% ammonium sulfate removed all the ID antibody, indicated its belonging to the globulin class. Adsorption of the antisera with either WN or WEE, a heterologous sandfly fever virus, or a combination thereof, removed the common line, and the specific line remained. Studies carried out thus far indicate that the common line may be due to an antibody or antibodies produced against a viral-induced modified brain antigen which reacts with most, if not all, arbovirus infected mouse brain antigens.

In order to determine if the reactions were specific to arbovirus or due to non-viral inflammatory reactions, brains of mice which had been infected with Coxsackie virus or inoculated with starch or bovine albumin were tested with the rabbit sera. No reaction appeared, suggesting that the cross-reacting (?) antibody appears to be in response to arbovirus modified brain antigen. Further studies are under way to elucidate this hypothesis.

(B. H. Sweet, A. Ibrahim, J. Hatgi)

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

The first year of arbovirus studies in the metropolitan area of Houston, Texas has been largely devoted to the selection of mosquito collection sites and to attempted virus isolation from mosquitoes collected from these sites. The principal study area selected encompasses 4.5 square miles in northwest Houston and contains approximately 6,000 residents. This area has no municipal water supply or sewer system. Dogs and chickens are present in large numbers as well as pigs, cattle, sheep, goats and horses. A second study site is the more centrally located Houston Arboretum. In addition to these two areas, mosquito resting sites widely scattered throughout metropolitan Houston were also regularly sampled. Mosquitoes were collected with the use of miniature CDC light traps baited with dry ice, by resting capture, and by human-biting capture. While all mosquito species collected were processed for virus isolation, only those collected in large numbers and yielding virus isolates will be described.

A total of 88,828 Culex quinquefasciatus (including 69,776 from June through September, 1969) was collected and processed in 1,772 pools from September, 1968 through September, 1969. Of these 1,772 pools, 47 contained Flanders virus. Flanders virus was also isolated from 6 pools of Culex restuans, 1 pool of Mansonia perturbans, and 2 pools of Culex salinarius.

From September, 1968 through September, 1969 8,246 Aedes atlanticus-tormentor were processed in 185 pools. Of these, 7,325 were collected in May and June, 1969. Virus belonging to the California complex was isolated from 21 of these pools. Crude antigen from each of these strains fixed complement in the presence of San Angelo hyperimmune ascitic fluid. One strain selected as a prototype reacted identically to a California strain isolated in Houston in 1966 in reciprocal cross complement fixation tests utilizing sucrose-acetone antigen and hyperimmune ascitic fluid. Ten of the eleven positive mosquito pools for the month of May were from 3,484 Aedes atlanticus-tormentor collected in dry ice baited light traps which were operated in the Arboretum the night of May 8-9. On May 8 a human biting capture represented by 4.6 man hours was also made in the Arboretum. During this time a total of 363 mosquitoes came to feed on man, and of this number 326 were Aedes atlanticus-tormentor. The high California virus infection rate in mosquitoes and the avidity of this species for man suggests

that residents and visitors to the area are exposed to relatively high risk of acquiring disease.

Two isolates of Cache Valley-like virus were made from a group of 3,159 Culiseta inornata processed in 41 mosquito pools. One was from a pool of mosquitoes collected in October and November, 1968, and the second from a pool collected in February, 1969. In addition, one Cache Valley-like strain was isolated from a pool of 56 Anopheles quadrimaculatus collected during August, 1969.

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

Arbovirus research is now underway at the new Department. Owing to limitation of funds no field projects have been as yet initiated. Fundamental investigations on the biochemistry of St. Louis encephalitis (SLE) virus (conducted for the previous 4 years at Brigham Young University by Dr. Dennis Trent) are now in progress here.

SLE virus infection of BHK-21/13 cells results in an inhibition of cellular RNA and protein synthesis. Virus-specific RNA synthesis is membrane-bound and has a biphasic cycle of replication. Most of the RNA synthesized prior to the onset of virion maturation is incorporated into virus-specific free cytoplasmic ribosomes which are active in the synthesis of viral proteins. Virus-specific polysomes begin to appear in the cytoplasm of the infected cells as early as 6 hr PI and are demonstrable as late as 18 hr PI. SLE viral polysomes are slightly heavier than those found in uninfected cells and are labile to treatment with ionic and non-ionic detergents. Three species of viral-RNA are associated with the virus-specific polysomes; a 43S ribonuclease-sensitive form identical to virion RNA and the 20S and 26S ribonuclease-resistant species which are presumably the replicative forms.

SLE virus labeled in the protein moiety with ^{14}C-amino acids was prepared in roller bottle cultures of PS cells. Virus was concentrated and purified by ammonium sulfate precipitation, magnesium pyrophosphate gel adsorp-

tion and collection of the virus on a sucrose interface after high speed centrifugation. Isopycnic gradient centrifugation of the virus in potassium tartrate resulted in separation of virus particles with 2 different densities. More than 99% of the infectivity and radioactivity was associated with the more rapidly sedimenting complete virions at a density of 1.228. Very little infectivity was associated with the slower sedimenting incomplete virions which have a density of 1.197. Complete virus dissociated with SDS, mercaptoethanol, and urea was analyzed by electrophoresis in 8.5% acrylamide gels. Electropherograms of ^{14}C-proteins from complete virions contained 3 peaks of radioactivity, 2 small rapidly moving peaks (VP-2, 3) and a larger slower moving protein VP-1. Approximately 46% of the radioactivity was found in VP-1, 34% in VP-2 and 22% in VP-3.

Complete purified SLE virus, doubly-labeled with ^{14}C-amino acids and ^{3}H-uridine, was treated with DOC to solubilize the envelope and subjected to isopycnic centrifugation. After DOC-treatment over 70% of the radioactive viral protein and 95% of the labeled RNA cosedimented with the "core" particle at a density of 1.301. Approximately 30% of the ^{14}C-amino acid label from the complete virus was released by DOC treatment and sedimented near the top of the gradient. The location of the structural proteins was determined by coelectrophoresis of ^{14}C-labeled proteins from the core particle with tritium-labeled dissociated proteins from complete virus. The ^{14}C-labeled proteins from the "core" particle comigrated with the 2 more electrophoretically mobile proteins from the complete virus. Treatment of complete virus with DOC solubilized the VP-1 containing lipoprotein envelope which is responsible for the biological activities of HA and adsorption to animal cells.

We have previously reported that 8 hr after infection the rate of protein synthesis exceeded the rate in the non-infected controls; however, identification of virus-specific proteins was impossible because of the background of cellular synthesis. Protein synthesis in virus-infected and non-infected cells was inhibited by more than 90% in 30 min by cyclohexamide. Synthesis of cellular proteins in actinomycin-treated infected and non-infected cultures was irreversibly inhibited by cyclohexamide. Addition of cyclohexamide for 1 hr at any time during the first 11 hr prevented the formation of complete virus or significant amounts of viral RNA or protein. When cyclohexamide was added after the onset of virus maturation the synthesis of viral RNA and protein were reversibly inhibited although virion formation was irreversibly inhibited by 99%. Cultures were infected in the presence of actinomycin and at 11 hr PI pulse-inhibited for 1 hr with cyclohexamide and then labeled for 6 hr with ^{14}C-amino acids. Cytoplasmic extracts from infected and non-infected ^{3}H-amino acid labeled cultures were mixed,

solubilized and analyzed by electrophoresis. The electrophoretic pattern of ^{14}C-labeled proteins from SLE infected cells did not correspond to any of the proteins from the infected cells. Seven major bands of radioactive protein were reproducibly detected in the cytoplasm of the infected cells. The identification of the viral core and membrane proteins in the infected cells was accomplished by coelectrophoresis. Coincidence of the ^{14}C-labeled cell-viral structural and ^{3}H-labeled cellular protein peaks was obtained. At least 6 proteins observed in the infected cells differ in their electrophoretic mobility from the virion structural proteins. The total amount of virus structural protein synthesized from 12 to 18 hr after infection was approximately 7% of the total protein synthesized in the infected cell. From the estimated molecular weight of each protein the total molecular weight of the polypeptides coded for by SLE RNA is estimated to be 5.3×10^5 daltons. This value is approximately that which theoretically could be encoded for by the viral RNA. Recent experiments using pulse-periods of varying length, pulse-chase techniques and protein inhibitors indicate that the polypeptides found in the cytoplasm of SLE infected cells are stable primary products of translation.

Other arbovirus projects are being initiated, including an inquiry into the morphogenesis of group B viruses.

(D. Trent, A. Shelokov)

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

Arboviral encephalitis remained at a low level of incidence in 1969. Laboratory confirmed or highly presumptive cases in the past 3 years since the SLE outbreaks in 1966 are shown as follows:

Year	WE	EE	SLE	CEV	VE
1967	4		2		
1968	5		1		
1969	2				

In assisting in the clinical differentiation of arboviral encephalitis and enterovirus "associated encephalitis" in infants or children. Coxsackievirus B3, Echovirus 9 and Echovirus 30 were incriminated; the latter was recovered from brain tissue.

From pools of approximately 102,000 mosquitoes (mostly culicines) 46 viral isolates obtained from May through August are shown as follows:

	No. of Isolates	Locality	Month
Flanders (Hart Park)	40	Scattered	May-July
Bunyamwera group (Tensaw)	1	Dallas	July
WE	2	Lubbock	July-August
Turlock	2	Lubbock	July-August
CEV*	1	Houston	May

* Mosquitoes were collected by the Harris County Mosquito Control District and tentative viral identification was done by the City Health Department Laboratory, Houston, Texas.

Chicken and other bird sera from Dallas county were tested for HI antibodies. Serologic conversion was negligible.

WE antibodies were found in horses from a few scattered areas. Three WE infections of horses were confirmed by significant conversion in HI or CF tests. Two moribund unvaccinated horses near Beaumont in Southeast Texas in October showed 1:80 and 1:320 HI titers for EE. No similar presumptive or confirmed cases of EE had been found since 1957. No evidence of the presence of VE virus has been found.

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

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

Serum specimens, usually paired, obtained in 1968 from 100 suspected arboviral encephalitis cases in Colorado and 34 cases in Hale County, Texas, were tested for the presence of neutralizing antibodies against the LaCrosse strain of California encephalitis virus (CEV). A plaque reduction technique in VERO cell monolayers was utilized for this purpose. Fifteen of the Colorado patients (15.0%) and two of the Hale County patients (5.9%) had antibodies. In no instance was evidence found of an increase in antibody titer in convalescent-phase serum as compared with the corresponding acute-phase serum.

Attempts also were made to obtain CEV isolates from mosquitoes collected during 1968 in Hale County, Texas. For this purpose, mosquitoes with known preference for mammal feeding were tested by plaque-production methods using VERO cell monolayers. One CEV isolation was obtained from 108 pools (5,089 individuals) of Aedes nigromaculis and one from 29 pools (1,312 individuals) of Psorophora signipennis; testing of mosquito pools is incomplete. The two isolates were strongly neutralized by mouse immune ascetic fluids (MIAF) prepared with CEV types LaCrosse and BFS-283. Only slight neutralization was achieved with MIAF prepared with either Jamestown Canyon or Trivitattus types.

Two Bunyamwera Group viruses were isolated from 29 P. signipennis pools. Based on plaque reduction tests, each isolate appears closely related to Main Drain virus. The latter agent previously has been isolated only from Culicoides variipennis and Lepus californicus in California. No virus was recovered from 172 pools (8,158 individuals) of Aedes vexans, or 14 pools (476 individuals) of Culiseta inornata.

A total of 473 nestling house sparrows (Passer domesticus) were bled for virus isolation. Twenty-four WE and three Turlock virus isolates were obtained along with an unidentified virus.

Dr. Michael P. Earnest, EIS Officer, Arboviral Disease Section, headed a team of researchers in a cooperative follow-up study of WE cases in Hale County, Texas. Twenty-three cases and 23 matched controls underwent extensive neurologic, psychologic, and intelligence testing to ascertain if there were subtle residua that affect learning ability. Preliminary results indicate that at least five of the cases suffered significant residual brain damage, and that the affects probably are of an "all or none" nature. Dr. Harold A. Goolishian, Chairman, Division of Psychology; Dr. Linda J. Smith, Neurology Department, and three graduate students of the University of Texas Medical Branch in Galveston, worked with Dr. Earnest in facilities provided by the Plainview-Hale County Health Department from June 23-27, 1969.

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

In order to answer the various questions raised since the successful isolation of EEE from a South Dakota pheasant encephalitis outbreak in 1967, we expended our research efforts to determine various arbovirus activities in South Dakota. Due to flood in plains in 1969 summer we expected a larger hatch of mosquitoes and we did trap 100,000 mosquitoes in the summer of 1969. Mosquitoes were trapped from July 14, 1969 to September 17, 1969 (summer is short in South Dakota). There were 16 separate collection sites in the vicinity of Brookings, Sioux Falls, Huron, Chamberlain and

Watertown at which we trapped mosquitoes using CDC mosquito trap with CO_2 baiting. There were 40 different trap nights on which mosquito traps were set in the various Eastern South Dakota farmsteads and near Sioux river on farms with horses, chickens, cows and pigs. With the assistance of Dr. John Rowe, Chief of NCDC services for region 6 at Kansas City, Mo., we have now identified 13,397 mosquitoes. (See Table 1.) Nineteen species of mosquitoes have been detected. Aedes trivitattus (37%), Aedes vexans (22.5%), Culex tarsalis (30.6%) constitute 3 major predominant species of Eastern South Dakota, making a total of 90% of mosquitoes identified. So far, we have prepared 255 mosquito pools and out of which have inoculated newborn mice (I.C.) with 61 pools and have tentatively found viral agent in 10 pools. These isolated agents need to be identified and characterized. Further research work in mosquito identification and virus isolations from mosquito pools is under investigation.

Epizootic Hemorrhagic Deer Disease Study

Epidemiological research on Epizootic Hemorrhagic deer disease (EHD) was conducted during 1966, 1967 and 1968 to obtain further information on: (1) distribution of the Disease in South Dakota according to counties, (2) occurrence in deer according to sex and age.

Analysis of EHD Serological Results for 1966, 1967, 1968

Among 380 samples of blood serum (one sample per animal) collected during the three year period (1966-68) from white tailed (WT) and mule deer (MD), 79 or approximately 20 per cent tested were positive for EHD (see Table 2). This indicated that, on the average, one among every five animals had contracted the disease in the field.

When all of the three-year WT and MD data for adults and young are combined (Table 2), we find a lower percentage (12%) of young deer (less than one year of age) than adults (22%) containing EHD antibodies. These results have two possible implications, first in a majority of cases, young deer may not acquire the disease until they are one year old; and second, the mortality rate in young deer is high and therefore sera samples show a lower percentage of young deer positive.

During the three-year period, 380 deer (WT & MD) sera samples were collected in 18 counties. EHD was present in 14 among 18 South Dakota counties according to deer sera tests. Pennington (55/14) and Harding

TABLE I

SOUTH DAKOTA MOSQUITO IDENTIFICATION AND PREPARATIONS FOR V

NAME	TOTAL	% OF TOTAL
1. Aedes trivittatus	5001	37.34
2. Culex tarsalis	4096	30.58
3. Aedes vexans	3012	22.49
4. Aedes species	868	6.48
5. Culex salinarius	119	.89
6. Aedes dorsalis	98	.73
7. Aedes sticticus	56	.42
8. Aedes triseriatus	37	.28
9. Anopheles punctipennis	27	.20
10. Culex restucins	25	.19
11. Culex species	24	.18
12. Aedes nigromaculus	6	.05
13. Anophelis walkeri	7	.05
14. Culiseta inornata	6	.04
15. Aedes flavescens	5	.03
16. Culex pepens	2	.01
17. Anopheles quadramaculatus	1	.01
18. Mansonia pubertans	1	.01
19. U-SAPPH Uranotenia sapphirima	1	.01

Total 13,393 Mosquitoes

TABLE 2

DETECTION OF EHD ANTIBODY IN SOUTH DAKOTA

1966, 1967 and 1968 White-Tail and Mule Deer Sera

	Young		Adult		
County	Male	Female	Male	Female	Total
Beadle			1/1		1/1
Brown	3/0	6/0	3/0	10/0	22/0
Brule		1/0	3/3	3/1	7/4
Buffalo			3/1		3/1
Butte			10/3	4/2	14/5
Custer			2/1	20/4	22/5
Day			4/1	1/0	5/1
Fall River	2/0	3/0	5/1	5/1	15/2
Hand			4/0	2/0	6/0
Harding	2/0	2/0	16/5	22/5	42/10
Lawrence	10/2	13/2	31/5	29/6	83/15
Lyman		1/0	1/0	4/0	6/0
Marshall		1/0		1/0	2/0
Meade	1/0	1/0	5/1	8/3	15/4
Pennington	2/1	4/1	14/4	35/7	55/14
Perkins	5/0	8/2	35/6	21/4	69/12
Sanborn			3/3	5/2	8/5
Walworth	1/0		2/0	2/1	5/1
Total	26/3 (11.5)	40/5 (12.5%)	142/35 (24.6)	172/36 (20.9)	380/79(2
	MALE	168/38 (22.6%)		YOUNG 66/8	(12.0%)
	FEMALE	212/41 (19.2%)		OLD 31.4/71	(22.25%)

No. of Samples Tested/No. of Samples EHD Antibody Positive

(44/10) counties had a higher percentage of EHD infected deer than Lawrence (83/15) and Perkins (96/12). (Table 2.) In addition to the above four counties, EHD infected deer were found in Butte, Custer, Fall River and Meade counties. Among South Dakota white tailed deer, EHD was most prevalent in Custer, Lawrence, Pennington and Perkins counties whereas among mule deer, the disease was more common in Harding (33/9), Pennington (16/14) and Perkins (119/1) counties.

(G. C. Parikh)

REPORT FROM THE ROCKY MOUNTAIN LABORATORY OF THE NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Argasid-tick viruses

1) An agent, RML 52451, was isolated from Argas cooleyi associated with swallows and bats in Texas. Six of 10 pools of this species yielded viruses in Vero cell culture, whereas viruses were recovered in suckling mice from only 4 of these pools. These isolates produce plaques in Vero cell monolayers, which are neutralized by antisera to Ar 861 from California and Ar 883 from Peru (both isolated from argasid ticks by Dr. Harald Johnson). Because of these facts cell culture appears to be a more efficient system than mice for the isolation and identification of this agent. The occurrence of this arbovirus of the Chenuda-Kemerovo group in the inland U. S. and well removed from colonies of marine birds is unique. This work is being done in collaboration with Dr. John George, Texas Technicological College, Lubbock.

2) A very large colony of Ornithodoros sp. nr. denmarki and a small one of Ixodes uriae was discovered on an island bird refuge off the northern coast of Oregon. This represents the northernmost distributional record in North America for members of the O. capensis group, which are vectors or reservoirs of viruses of the Hughes and Quaranfil groups and of other ungrouped ones such as Upulu Cay and a Nyamanini variant. Whereas no viruses could be recovered from the Ornithodoros ticks on this island, an

unidentified agent was isolated in suckling mice from the Ixodes uriae. This agent, RML 52481, is now being characterized. It fails to affect Vero or BHK-21 cells but is pathogenic for suckling mice by the i.p. and i.c. routes and for adult mice upon i.c. inoculation.

3) Twenty-one tick-borne viruses, mainly from argasid ticks, are being studied for ability to produce CPE or plaques in vertebrate cell cultures. Both CPE and plaques were produced in Vero cells by Hughes, Raza, Nyamanini (2 strains), Midway, Johnson Atoll, Quaranfil, RML 52451, and Chenuda viruses. Green Kure, Metucare and Ar 861 viruses caused slight to moderate CPE, but not plaques, in Vero cells. Ar 888 and Ar 883 viruses produced plaques in Vero cells, but CPE was not evident in liquid phase cultures. Sapphire and RML 52481 viruses failed to visibly affect Vero cells. Studies of these and other tick-borne viruses are being continued in a porcine kidney cell-line obtained from NBL, Oakland.

(C. M. Clifford and C. E. Yunker)

REPORT FROM THE DEPARTMENT OF MEDICAL MICROBIOLOGY, UNIVERSITY OF UTAH, SALT LAKE CITY, UTAH

California Encephalitis Virus

Plaque Cloning and Some Growth Characteristics in Primary Chick Embryo Cells

After a strain of California encephalitis virus (CEV-DI) was cloned to a point where a single plaque type was produced, stock mouse brain suspensions were prepared and titered by suckling mouse LD_{50} and also by plaque assay. Neutralization tests showed complete reduction of plaques. As a preliminary source of information a growth curve was determined on chick embryo cell monolayers. The results of the experiment provided positive evidence that at least one member of the California group is able to replicate in primary chick embryo cells with subsequent release of progeny virus into the surrounding medium.

Materials and Methods

Virus: The virus strain employed was isolated by personnel at Dugway Proving Ground and identified serologically as a CEV member; this was also confirmed by the National Communicable Disease Center (CDC) at Atlanta. The virus was designated CEV-DI.

Antisera: Rabbit antiserum against CEV-DI and BFS-283 was obtained from Dugway Proving Ground and goat antiserum against the prototype BFS-283 was obtained from CDC.

Mouse brain suspension: The technique of Clarke and Casals (1958) was employed and has been described previously (Crookston, 1968).

Monolayer preparation: The treatment of chick embryos for monolayer preparation was described previously (Crookston, 1968) and was the same with one exception: The cells were brought to a final concentration of 1×10^6 cells per ml and 5 ml added to 60 mm plastic Petri dishes (Falcon); 15 ml of this same cell suspension was added to 160 ml capacity milk dilution bottles (Pyrex) for monolayer production in these containers. The cell cultures were used 48 hours after preparation.

Overlay medium: Eagle's Minimum Essential Medium (MEM) with 5 percent calf serum was used in a 2X concentration and mixed with an equal volume of agarose at 0.8 percent (final concentration, 0.4 percent). The final concentration of sodium bicarbonate was 0.35 grams per liter.

Cloning procedure: Plugs were removed from plaque centers by sterile capillary pipette and mixed with 0.4 ml phosphate-buffered saline with 0.2 percent bovine serum albumin. This mixture was frozen, thawed rapidly at 37°C and inoculated intracerebrally into 2-3 day old suckling mice. 10 percent suspensions were prepared from the infected mouse brains and this material was diluted and plated out for plaque production. This procedure was repeated three times before litters of suckling mice were inoculated for subsequent preparation of stock virus.

Neutralization tests: Virus dilutions containing approximately 40 and 400 plaque forming units (PFU) per 0.5 ml were mixed with equal volumes of 1:10 dilutions of heat-treated (56°C for 30 minutes) normal and immune sera; these mixtures were incubated in a water bath for 30 minutes at 37°C before inoculation.

Growth characteristics studies: Cells in five milk dilution bottles were washed twice with cold PBS containing 100 units penicillin and 100 micrograms streptomycin before infection. An inoculum of 1×10^7 PFU contained in 1 ml was added to each bottle to give an approximate multiplicity of infection of one. After an adsorption period of 60 minutes the infected cells were washed once with PBS and antibiotics and then 10 ml of fresh Melnick's growth medium with 5 percent calf serum were added. At various times, a 0.1 ml sample was removed from each bottle, pooled, diluted in 4.5 ml of PBS with bovine serum albumin, and frozen for subsequent assay. The experiment was terminated 50 hours after infection.

Results

The plaques observed before cloning were predominantly 1.0-1.5 mm. in diameter after 60 hours of incubation; a small number of plaques were always present with diameters of 4.5-5.0 mm. Virus from these larger plaques were chosen for cloning. During cloning procedures, plates were incubated for 48 hours instead of 60 hours and the plaques were 3.0-3.5 mm in diameter. After preparation of a 10 percent mouse brain suspension as stock virus a comparison was made between suckling mouse titration (LD_{50} determinations were made by the Reed-Muench method (1938) and plaque assay of the same material; the former giving a value of 2.15×10^8 $_{50}$ per ml and the plaque assay showing 1.95×10^8 PFU per ml.

Neutralization tests were run with rabbit antisera to CEV-DI and BFS-283 and goat antisera against BFS-283. Controls were maintained with normal rabbit and goat sera. CEV-DI was neutralized by all three antisera to the same extent, indicating the possibility of a close antigenic relationship to the prototype BFS-283.

The results of the preliminary growth curve studies indicate that the virus is capable of propagating in chick embryo cells. Table 1 lists the sampling schedule and the PFU's detected in the growth medium at each time. Figure 1 illustrates the characteristics of growth over a 48-hour period.

Discussion

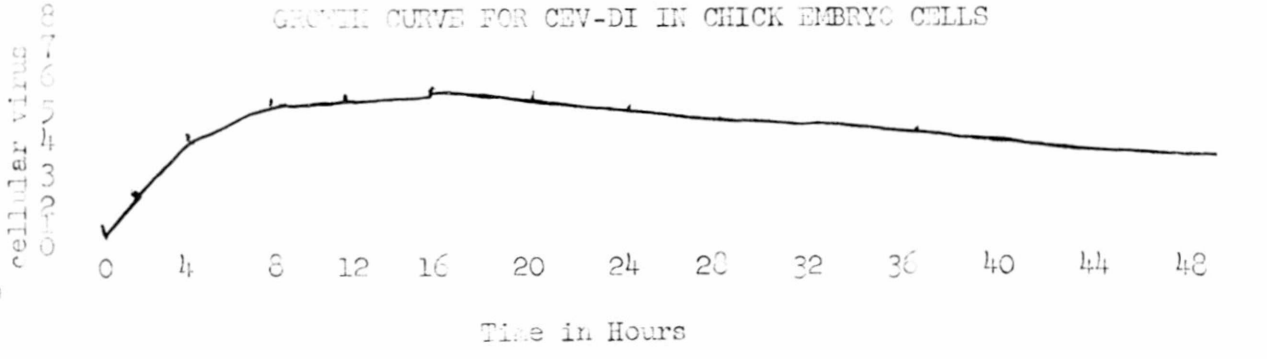
The infection of monolayers by CEV-DI served as a source of preliminary data regarding the behavior of the virus in primary chick embryo cells. The information is of limited significance, quantitatively, because the number of cells comprising a monolayer in the milk dilution bottles varied greatly, e.g., 5×10^6 - 1×10^7 cells per monolayer. The growth curve for CEV-DI showed a strong resemblance to the curves produced by members

Table 1

VIRUS CONCENTRATION DETECTABLE IN THE LIQUID OVERLAY OF INFECTED MONOLAYERS AT INDICATED TIMES AFTER INFECTION

Sample time*	Virus concentration (PFU/ml)
30 minutes	1.15×10^3
60 minutes	8.85×10^2
2 hours	1.38×10^3
4.5 hours	2.39×10^5
8 hours	2.86×10^6
12 hours	6.58×10^6
16 hours	8.05×10^6
20 hours	7.15×10^6
24 hours	2.45×10^6
36 hours	2.28×10^6
48 hours	9.5×10^5

Figure 1



* Time zero considered to be the time growth medium was added.

of the group A arboviruses (Dalrymple, 1968). Further comparison with curves produced by group B arboviruses (Yuill et al., 1968) lends evidence that the behavior of at least one of the California group members is more analogous to group A arboviruses than group B. After a lag or latent period of 1.5-2 hours there was a rapid rise in titer of extracellular virus which approached maximum titer at 12 hours. The virus appeared to be quite stable inasmuch as less than a log in titer was lost during the 36 hours after maximum titer had been reached. The next experiments to be undertaken will make use of infective center assays. Also, there is evidence that successive passes through chick cells results in an approximate one log decrease in titer after each pass. In a subsequent series of experiments Actinomycin D will be incorporated in an attempt to eliminate any cell-directed interference.

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(D. W. Hill)

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

There were 10 cases of Colorado tick fever (CTF) diagnosed in California during 1969, 4 were persons exposed in known endemic areas of California, 3 exposed in Nevada, 2 in Colorado, and 1 in Idaho. In all cases, the diagnosis was confirmed by isolation of the virus in suckling mice and by fluorescent antibody staining of blood smears, using specific immune serum. The latter method is of great value in making a rapid diagnosis of the disease. In one case, the virus was isolated from the washed blood cells from heparinized blood taken 41 days after onset of symptoms, again documenting the unusually long viremia in this disease. Viremia persists long after complete recovery from symptoms and development of antibody. In previous years, viremic periods of up to 102 days have been confirmed in cases of CTF. The virus occurs intracellularly, largely in the erythrocyte fractions of the blood, and circulates for a long period, protected from neutralizing antibody.

A study was completed comparing CF, HI, plaque-reduction, and indirect immunofluorescent (IFA) serologic tests for the diagnosis of Colorado tick fever. The IFA test is simple, rapid, and accurate and is a useful supplementary test in a diagnostic laboratory. Preliminary results using this method for the diagnosis of St. Louis encephalitis, western equine encephalomyelitis, lymphocytic choriomeningitis, and other arbovirus infections are promising.

A field study on Colorado tick fever virus is in progress in Shasta County, California, at the edge of the known endemic area, to attempt to determine why the virus remains limited in distribution to the areas where Dermacentor andersoni occurs.

A major epidemic of arthropod-borne encephalitis was considered possible in California during 1969, because of record snowfall and precipitation during the winter and early spring and the subsequent flooding with increased mosquito breeding. Extraordinary efforts were made at mosquito control, concentrating especially on areas with the largest human populations and where mosquito breeding was found to be at a high level. Special surveillance efforts to detect human and equine cases and virus-positive mosquitoes were conducted.

Mosquito collections were made throughout the San Joaquin and Sacramento Valleys by California State Department of Public Health and National Communicable Disease Center field teams. In our laboratory, 442 pools (22, 260 mosquitoes) of Culex tarsalis, and 305 pools (13, 224 mosquitoes) of other species were tested in suckling mice. Two isolates of Hart Park virus were made from C. tarsalis collected June 4 in Kings County, and 13 isolates of SLE virus were made from C. tarsalis collected from August 18 to September 11 in Tehama, Shasta, Sutter, and Solano Counties. Fluorescent antibody staining of mouse brain smears was useful in rapidly identifying the SLE isolates. All isolates were confirmed by specific neutralization tests in suckling mice.

A total of 57 horses were reported as clinically suspicious for WEE (or for rabies or other CNS disease) and specimens were obtained from the majority for serologic tests (30 cases) or inoculation of horse brain into suckling mice and fluorescent antibody staining of horse brain for rabies and for WEE virus (22 cases). Two confirmed cases of WEE in horses occurred, one in Tehama County on August 4, and one in San Joaquin County on October 2. Five additional cases with high stationary antibody titers, with no history of vaccination, occurred in Kern, Glenn, Sonoma, and Fresno Counties, and three cases (Mendocino, Santa Barbara, and Fresno Counties) had single CF titers of 1:64, but had been vaccinated previously. Previous experience indicates that WEE vaccine alone rarely induces CF titers this high, but the status of these cases is inconclusive at present.

Although WEE virus has been isolated in previous years from the brains of sick squirrels in WEE endemic areas, of 170 squirrel brains tested in 1969, all were negative for virus. In addition, 129 other wildlife specimens, 8 dogs, and 10 cats from endemic WEE areas and found dying of apparent CNS disease were tested and were negative for virus.

Approximately 400 human cases of suspected meningitis or encephalitis were known to occur in California from April 1 to November 1 and a majority of them were tested serologically (over 300 cases) or by virus isolation attempts on brain tissue (32 cases) for arboviruses. No cases of WEE or California encephalitis were detected. To date, 5 cases of St. Louis encephalitis have been confirmed, all with complete recovery: a 66 year old man in Sutter County, onset September 4; a 42 year old woman in Sacramento County, onset September 8; a 14 year old boy exposed in Sacramento County, onset September 12; a 35 year old man in Glenn County, onset September 15; and a 36 year old woman from Glenn County, onset September 21. All cases occurred in the area of the State where SLE virus strains were isolated from mosquitoes.

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

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

This report reviews field and laboratory studies on arboviruses during the period May 1, 1968 through April 30, 1969.

The summer of 1968 was a period of normal temperatures and river flow. There was less than normal rainfall in the preceding winter and spring. Population levels of Culex tarsalis were comparable to those of 1967 in most areas and peaked in July or early August. At one study site, an unusual water usage pattern resulted in a peak vector population in June. At each study site there was a high correlation between the period of peak vector population and the time when antibodies to western equine encephalitis (WEE) virus first appeared in sentinel chicken flocks. Antibody conversions to WEE virus were detected in 10 of 12 flocks maintained in rural community and rural agricultural environments but not in 5 flocks maintained in urban, foothill, and desert environments. St. Louis encephalitis (SLE) virus was isolated from 2 pools of C. tarsalis collected in early and mid-August. This was the first isolation of SLE virus from Kern County since 1964. The location of these isolations were correlated with sites where SLE antibody developed in 2 sentinel chicken flocks. The sites where SLE virus reappeared were in areas with high population levels of C. tarsalis by midsummer.

Turlock virus was isolated from 3 pools of C. tarsalis and antibody conversions to this virus were detected in 8 of 17 chicken flocks.

Forty-seven cases of encephalitis and 15 cases of aseptic meningitis were reported in persons who resided in Kern County in 1968. Two cases were confirmed as WEE and 1 as SLE. The 2 WEE cases were the first detected since 1963, and the SLE case was the first confirmed from this area since 1958. It is of considerable concern that these 2 diseases reappeared after a long absence, and it is believed their reappearance reflected a degree of resurgence of the C. tarsalis population at some localities.

Twenty-two suspect cases of encephalitis in horses were reported from Kern County in 1968. Diagnostic specimens were received from 10 cases and 6 cases were confirmed to be WEE. Tests on 7 of the paired sera with a bank of HAI arboviral reagents led to the interesting finding that 5 horses had stationary antibody titers to Main Drain virus and 1 had converted from negative on the acute phase to a titer of 1:20 on the convalescent phase serum. Confirmatory CF tests will be done.

An intensive study of wild mammal and bird populations at 3 localities over a 5 year period was discontinued in March 1968, and the extensive ecologic data are being analyzed. Peromyscus maniculatus, Mus musculus and Reithrodontomys megalotis bred year round. The breeding season of Dipodomys nitratoides and Dipodomys heermanni was quite variable in different years as in some years they produced single litters in the early spring and in other years as many as 3 litters in the spring and summer. Ammospermophilus nelsoni and Citellus beecheyi had only 1 litter per year, usually in late February or early March.

Life table analysis of data on D. nitratoides indicated a possible 76 percent survival rate through each month and 19 percent survival after 6 months. These data convert to a survival of 37 animals after 12 months and 1 after 2 years from a cohort of 1,000 animals.

Data on habitat associations of the principal mammalian species in the principal study area indicated the following preferences. Dipodomys nitratoides and Onychomys torridus preferred saltbush (Atriplex polycarpa) dominated habitats. Dipodomys heermanni, P. maniculatus, and M. musculus preferred quailbush (Atriplex lentiformis) dominated habitats. Ammospermophilus nelsoni frequented habitats with stands of moderate density saltbush. Habitat usage by various species at the other 2 study sites was generally similar except for changes due to differences in the predominating plant types.

Virologic and serologic studies on blood samples collected from animals at 3 study sites in the San Joaquin Valley area in 1968 can be summarized as follows. Only 2 of 182 wild rodent sera collected in September 1968 were positive in HAI tests, and these were from 2 P. maniculatus that had antibody to Modoc virus. Lepus californicus appeared to be the primary vertebrate host for Buttonwillow (BW), Lokern, and Main Drain viruses; and the monthly prevalences of HAI antibodies indicated that these 3 viruses were active at different times of the summer at all 3 study sites in the San Joaquin Valley portion of Kern County. An increase in the proportion of

jackrabbits with antibodies occurred in May and June for BW virus, in July for Main Drain virus, and in August for Lokern virus. A diphasic increase in BW antibody prevalence occurred at all 3 sites, the first increase in May and June and the second in late summer. There was a high correlation between the time when the 3 viruses could be isolated from Culicoides variipennis and the subsequent time when increases in antibody prevalence occurred in jackrabbits. Comparative studies at a higher elevation site in the Mojave Desert indicated that the 3 viruses were active about 1 month later than at the San Joaquin Valley sites, and there was no diphasic pattern in the occurrence of BW antibodies. It is of considerable interest that 3 viruses utilize the same vector and vertebrate host species but are active at different times during the summer. This phenomenon will be the subject of continuing studies.

There was serologic evidence that WEE virus extended from the bird-C. tarsalis cycle to the jackrabbit population during July and August of 1968, but there was little or no serologic evidence of SLE or California encephalitis (CE) virus infection in these rabbits.

Serologic studies of 145 wild bird bloods confirmed the importance of these animals as hosts of WEE and Turlock viruses. The prevalence of SLE antibodies was surprisingly low, in view of the evidence that C. tarsalis and chickens were infected in the same area.

A study of blood samples collected from 694 cold-blooded vertebrate hosts that were collected in 1967 and 1968 provided little evidence of arboviral infection. HAI antibodies were detected to WEE virus in 1 serum from a western toad, to SLE virus in 2 sera from western bullfrogs, and to both Modoc and CE viruses in one serum from a king snake. All sera were negative in antibody tests for Powassan, BW, Lokern, Main Drain, and Turlock viruses. No virus was isolated in tests of these bloods.

The discovery that C. variipennis is a primary vector of 3 new arboviruses (BW, Main Drain, and Lokern) led to an intensive 12 month field study of this midge in 1967-1968. The midge population had 2 peaks, one in April-May and a second higher peak in September-October. Only a few adult specimens could be collected in midwinter, which probably reflected the suppressive influence on the population of low temperatures and rain. An evaluation of parity rates revealed that they peaked when populations were largest and that the periods of highest parity rates were related to the seasonal patterns of viral infection in the vector population and jackrabbits. Field collections of C. variipennis were segregated into 258 pools of parous

and 257 pools of nulliparous specimens. Thirty-six viral isolations were made from the parous pools and none from the nulliparous pools. The survival rates of C. variipennis were estimated on the basis of parity data and indicated that with a 3 day gonotrophic cycle about 37 percent of females would survive 3 days and 14 percent would survive 6 days. With a 4 day gonotrophic cycle about 37 percent would survive 4 days and 14 percent 8 days. Comparative analysis of parity data for C. tarsalis indicated that this mosquito if it had a 4 day gonotrophic cycle would have about 50 percent survival at 10 days and with a 5 day cycle would have nearly 50 percent survival at 11 days.

A bank of 1,592 sera was collected in 1968 from domestic mammals that were resident in representative areas of California that extended from Modoc County in the north to the Antelope Valley in Los Angeles County to the south. These sera were tested for HAI antibodies to 9 arboviruses (WEE, SLE, Powassan, Modoc, CE, BW, Lokern, Main Drain, and Turlock). The prevalence of antibodies was very low in bovine sera, except that 22 percent of animals from the Merced-Stockton area reacted to Lokern antigen. A high proportion of horse sera reacted with WEE, Main Drain, SLE, Turlock, Lokern, and CE antigens. Ovine sera reacted most frequently with Main Drain and Lokern antigens. Porcine sera reacted most frequently with SLE, Turlock, and WEE antigens. Dogs had a high frequency of positives to all viruses except Powassan and Modoc. It was of interest that BW virus infection appeared to be very limited. Of the 9 viruses, Main Drain, Lokern, and CE viruses were more active than were the other 6 viruses in Modoc County. SLE and Turlock antibodies had the highest prevalence in samples from the Sacramento Valley area. Main Drain antibodies were prevalent in all areas sampled.

A new area in Butte and Glenn Counties in the Sacramento Valley has been selected for intensive field studies for at least the next 5 years. Chicken sera were collected from farm flocks in this area in November, 1968. Serologic test results allowed a retrospective interpretation that WEE, SLE and Turlock viruses had been active in both Butte and Glenn Counties during 1967 and 1968 and that sites with the highest population indices of C. tarsalis had the highest levels of viral activity.

A study of the blood-feeding habits of the mosquitoes of Hawaii was completed in May, 1968. Of the 10,864 blood meals identified from Culex quinquefasciatus, 52 percent were from chickens, 8.8 percent from passeriform birds, 2.7 percent from Boobys, 6.5 percent from miscellaneous birds, and 31 percent from mammals. When Aedes aegypti and Aedes albopictus were collected from the same domestic habitats, they had

fed on man at about the same rate, 56.0 percent and 53.7 respectively. When A. albopictus were sampled from sylvan habitats, they had fed on a wide range of mammals and occasionally on birds. Aedes vexans nocturnus had fed exclusively on mammals. It is concluded that the host-feeding patterns of the 4 mosquitoes indigenous to Hawaii could be conducive to transmission of a variety of arboviruses if viral importation occurred.

A study was completed of the blood-feeding habits of 5 species of Deinocerites mosquitoes in Panama. Deinocerites dyari and Deinocerites melanophyllum had fed almost exclusively on lizards. Deinocerites pseudes and Deinocerites epitedeus fed frequently on mammals and birds and included lizards in their host selection. Deinocerites cancer had fed primarily on birds. No mosquito genus or species that we have studied has shown this broad host range, which included all 4 classes of land vertebrates.

A limited study of engorged mosquitoes from Saskatchewan revealed that C. tarsalis, Aedes vexans, Aedes campestris, Aedes flavescens, Aedes fitchii, and Anopheles earlei fed on mammals and chickens. Culiseta inornata and Aedes dorsalis had fed almost exclusively on mammals. A limited evaluation of the blood-feeding patterns of 7 species of mosquitoes collected from Southeastern Illinois, coincidental to the recurrence of St. Louis encephalitis in humans, did not reveal any significant deviations in host preferences from earlier and more extensive studies on the same mosquito species in nonepidemic periods.

A new and limited study on the blood-feeding habits of mosquitoes from the Houston, Texas area revealed that 54 percent of C. quinquefasciatus had fed on mammals, in contrast to 30 percent of mammalian blood meals in earlier studies of this mosquito collected in Hawaii and Florida. Data are too limited to allow an interpretation of the significance of this deviation.

An interesting series of engorged mosquitoes were provided us from Malaya. All Uranotaenia lateralis had fed on "skipper" fish, which are semi-terrestrial. Several unnamed species of Aedes also had fed on "skippers". Culex quinquefasciatus had fed principally on chickens.

Previous studies indicated that storage and cell cultural conditions influenced the ability of Vero cells to form plaques after infection with several group B arboviruses. Thus, 2 stocks of Vero cells were cloned to obtain a cell population with uniform susceptibility to SLE, Powassan, Modoc, and Rio Bravo viruses. Of the 6 clones that were obtained, 2 plaqued all 4 viruses, 2 plaqued only 1 or 2 of the viruses, and 1 plaqued none of the viruses. Recloning of 2 susceptible clones provided 4 subclones that

allowed plaquing of the 4 group B viruses. The failure of Vero cell clones to form plaques with group B arboviruses was not related to contamination with mycoplasma or to an inability of the cells to support viral replication.

Attempts were continued to increase hemagglutinin titers of viruses that usually produce low-titered hemagglutinins or none. Treatment of sonicated antigens with trypsin or DEAE-dextran failed to provide hemagglutinins for Hart Park and blue tongue viruses. Brains from suckling hamsters infected with Jerry Slough virus yielded a higher titered hemagglutinin than did infected brains from suckling mice. Untreated fluid media from primary hamster kidney cell cultures that were inoculated with pools of C. tarsalis infected with WEE virus had hemagglutinin titers of 1:32 to 1:256. This technique allowed rapid identification of newly isolated strains of WEE virus but not of SLE virus.

Eight unidentified viruses that were inactivated by sodium deoxycholate have been isolated during the past 6 years from bloods or organs of wild rodents. One agent (A 10038) that was isolated from the blood of an A. nelsoni is identical or closely related to an agent (A 13291) isolated from the blood of a Perognathus californicus. Also, 2 viruses from kidneys of Peromyscus boylei and Peromyscus truei are reciprocally cross neutralized by immune sera.

Studies were continued on the pathogenesis of arboviruses in wild birds and mammals. On the basis of avian and mammalian susceptibility, 9 arboviruses were separated into 3 groups. WEE and SLE viruses infected both birds and mammals. Turlock virus was more infectious for birds than for mammals, whereas Powassan, Modoc, Rio Bravo, CE, BW, and Main Drain viruses infected primarily mammals. Leporid species were more susceptible to infection with BW and Main Drain viruses than were rodent species.

Six of 6 L. californicus and 4 of 6 Zenaidura macroura became infected after inoculation with WEE virus. Viremias were detected in L. californicus on post-inoculation days 1-4 and peak titers occurred on day 2. Citellus beecheyi were refractory to infection. Western bullfrogs and western toads failed to develop HAI antibodies after inoculation with 10^4 to 10^6 plaque forming units of virus.

Viremia responses were determined for wild birds and mammals that developed HAI antibodies after inoculation with Modoc, Rio Bravo, or Powassan virus. Viremias in A. nelsoni and D. nitratoides infected with Modoc and Rio Bravo viruses usually persisted for 4-7 days and peaked on

postinoculation days 3 through 5. Ammospermophilus nelsoni developed lower titered viremias after inoculation with Powassan virus than they did after inoculation with Modoc and Rio Bravo viruses. Only 3 of 9 P. maniculatus infected with Modoc virus developed detectable viremias. No viremia was detected in Z. macroura or Sylvilagus auduboni, although they developed HAI antibody after infection with Modoc or Rio Bravo virus.

Sixty-six of 70 mammals became infected after inoculation with CE virus, whereas none of 40 birds became infected. Viremias were demonstrated in 11 of 11 leporids and 14 of 30 rodents that developed HAI antibody and were tested for virus. Viremias usually were present on postinoculation days 1 through 4 and peak titers occurred on days 2-3. All animals that became infected developed high levels of HAI antibody that persisted for at least 8 weeks.

Leporids were highly susceptible to experimental infection with Main Drain virus, rodents were partially refractory, and birds were completely refractory. Viremias in L. californicus were limited to postinoculation days 2 and 3.

Selected species of mosquitoes, biting midges, and ticks were evaluated for their ability to become infected with and transmit virus after ingestion of virus from pledgets or hosts. Ornithodorus parkeri and Argas sanchezi became infected after ingestion of WEE virus from viremic chicks. Dermacentor parumapertus and C. variipennis failed to become infected with WEE virus.

Culex tarsalis, Culiseta inornata, Anopheles freeborni, Aedes melanimon and Aedes nigromaculis became infected when fed on pledgets soaked with high concentrations of CE virus. Aedes melanimon, A. nigromaculis, and A. freeborni, but not C. tarsalis, were infected by feeding on viremic S. audoboni. Anopheles freeborni transmitted virus on one occasion. No virus was recovered from C. variipennis, O. parkeri or D. parumapertus that had fed on infected pledgets or hosts.

Jerry Slough virus was recovered from A. nigromaculis, A. freeborni, C. tarsalis, and C. inornata, but not A. melanimon, after feeding on pledgets. Culiseta inornata also became infected when fed on a viremic S. auduboni but failed to transmit virus in 1 attempt.

Three of 8 pools of pledget-fed C. tarsalis became infected with Jamestown Canyon virus. Limited attempts to infect A. nigromaculis and A. melanimon with similar concentrations of virus were unsuccessful.

Anopheles freeborni and C. variipennis became infected when fed on pledgets soaked with high concentrations of BW virus, while A. melanimon, A. nigromaculis, and C. inornata could not be infected by the same technique. Attempts were unsuccessful to infect A. freeborni, C. tarsalis, C. variipennis, and D. parumapertus on viremic S. auduboni.

Preliminary results indicated that Main Drain virus had a wide vector range. Pledget feedings demonstrated that A. melanimon, A. nigromaculis, A. freeborni, C. tarsalis, C. inornata, and C. variipennis could be infected with this virus. Anopheles freeborni, C. inornata, and C. variipennis, but not C. tarsalis and D. parumapertus, became infected when fed on viremic S. auduboni. Virus was recovered from 4 of 10 pools of O. parkeri 24 days after ingestion of virus from a viremic S. auduboni, but none of 9 pools from the same lot were infected 100 days after ingestion of virus. Transmission of virus was achieved with C. variipennis on 4 occasions after 6 to 13 days extrinsic incubation.

Limited pledget feedings with Lokern virus demonstrated that A. nigromaculis and C. variipennis, but not A. melanimon and A. freeborni, could be infected with this virus.

Preliminary studies were initiated to compare the pathogenesis in hamsters of a mosquito strain of WEE virus with that of a rodent strain of WEE virus because in previous studies these strains differed in their pathogenesis for kangaroo rats and White-crowned Sparrows. In hamsters, the mosquito strain usually produced higher titered viremias, higher mortality rates, and shorter average survival times than did the rodent strain. These differences were most evident in weanling hamsters. Paralysis was observed in hamsters that died after infection with the rodent strain, whereas no overt symptoms of disease were evident in hamsters that died from infection with the mosquito strain.

The effect of temperature on the replication of WEE and SLE viruses in mammalian and avian cells was evaluated to determine if there was a relationship between the temperature dependence of these 2 viruses and the seasonal occurrence of peak infection rates in C. tarsalis in Kern County. In continuous lines of bat lung cells, both WEE and SLE viruses replicated at 24, 36, and 42 C; but, as expected, WEE virus multiplied at a faster rate than SLE virus at all 3 temperatures. In duck embryonic cell cultures, WEE virus replicated at 18, 24, 30, and 36 C but not at 12 C; whereas, SLE virus failed to replicate at 12 and 18 C and replicated quite slowly at 24 C.

Highly specific antisera have been prepared to purified serum albumins from blacktailed jackrabbits, desert cottontails, House Sparrows, House Finches, Tricolored Blackbirds and White-crowned Sparrows. These antisera were employed in the micro-complement fixation procedures to identify blood meals from mosquitoes that were fed on known host species. Of 48 field collected mosquitoes, 7 were identified as having fed on English Sparrows, 9 on House Finches, and 7 on Tricolored Blackbirds. Double feedings were found in 2 instances.

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

(W. C. Reeves)

REPORT OF 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

Epidemic Dengue in Malaysia

An epidemic of mild, classical dengue occurred in Malaysia between June 1967 and March 1968. When compared to the 1962 - 1964 outbreak of hemorrhagic dengue in Penang, Malaysia, it was concluded on epidemiological grounds that Aedes albopictus was the principal vector of the recent mild dengue episode, while A. aegypti was the principal vector of the earlier severe hemorrhagic fever epidemic in Penang.

Another dengue outbreak began in April 1969 and new cases continued to appear through September (the time of writing this report). In contrast to the 1967 - 1968 epidemic, the current episode includes a number of cases of hemorrhagic dengue, some exhibiting a typical shock syndrome. None of the dengue isolates from this epidemic have been typed yet.

Almost all the 106 dengue isolates from the 1967 - 1968 epidemic have been identified to type by plaque reduction neutralization tests as shown in the following table.

Dengue I:	Human origin	4
Dengue II:	Human	23
	Aedes aegypti	2
	A. albopictus	2
Dengue III:	Human	3
Dengue IV:	Human	57
	A. aegypti	2
	A. albopictus	1
Untyped:	Human	2
	Total:	106

The dengue virus isolated from a sentinel monkey in the Tanjong Rabok study area (reported in the last newsletter) has been identified as dengue type III.

Malaysian Virus Isolates

A strain of Umbre virus was isolated from a pool of 96 Culex annulus mosquitoes collected at domestic animal shelters near Kuala Lumpur. This virus has not been reported previously from Malaysia, nor anywhere else outside India.

A strain of Bakau was isolated from the blood of a wild Macaca irus trapped in Mangrove Swamp on Carey Island. Bakau neutralizing antibody was demonstrated in the sera of 8/38 other wild monkeys trapped in the same area. Bakau virus was initially isolated from a pool of Culex lophoceraomyia mosquitoes collected from Coastal Nipah palm-Mangrove Swamp near Klang, Malaysia. A second isolation was made some years later from a pool of 9 Culex sp. collected from a freshwater peat swamp forest approximately 50 miles north of Kuala Lumpur.

A virus belonging to Sero-Group B was isolated from a pool of Aedes (Cancraedes) spp. mosquitoes collected in a mangrove swamp on Carey Island. It is antigenically distinct from any of the known Malaysian Group B arboviruses. Preliminary neutralization tests results suggest it may be related to Uganda S virus.

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

REPORT FROM THE RESEARCH STATION, RESEARCH BRANCH CANADA AGRICULTURE, UNIVERSITY CAMPUS SASKATOON, SASKATCHEWAN

Last summer we had to stop our mosquito collections and virus surveillance at the end of July for lack of funds, but we did manage to keep the sentinel flocks in the field until the end of September.

No human cases of WE were reported in Saskatchewan last summer. Forty clinical equine cases were reported but so far only two of these have been confirmed. To date, about 500 mosquito pools out of about 1000 have been negative; 15 out of 25 chickens in our most southerly flock at Estevan converted between the end of July and the end of September and 4 out of 25 at Outlook. There was definitely an increase in WE virus activity in the Province in 1969 over the preceding two years.

(J. McLintock)

REPORT FROM THE UNIVERSITY OF BRITISH COLUMBIA, VANCOUVER

1. Field Investigations, Summer 1969

Between 6 April and 12 August 1969 near Penticton, B. C. (119°30'W, 49°30'N) at altitudes ranging from 1100 to 5200 feet, 833 small wild rodents were surveyed for arbovirus antibodies. Group B arbovirus antihemagglutinins were detected in 133 sera including 54 which reacted exclusively with Powassan (POW) antigen, 22 with St. Louis encephalitis (SLE) antigen solely, and 57 with both antigens. Sera from 94 of 422 marmots (Marmota flaviventris), which was the dominant vertebrate species collected, inhibited hemagglutination by one or more group B arboviruses, but HI antibodies were also found in 19 of 78 squirrels (Tamiasciurus hudsonicus), 10 of 225

chipmunks (Eutamias amoenus) and 10 of 108 other species. Of 526 sera collected during April, May and June which have been examined by neutralization tests, 18 neutralized POW exclusively, 4 neutralized SLE and 7 neutralized both viruses. Antibodies to western equine encephalomyelitis (WEE) virus were not detected in these sera by HI.

No virus was isolated from adult and nymphal Dermacentor andersoni ticks removed from 25 marmots. An additional 9 pools of flat D. andersoni ticks collected by dragging the underbrush during April and May did not yield virus by inoculation of newborn mice.

Mosquitoes comprising Aedes canadensis and A. vexans which were collected on 14th June 1969 yielded the Gray Sage isolate, by inoculation of newborn mice. This isolate was neutralized by antiserum to the BFS-283 strain of California encephalitis (CE) virus, and antiserum to the Gray Sage strain neutralized and fixed complement in the presence of the homologous virus and the La Crosse isolate of CE virus. Thus the Gray Sage virus was a member of the CE complex of arboviruses. This was the only virus isolation achieved from 26 mosquito pools collected from 6 sites between 8th June and 4th August. Sera from 2 of 130 mammals collected between 1st and 18th June neutralized the Gray Sage isolate.

(Donald M. McLean)

2. Growth of Powassan Virus in Dermacentor andersoni Ticks

All stages of D. andersoni ticks have become infected with Powassan virus by feeding on rabbits rendered viremic by intravenous injection of high concentrations of virus 2 to 3 days after ticks first became attached. Virus has been transferred transstadially from larvae to nymphs and from nymphs to adults and virus transmission has been obtained during bites of vertebrates by nymphs and by adults which were infected as larvae. Virus was localized principally in the gut of larvae, but it invaded nymphal salivary glands during ecdysis. Infection was localized in the salivary glands of nymphs, and to a lesser extent in the gut. In adults, virus was detected principally in the salivary glands, and minimal quantities were found in the gut. In male adults the virus titer in salivary glands remained high throughout their life, but in female adults the virus titer in salivary glands declined steadily following another blood meal, but the virus titers in Gené's organ glands increased considerably. Immunofluorescent foci due to Powassan virus in the cytoplasm were seen regularly in tick tissues

containing virus. Powassan virus was also detected in salivary gland secretions of infected adult ticks.

(Max A. Chernesky)

REPORT FROM THE UNIVERSITY OF SAN CARLOS MEDICAL SCHOOL GUATEMALA, GUATEMALA, C. A.

The Area of Biological Sciences of the School of Medicine, University of San Carlos and The Department of Microbiology Cornell University Medical College, have conducted studies on the Ecology of Arbovirus with special reference to VE virus, for the past 4 years. These studies have been carried out in Guatemala (north and south coast), north coast of Honduras and British Honduras. During the summer of 1967 and again in 1968 the presence of VE and other viruses was established in some parts of the study area, by the use of sentinel hamsters.

In the middle of May of this year an epizootic disease in horses appeared in the south eastern part of Guatemala. The outbreak extended to the neighboring country of Salvador and later probably to southern Honduras and Nicaragua, along the Pacific coast. It also extended into the Guatemalan uplands involving several small valleys along the Salvador and Honduras bordors, reaching the large Motagua River valley and extending into the south east part of the Department of Peten in late September. The description of the extention of the epizootic is based on horse deaths, not always confirmed by virological studies.

Rough estimates show about 15 hundred horse deaths, in the country. This represents about 30% mortality as observed in several horse populations involved. The horse data reported was obtained by direct inquiry of persons in the affected areas, by exchange with the different groups working on the problem and in the case of the Department of Peten, by newspaper reports.

Our group was mainly interested in studying the human aspects, as well as establishing the correct diagnosis of the horse disease.

It was established then that a human outbreak was also taking place. The clinical picture has been one with sudden onset of fever (as high as 40°C.), headache, generalized aches and pains. Many cases suffered abdominal pain, nausea and vomiting but not diarrhea. Coryza has been rare; sore throat almost absent. In other words it has been some what different than a "flulike syndrome". On the other hand very few patients have shown encephalitic involvement and no well documented fatalities have been found.

The virological studies of the human disease have been of several kinds. In one area acute samples for virus isolation were collected and processed some in chicken embryo tissue culture and some in suckling mice for virus isolation. Serum samples from the acute and convalescent phase, will also be examined for antibodies.

Two small towns (about 500 people each), one on the south coast and one on the lowlands towards the Atlantic coast, were also selected for study. In these two towns, the disease will be monitored as it appears. Pre-bleeds were taken in about 20% of the population and a second sample will be looked for later on.

Up to the present time (October 1969) 2 viruses have been isolated from the blood of patients in the acute phase. One virus isolated from the blood of a sick horse, all three identified as VE. Two more isolates from humans and one from a horse, are awaiting identification.

Preliminary tests showed the presence of HI and CF antibodies in some patients. The rate of clinical disease in exposed populations could be as high as 10%, maybe somewhat higher.

More detailed studies are under way, concerning the epidemiology of this disease.

(José Victor Ordóñez)

Venezuelan Equine Encephalitis Virus in Central America

During the past two years, we have obtained multiple isolates of VEE virus from all the Central American countries except El Salvador. When tested by kinetic HI, these have proven identical to strains previously tested from Mexico and western Panama, indicating the continuous distribution of a single antigenic variant of VEE over the whole of Central America.

However, isolates from the 1969 epizootic in Guatemala, while definitely VEE, are clearly distinguishable from the previously known variant. This suggests that the new variant responsible for the current epidemic either arose as a spontaneous mutation in Guatemala or was somehow transported from some area in South America, possibly Ecuador.

Further studies comparing the epidemic Guatemala isolate with other South American strains are underway. We also plan experimental infections with various VEE strains in equines, comparing levels of viremia and pathogenicity. An interesting and unexplained phenomenon is that, in endemic areas such as Panama, large populations of equines of all age groups develop neutralizing antibody to this virus with little or no apparent morbidity, in contrast with the high fatality rates in susceptible horses during epidemics.

(Peter T. Franck)

Ecologic Studies of Vesicular Stomatitis Virus

In 1961, six isolations of VSV-Indiana were made from Phlebotomine sandflies collected in Almirante, Republic of Panama. In addition, serologic studies have suggested an association between cutaneous leishmaniasis and VSV-Indiana infection. To investigate further the role of sandflies in the ecology of the virus, in January 1969 we began regular monthly collections of sandflies for virus isolation in an area of VSV activity. Phlebotomines were collected by hand aspirators, in light traps, and from human bait and were separated according to the method of collection and by sex into pools containing 50 to 100 flies. Insect pools were triturated in borate saline

solution (pH 8.0) containing 25% rabbit serum and 2% penicillin and streptomycin. Each pool was inoculated intracerebrally into suckling mice and into Vero cell cultures. Caged sentinel animals (monkeys and hamsters) were also exposed in the forest adjacent to the study area.

To date, 38,947 sandflies (23,466 females and 15,481 males) have been collected and processed for virus. Twenty-four virus isolates have been obtained. Five have been positively identified as VSV-Indiana; the remaining 19 isolates are under study. The five VSV isolates were all obtained from female sandflies captured in August; three of the positive pools were from human bait collections; the remaining two were from flies aspirated from tree buttresses. All five VSV isolates were obtained from sandflies collected at a single site on two consecutive nights, suggesting that the occurrence of virus in the forest was brief and focal. One of five sentinel monkeys exposed in the forest canopy developed VSV-Indiana neutralizing antibodies during the same period, but none of 12 sentinel hamsters were infected.

(Robert B. Tesh and Byron N. Chaniotis)

REPORT FROM THE UNIVERSITY OF THE WEST INDIES KINGSTON, JAMAICA

Dengue Virus Infection in Jamaica 1969

Following the epidemic of dengue fever (Sept. 1968 - Jan. 1969) which peaked in November, when just under two hundred (200) cases were reported, dengue fever occurred sporadically until around July when an outbreak occurred in Port Antonio. Over one hundred (100) cases were reported from this area.

About sixty percent of the cases investigated during the former epidemic were confirmed in the laboratory, and of nineteen (19) isolates from acute sera, five have been confirmed as dengue viruses by Dr. Russel of the Walter Reed Army Institute of Research in Washington. Three (3) isolates were similar to dengue 3 and one (1) to dengue 2. One (1) was untyped.

From the Port Antonio outbreak fourteen (14) of thirty (30) cases with single or paired sera had current or recent dengue virus infection. So far only one viral isolate was obtained from the acute sera of a visitor to this area who contracted dengue fever.

During the periods following the above two outbreaks, at least one hundred and two (102) cases of dengue fever have been reported to our laboratory. Sixty-seven (67) cases, with sufficient data and the required number of serum samples, were investigated. Of these thirty nine (39) were serologically confirmed, with two thirds showing a 'secondary type' antibody response. Virus isolation is still in progress and so far only one (1) isolate has been made from this group of sporadic cases.

REPORT FROM THE VIRUS DEPARTMENT OF THE CENTRAL LABORATORIUM PARAMARIBO, SURINAME

In the first three quarters of the year 1969, 12, 634 mosquitoes were caught. Only two localities were frequented, MaRetraite in the coastal region and Matta in the Savannah belt.

Besides these 71 litters of sentinel swiss albino infant mice were exposed all in Matta.

From the mosquitoes 6 viruses were isolated; another 8 from the litters.

The bulk of these viruses belong to group A. Some, however, give cross-reactions with group C and the Guama group. We gratefully acknowledge the Belem Virus Laboratory for their willingness to help us with the identification of these viruses. Two viruses belong to the Guama group appear to us to be Guama viruses.

(R. A. de Haas)

REPORT FROM THE DEPARTAMENTO DE VIROLOGIA OF INSTITUTO VENEZOLANO DE INVESTIGACIONES CIENTIFICAS - I.V.I.C.

About a thousand sera were collected from Guaica Indians in the Territorio Amazonas. In cooperation with Dr. James V. Neel from the University of Michigan, these were checked against 17 arbovirus antigens. The majority of positive reaction were given by group B virus.

Piry virus was investigated. Multiplication in BHK cells, Aedes aegypti and Aedes albopictus cells, and Aedes aegypti mosquitoes were studied. Virus particles were multiplied in BHK cells and concentrated by ultracentrifugation. The Piry virus particles were found to be rod shaped, typical of the rhabdovirus group. In sections of infected BHK cells predominantly rods were observed, whereas, in purified preparations many more spherical particles than rods are present. Separation of the two types of particles by density gradient centrifugation were not successful.

The Nariva virus is being investigated. On morphological evidence in sections, it does not appear to belong to any of the known arbovirus groups.

Dr. Ian Holmes, from Melbourn University has commenced his sabbatical year in our department.

(G.H. Bergold)

REPORT FROM THE VIROLOGY SECTION OF THE INSTITUTO DE INVESTIGACION CLINICA, FACULTAD DE MEDICINA, UNIVERSIDAD DEL ZULIA, MARACAIBO, VENEZUELA

Human Sera Survey

In the July 1968 issue of the Information Exchange we reported the VEE HI results of an arbovirus serological survey of the State we conducted during 1967. We have subsequently completed the neutralization tests on Group A arbovirus and the results are summarized as follows:

VEE	Mayaro	EEE	WEE
$105/116$*	$29/117$	$1/50$	$0/50$

*number positives/number tested.

VEE antibodies were found mostly in the Guajira Peninsula where cases of suspected or confirmed VEE have been reported periodically since 1936. Fifty-two percent of the population of the area above 6 years of age showed antibodies while no antibodies were found in children born after 1962's epidemic. Mayaro seems to be prevalent in areas of tropical forest in the southwestern part of the State. Only one serum showed neutralizing antibodies against EEE virus. This case came from Concha, located south of the lake of Maracaibo. This finding, the first reported in Venezuela, will be more intensively studied because of its epidemiological implications. No WEE virus activity was obtained.

Information on Group B arboviruses activity have been obtained by HI tests. Seven hundred and ninety four sera samples were tested against four dengues, SLE, YF, Ilheus and Bussuquara antigens. Forty-eight percent of the specimens tested were found with antibodies for one or more Group B antigens. Combinations most frequently found were:

All viruses tested	234
YF	83
SLE-YF-Ilh-Buss	13
Deng-SLE-Ilh-Buss	10

Dengues were prevalent in the major cities. Antibodies for SLE only were found on three occasions from three different locations. Neutralization tests are pending.

VEE Outbreak

In October 1968 an outbreak of encephalitis among humans and equines was reported from the Guajira Peninsula. Based on previous data VEE virus was suspected and confirmed by isolation of the agent from human sera in 14 instances. Identification was carried out by NT in newborn mice inoculated i.c. Twenty paired sera (acute and convalescent) were tested for VEE HI antibodies and the results showed a four-fold or greater increase in titer in all cases. Attack rate was 110 per 1.000 inhabitants in children under 6 years of age as compared with 41 per 1.000 in the population above 6 years of age.

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

The Rockefeller Foundation, in pursuance of its present program of concentrating activities in areas other than arbovirus research, will terminate its support for this laboratory at the end of 1970. Any institution which might be interested in taking over a productive tropical field laboratory, under agreement with the Brazilian Government, is invited to contact the Director (who will also be leaving at the end of 1970).

Pacuí Virus Epidemiology

Pacuí virus was first isolated in 1961 by Ottis Causey and co-workers from serum of a forest rat, Oryzomys capito, trapped at Kilometer 92 of the Belém-Brasilia highway in Pará State. In 1962 four more strains were forthcoming from Oryzomys taken along the highway and in the Utinga Forest near the city of Belém. Again in 1963, two rodent strains were recovered from the Utinga Forest. Thereafter, virus activity was not evident in Brazil until October 1968. Meanwhile, Trinidadian workers investigating the epidemiology of rodent-associated viruses in Bush Bush Forest, isolated

two strains of Pacuí virus in 1961 from the sera of Oryzomys laticeps and Zygodontomys brevicauda. Throughout the years, many thousands of blood-sucking arthropods (including mosquitoes and phlebotomine flies) in both countries were tested, but none yielded the virus of Pacuí.

In October 1968, the opportunity presented itself to test phlebotomine flies caught in the castor-oil-painted trays of Disney traps exposed in the Utinga and Catú Forests near Belém (Disney, R.H. 1966, Bull. ent. Res. 56:445). Several thousand phlebotomines of the readily recognizable species Lutzomyia flaviscutellata were picked into Petri dishes of cold physiological saline, and sorted into pools of males, unengorged females and engorged females for subsequent grinding and inoculation into baby mice. During the last three months of 1968, 12 strains of Pacuí virus were isolated from pools of engorged and unengorged female flies; 23 additional strains have been isolated from the same sources through September 1969.

As various workers have repeatedly failed to demonstrate an invertebrate carrier of this virus in nature, the present success may seem strange. Phlebotomine flies, being small, fragile insects, are readily susceptible to desiccation. Unless special precautions are taken, collecting techniques may result in desiccated flies and possible loss of virus. In the case of flies trapped in oil, the insects are enveloped in a fine film which may not only preserve them from desiccation but also maintain the tissues in a viable condition for many hours. Thus any virus present has a greater opportunity for survival. Viruses have also been isolated from the few mosquitoes caught in the oil film: Catú, Oriboca, and BeAn 109303 (Gr. Guama). But the numbers of mosquitoes taken are small.

Pacuí appears to be a rodent-associated virus. In addition to repeated isolation from forest rats, high antibody rates have been found in Oryzomys (38%) and Proechimys (31%) (Woodall, J.P. - "Virus Research in Amazonia". Atas Simp. Biota Amazonica, 6:31-63, 1967). Lutzomyia flaviscutellata is primarily a rodent-biting fly. Distribution of Pacuí virus in nature may well be spotty, with small pockets of infection scattered through the forest, the result of limited activity patterns of both rodent reservoir and phlebotomine vector.

SLE Activity in 1969

This year has already been exceptional in the 15-year history of the laboratory for the number of SLE isolates obtained. They have come from sentinel chickens, wild birds and a marsupial. Paradoxically, mosquitoes and sentinel mice have so far been negative, and no human cases have been

recognized. During 1968 only 2 SLE isolations were made, from Culex coronator in August and the bird Galbula albirostris in October. The 1969 list is as follows:

Jan.	3	Pyriglena leucoptera (bird)
	6	sentinel chicken PT 317
	6	" " PT 319
	21	Chiroxiphia pareola (bird)
Feb.	1	sentinel chicken PT 318
	6	Formicarius analis (bird)
	13	Philander opossum (marsupial)
	27	Pipra pipra (bird)
Mar.	11	sentinel chicken PT 329
	27	" " PT 332
Apr.	2	" " PT 339
May	16	" " PT 353
June	28	" " PT 362
July	28	" " PT 365
Aug.	4	" " PT 376
Sept.	17	" " PT 382

There were also a number of HI conversions in sentinel chickens which did not yield virus in their daily bleeds. This intense activity contrasts with a complete absence of SLE activity from Florida this year, and it will be interesting to see if this build-up in north Brazil is the precursor of another southern US epidemic.

REPORT FROM THE ARBOVIRUS LABORATORY, INSTITUTE ADOLFO LUTZ, SAO PAULO, BRAZIL

During 1966 a bird netting program was started in the field stations of Casa Grande and Itapetininga and in 1967 it was transformed in a bird banding project. Each station is visited every two weeks for two days and the birds are bled, banded and released. The blood is kept in ice and transported to the Laboratory for tentatives of virus isolations and serology. In this project 2146 birds were captured in 1967, 2018 in 1968 and 1445 were obtained up to July 1969. Part of the blood was already inoculated in baby mice and several agents were isolated. Some of them were identified as isolations of three different viruses and others are still unidentified.

From 1966 to 1968 we had 14 isolations of a virus whose prototype was SPAn 5245. Table I shows the virus isolations by species, date and station. SPAn 5245 is being considered an arbovirus because it was shown to be a DCA sensitive agent (more than 3 logs), it was isolated twice from pools of Anopheles (K.) cruzii and Culex sp collected in Casa Grande. Besides it crossed with Manzanilla Virus (Tr. 3587) from Simbu Group by HI and CF tests, but no crossings were observed with Oropouche and Utinga viruses from the same group. Studies are in progress to verify if SPAn 5245 is a strain of Manzanilla Virus.

The second virus recovered from birds (Table I) was identified as a strain of SLE virus. We isolated SLE from birds, sentinel mice and wild rodents in a total of 8 isolations. No isolation came from mosquitoes up to now.

Since 1961, several serological surveys performed by this laboratory showed that a certain part of the population would react with the B Group antigens being SLE that which reacted in higher titers. Neutralization tests performed with two different strains of SLE virus (Parton and Tr 9464) showed consistent negative results, suggesting that the HI reactions observed were not due to a SLE infection.

We examined by NT tests in adult mice 13 paired sera obtained from human residents in Casa Grande in 1964 and 1965 which had shown serological conversions for the B Group in HI tests. The results of the NT tests were similar to those obtained previously, that is, no clear protection was

Virus Isolations from Birds During 1966-1969 in [illegible]

Number	Date	Local	Species	Identification
SPAn 5245	6-10-66	Itapetininga	Conopophaga lineata	Prototype
SPAn 5560	8-19-66	Casa Grande	Conopophaga lineata	= SPAn 5245
SPAn 5632	8-26-66	Itapetininga	Xanthomyas virescens	"
SPAn 5742	9- 9-66	Casa Grande	Schiffornis virescens	"
SPAn 6109	11-24-66	Itapetininga	Dendrocolaptes platyrostris	"
SPAn 6142	12- 1-66	Casa Grande	Dysithamnus mentalis	"
SPAn 6212	12- 9-66	Itapetininga	Sporophila caerulescens	"
SPAn 6236	12-16-66	Casa Grande	Sittasomus griseicapillus	"
SPAn 6241	12-16-66	Casa Grande	Basileuterus auricapillus	"
SPAn 6291	12-23-66	Itapetininga	Platyrinchus mystaceus	"
SPAn 6789	3- 3-67	Casa Grande	Turdus albicollis	"
SPAn 6829	3-10-67	Itapetininga	Emberezoides herbicola	"
SPAn 7126	4- 6-67	Itapetininga	Platyrinchus mystaceus	"
SPAn 10590	6-28-68	Guaratuba	Crypturellus noctivagus	= An 11916 (SLE)
SPAn 11823	1-17-69	Itapetininga	Thraupis sayaca	"
SPAn 12607	4-15-69	Guaratuba	Chiroxiphia caudata	= Ar 11921 (G. Phlebotomus)
			Ramphocelus bresilius	" "

observed. We bled the Casa Grande people again and the results will be reported in the future.

The third virus isolated from birds belongs to the Phlebotomus Group and it was recovered only in 1969. This agent was shown by CF, HI and NT tests, to be closely related to Icoaracy virus. This virus was also isolated from different species of Anopheline and Culicine mosquitoes in a total of 13 isolations from mosquitoes but no isolations came from wild mammals or sentinel mice or hamsters exposed in the collecting station.

If this virus is able to infect the residents from the collecting stations or not will be reported in the future. From all these agents isolated from birds as described we obtained good HA antigens and HI tests performed with the avian sera extracted with acetone showed some reactions, specially from SLE antigen, and the results are being tabulated to be reported.

(O. S. de Souza Lopes, I. M. Fonseca, L. A. Sacchetta)

REPORT FROM THE INSTITUTO DE VIROLOGIA CORDOBA, ARGENTINA

During this year the endemic area of Argentine Hemorrhagic Fever in Cordoba Province has apparently expanded to the North, through Laboulaye city (12,000 inhabitants). Reported cases occured in farms up to 20 km North from this city.

At least 6 human cases were confirmed by virus isolation or serological conversion in Laboulaye city, among individuals that did not go to the field 20 days before the onset of the illness.

From the urban and suburban blocks, 126 Mus musculus, 19 Calomys musculinus, 1 Calomys laucha and 1 Rattus sp. were trapped. From the railroad track crossing the city, 102 Mus musculus, 45 C. musculinus, 8 C. laucha, 1 Rattus sp. and also 3 Akodon azarae and 1 Oryzomys flavescens were collected.

Junín complement fixation antigen was detected in the brain of 3 C. musculinus trapped in the railroad. We have evidences from previous work that this rodent species is a reservoir of Junín virus.

(M. S. Sabattini)

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

Purification of Dengue Virus by Gel Filtration

BHK-21 cell monolayer cultures (cultivated with a medium consisting of 5% bovine serum in Eagle MEM) were infected with type 1 dengue Mochizuki strain virus. Two hours post infection the cultures were washed twice with Earle solution (pH 7.4), and then "virus growth medium" consisting of 0.4% bovine serum albumin in Eagle MEM was added. Temperature of incubation was 37° C throughout. Maximum titers of virus in the fluid phase (10^6 - 10^7 PFU/ml) were obtained at the 4th day after infection, and this level of infectivity continued for 5 or 6 days thereafter by replacing the medium every day. The culture fluid harvested from the 4th to 10th days and pooled was used as starting material.

The sample was centrifuged at 1,000 g for 10 minutes, and the supernatant was added with 2M zinc acetate at a final concentration of 0.04M, at pH 6.8-7.2 adjusted with 1N NaOH. After being held at 4° C for 3 hours, the precipitate was collected by centrifugation at 1,000 g for 30 minutes and dissolved in saturated EDTA solution (pH 7.8 adjusted with solid tris-amino methane) of 1/50 volume of the starting material. This suspension was passed through a Sephadex G200 column (2.5 x 50 cm) twice, and a Sepharose 2B column (2.5 x 100 cm) once, which had been equilibrated with 0.13M-0.05M tris buffer (pH 7.8). The filtrate was concentrated in a collodion bag to a final volume of 1 or 2 ml. The virus recovery rate was 30-40%, and the purification was approximately 1,000 times per mg protein, with respect to HAU and PFU.

It was shown that the purified virus was of heterogeneous composition:

(i) Sepharose column chromatographic patterns showed a broad peak of HAU and PFU, suggesting the heterogeneity of virus particles.

(ii) Electron micrographs revealed three different particle sizes: Major particles (about 60-70% in number, based on the calculations of limited extent) were approximately 40 mμ in diameter, and minor particles (30-40% in number) were about 20 mμ in diameter. A few number of large particles which were two to four times larger than the major ones was also seen.

(iii) Sucrose density gradient centrifugation patterns showed two HAU peaks, the top fraction of which had no infectivity.

(iv) In the CsCl equilibrium density gradient centrifugation, three HAU peaks were noted, representing the buoyant densities (g/cc) of 1.24, 1.21, and 1.18, respectively; the lowest density fraction was non-infectious.

Further studies are now in progress to improve the purification techniques, and in addition other arboviruses including chikungunya, Japanese encephalitis and yellow fever viruses are being tested.

(Y. Yoshinaka, M. Takehara, and S. Hotta)

REPORT FROM THE DEPARTMENT OF PREVENTIVE MEDICINE
INSTITUTE OF MICROBIOLOGICAL DISEASES
OSAKA UNIVERSITY
OSAKA, JAPAN

An intracellular component associated with chikungunya virus-specific RNA was isolated from BHK-21 cells infected with the virus. This component, arbitrarily named "X", contained 45S RNA, together with 26S RNA. While the RNA from partially purified virus contained only 45S RNA. In both cases, infectivity of RNA was associated with the 45S peak. After glutaraldehyde treatment, "X" formed a single peak at a density of 1.32 g/cc in CsCl isopycnic density gradient sedimentation. While partially purified virus formed a single peak at a density of 1.24 g/cc without glutaraldehyde treatment. The "X"-component was shown to contain also virus-specific antigen, and the formation of "X" from free chikungunya virus-specific RNA seems to require de novo synthesis of protein, which is sensitive to cycloheximide. By morphological observation under an electron microscope, the fraction containing the "X" showed spherical particles of 28-32 mμ diameter. These particles have no outer envelope with projections, which can be observed in mature virus particles.

From these observations, it seems likely that the "X" is a nucleoprotein core of the virus accumulating inside the infected cells.

(A. Igarashi and K. Fukai)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY
SEOUL NATIONAL UNIVERSITY COLLEGE OF MEDICINE
SEOUL, KOREA

Isolation and Serologic Studies of Japanese Encephalitis Virus in Snakes

It has been confirmed that Japanese encephalitis virus (JEV) proliferates and forms antibodies irregularly in the non-poisonous snakes of Korea after inoculation of the virus.

Since 1965, the author has been studying on the relationship between JE virus and snakes and has found that snakes collected in the field contain HI antibody to JE virus in relatively high proportion and has isolated 2 strains of JE virus from the snakes collected in the field during epidemic season of the encephalitis. Statistics of Japanese encephalitis in Korea in recent years are as follows.

Japanese Encephalitis in Korea

Year	Reported cases	No. of death	Fatality
64	2, 955	971	32.8
65	752	284	37.8
66	3, 595	957	26.6
67	2, 691	810	30.1
68	1, 226	396	32.3

Isolation of JEV from snake plasma was done in primary chick embryo cells with agar overlay, HI antibody to JEV in snake plasma was detected by modified Buescher et al's method and plaque neutralizing antibody was detected in primary chick embryo cells.

In 1965, HI antibody to JEV in the snake plasma was detected for the first time and 45% of the 210 snakes collected (Table 1) were positive. In 1966, HI antibody was proved in 43% of 304 snakes collected in nature. As a result of plaque formation with snake plasma in primary chick embryo cells, Japanese encephalitis virus was isolated from one of 304 collected snakes. This JE virus was isolated from the snake (E. rufodorsata CANTOR) collected in Pupyung about 40 Km away from Seoul on October 19, 1966 and was named S-6-182 strain (Table 2, 6).

TABLE 1

Occurrence of HI Antibodies to JEV in Non-poisonous Snakes of Korea Collected in 1965

$$\frac{\text{No. of HI positive}}{\text{Total No. tested}} = \frac{95}{210} = 45\%$$

Species of snake	No. of HI positive / No. of tested	HI titer to JEV	No. of snake	% of HI positive
Elaphe rufodorsata CANTOR	87/155	< 10	68	56
		10	14	
		20	32	
		40	27	
		80	9	
		160	4	
		320	1	
Natrix tigrina lateralis BERTHOLD	7/50	< 10	43	14
		10	6	
		20	1	
Elaphe schrenckii STRAUCH	1/4	< 10	3	25
		10	1	
Dinodon rufozonatum rufozonatum CANTOR	0/1	< 10	1	

TABLE 2

Occurrence of HI Antibodies to JEV in Non-poisonous Snakes of Korea Collected in 1966

$$\frac{\text{No. of Positive}}{\text{Total No. tested}} = \frac{132}{} = 43\%$$

Species of snake	No. of HI positive / No. of tested	HI titer to JEV	No. of snake	% of HI positive
Elaphe rufodorsata CANTOR	128/184	<10 10 20 40 80	56 44 66 17 1	60
Natrix tigrina lateralis BERTHOLD	1/11	<10 20	10 1	9
Elaphe schrenckii STRAUCH	1/6	<10 10	5 1	17
Dinodon rufozonatum rufozonatum CANTOR	2/3	<10 10 20	1 1 1	67

$$\frac{\text{No. of Positive}}{\text{Total No. tested}} = \frac{215}{535} = \% \text{ of Positive} = 40\%$$

Species of snake	HI titer	Month of Year								
		March	April	May	June	July	August	September	October	November
P. rufodorsata CANTOR	<10		133	30	4	8	2	41	25	54
	10		11	10		3	2	18	10	14
	20		8	13		11		33	31	7
	40			2	1	11	3	16	5	1
	80		1			2				
N. tigrina lateralis BERTHOLD	<10	1	1	12		2		4	1	1
	10									
	20			1		1				
F. schrenckii STRAUCH	<10		3	1				1	3	1
D.R. rufozonatum CANTOR	<10		1							
No. of Positive / No. tested		0/1	20/158 (13%)	26/69 (37%)	1/6 (17%)	28/38 (74%)	5/7 (71%)	67/103 (60%)	46/75 (61%)	32/78 (28%)
Total No. tested		535								

Table 3

Table 4

Occurrence of HI Antibodies to JEV in
Non-poisonous Snakes of Korea Collected
in 1968

$$\frac{\text{No. of Positive}}{\text{Total No. tested}} = \frac{400}{540} = 72\%$$

Species of snake	No. of HI positive / No. of tested	HI titer to JEV	No. of snake	% of HI positive
Elaphe rufodorsata CANTOR	399/525	< 10	146	72%
		10	111	
		20	142	
		40	87	
		80	39	
Natrix tigrina lateralis BERTHOLD	0/8	< 10	8	0
Elaphe schrenckii STRAUCH	1/7	< 10	6	14%
		10	1	

Table 5

Occurrence of Plaque Neutralizing Antibody to JEV in Snakes of Korea Collected in 1968

$$\frac{\text{No. of Positive}}{\text{Total No. tested}} = \frac{9}{412} = 2\%$$

Species of Snake	No. of neutralizing antibody positive / No. of tested
Elaphe rufodorsata CANTOR	9/399
Natrix tigrina lateralis BERTHOLD	0/7
Elaphe schrenckii STRAUCH	0/6

Table 6

Isolation of JEV in Non-poisonous Snakes of Korea Collected in Nature

Species of snake	No. of JEV Isolation / Total No. tested	
	1966	1967
Elaphe rufodorsata CANTOR	1*/184	1**/510
Natrix tigrina lateralis BERTHOLD	0/11	0/24
Elaphe schrenckii STRAUCH	0/6	0/10
Dinodon rufozonatum rufozonatum CANTOR	0/3	0/1

*: JEV S-6-182 (10/19/66 Pupyung, Kyungdo)
**: JEV S-7-283 (7/16/67 Changwidong, Seoul)

In 1967, HI antibody to JEV was proved in 40% of the 535 snakes collected. The S-7-283 strain of Japanese encephalitis virus was isolated from E. rufodorsata CANTOR collected on July 16, 1967 in the suburb near Seoul. E. rufodorsata CANTOR is the most prevalent snake in the field and inhabits near or in rice paddies, the main breeding and living sites of JE vector mosquito C. tritaeniorhynchus.

As in Table 4, HI antibody levels of the snakes caught during epidemic season are much more higher than the snakes caught pre- and post-epidemic seasons of Japanese encephalitis.

In 1968, HI antibody was proved in 72% of 540 snakes collected and neutralizing antibody to JEV was positive in 2% of the snakes. As the tables show, HI antibody titer to JE virus was proved to be low in general, but the highest one was 1:320. Most of them belonged to between the range of 1:10 to 1:40.

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(H. W. Lee)

REPORT FROM THE WHO - JAPANESE ENCEPHALITIS VECTOR RESEARCH UNIT AND THE JAPANESE ENCEPHALITIS RESEARCH INSTITUTE OF THE REPUBLIC OF KOREA SEOUL, KOREA

Early in 1969 WHO established a Japanese Encephalitis Vector Research Unit (JEVRU) at the National Institute of Health (NIH) of the Ministry of Health and Social Affairs, Seoul, Korea. JEVRU also has a field station in an endemic area in Cholla Pukdo about 370 Km south of Seoul.

At the same time NIH established the Japanese Encephalitis Research Institution (JERI). JERI consists of an Administrative Section, JE Virology Division, Vector Division and Epidemiology Division. JERI and JEVRU cooperate in their studies of Japanese Encephalitis. JERI soon will move into new laboratory and office facilities at NIH.

In October 1969 WHO had a programme planning meeting in Seoul and outlined a research endeavour for the next several years. This programme will include research on Culex tritaeniorhynchus and other possible vectors of JE. Population assessments of larval and adult mosquitos will be made as well as life cycle, host preference, overwintering and other bionomic studies. The natural history of JE virus will be studied. This will include virus isolation attempts and serologic surveys of potential reservoir hosts and vectors. Research on control of vectors and the establishment of a surveillance system will be done. Also, in October a similar committee met in Taiwan to plan a programme for a JEVRU to be established soon in the Republic of China.

The number of human cases of JE reported from Korea in 1969 was very low. As of the end of September only about 50 cases were confirmed serologically. Virus isolation attempts were made from 18 pools of Culex tritaeniorhynchus collected from Sintaein, Korea. All were negative.

REPORT FROM THE ARBOVIRUS LABORATORY
PASTEUR INSTITUTE AND ORSTOM
PHNOM-PENH, CAMBODIA

Isolation From Human Cases

238 sera were inoculated and 29 strains were isolated: 2 were chikungunya virus, one from an European, 36 years, the other from a 5 year old Cambodian child who presented diphasic fever with thrombocytopenia. All the other strains belong to group B and are yet to be typed, but it seems that there are dengue virus, type 2 for the most and also type 4. The diseases appeared during the rain season, especially in May, June and July, and induced little outbreaks in European families. In the local population, they were isolated cases among children below 10 years. The disease was clinically characterized by diphasic fever, rash and also important arthralgia with chikungunya virus.

In 1969, physicians have observed few haemorrhagic fevers (from only thrombocytopenia to important gastrointestinal haemorrhage, preceded by a sudden chock or collapse and sometimes followed by death). 14 sera were collected, and one strain of chikungunya virus was isolated.

Isolation From Vertebrates

1. 548 specimens were collected:

312 snakes (Enhydris enhydris)

426 lizards (Gecko gecko, Hemidactylus frenatus, Calotes versicolor, Mabuya multifasciata),

157 frogs,

218 rodents (Rattus and Scincus),

132 sparrows,

303 bats (Cynopterus, Tadarida, Roussetus, Scotophilus, and Taphozous).

One strain was isolated, A - 38/69, from salivary glands and interscapular brown fat of 8 Cynopterus brachyotis angulatus. This strain, belonging to group B, seems unrelated to our reference strains (Chik, Getah, Dengue types 1, 2, 3, 4, JBE, West-Nile and Langat). A - 38/69 was sent to Dr. Downs for complete identification. Mosquitoes Culex pipiens fatigans and Aedes aegypti were infected by feeding on viremic infant mice inoculated with A - 38/69. It seems that transmission was possible with Aedes aegypti. No result with C. pipiens.

Isolation From Arthropods

46.000 arthropods have been pooled for isolation

(Table 1)

37.000 mosquitoes

9.000 bugs caught on bats.

4 strains were isolated, one from Culex tritaeniorhynchus was a JBE virus, 2 from Aedes nocturnus and Anopheles vagus were dengue type 2. The fourth was perhaps an arbovirus, ether resistant, desoxycholate sensitive, did not give hemagglutinin, but induced arboviral histopathological lesions in the brain of newborn mice.

Serological survey

293 human sera were tested with HI. 44 showed significant antibody conversion to dengue antigen (principally type 2) and only 2 to chikungunya virus.

The survey of vertebrate sera showed that 30% of animals (lizards, batrachians, birds, mammals) have antibodies against chikungunya and dengue viruses. 70% of snakes have the similar antibodies. Bats have principally antibodies against JBE and WN viruses. Only 3 bats of 55 tested have HI antibodies against A - 38/69.

TABLE 1

Species	Lots	Quantity
Aedes aegypti	23	8 691
nocturnus	1	209
mediopunctatus	1	100
autres	1	118
Total	26	9 118
Anopheles vagus	8	1 939
annularis	2	133
autres	4	222
Total	14	2 294
Culex pipiens fatigans	23	7 706
tritaeniorhynchus	27	9 816
brevipalpis	7	1 875
annulus	12	2 234
gelidus	10	1 345
pseudovishnui	1	487
autres	7	403
Total	87	23 866
Mansonia	3	889
Autres	15	586
Total général	145	36 753

SPECIES	CAUGHT	NUMBER OF POSITIVE REACTIONS	MAN	BOVID SHEEP	DOG	CAT	PIG	RAT	MAMMALS UNKNOWN	BIRDS	REPTILE AMPHIBIA	ASSOCIATIONS
AEDES AEGYPTI	INSIDE	178	174	1	2	1	0	0	0	0	0	0
	OUTSIDE	134	113	14	1	1	0	1	1	3	0	0
CULEX TRITAE-NIORHYNCHUS	INSIDE	214	1	156	10	0	35	0	2	6	0	4
	OUTSIDE	2.870	11	2.616	25	0	157	2	6	27	1	25
CULEX PIPIENS FATIGANS	INSIDE	616	380	4	64	2	1	1	12	136	2	14
	OUTSIDE	1.568	421	58	186	4	10	12	40	766	0	71
CULEX ANNULUS	INSIDE	229	5	197	8	0	5	2	0	8	0	4
	OUTSIDE	1.420	5	1.315	37	0	22	2	3	33	0	3
CULEX GELIDUS	INSIDE	55	0	53	0	0	1	0	1	0	0	0
	OUTSIDE	357	3	333	4	0	11	0	0	1	0	5

Identification of the Blood Meals of Mosquitoes

Over 10.000 identifications by test of diffuse precipitation in agar gel were performed (Table 2 for the 5 most important species).

(J. J. Salaun and J. M. Klein)

REPORT FROM THE VIRUS RESEARCH INSTITUTE DEPARTMENT OF MEDICAL SCIENCES BANGKOK, THAILAND

An Outbreak of Japanese Encephalitis in Thailand

In 1969 encephalitis has emerged as a serious public health matter in Chiang Mai Province, the largest city in the North. The number of cases were unusually increased in the third week of June, reached its highest peak in the second week of July and gradually declined from the beginning of August. There were altogether 185 cases with 45 deaths.

Age and sex distribution of encephalitis cases and death in Chiang Mai from April - 9 August 1969 is shown in Table 1.

Table 1 - Age and Sex Distribution of Encephalitis Cases and Deaths in Chiang Mai (April - 9 August 1969)

Age / year	Male	Female	Total
Under 1	1	2	3
1 - 4	22 (3)	8 (1)	30 (4)
5 - 9	34 (10)	19 (6)	53 (16)
10 - 14	38 (13)	24 (7)	62 (20)
15 - 19	13 (2)	6 (1)	19 (3)
20 over	10 (2)	7	17 (2)
Unknown	1	-	1
Total	119 (30)	66 (15)	185 (45)

( ) = Number of deaths

The illness begins quite acutely with fever, marked and increasing disturbances of the sensorium, signs of meningeal irritation and followed with cerebral manifestations progressing to coma. The cerebrospinal fluid almost always shows abnormalities, consisting of mild to moderate pleocytosis (mostly lymphocytes), and a raised protein level. The severe case usually dies about the end of first week. Convalescence is usually prolonged, with pre-existing weakness, lethargy, incoordination, tremors and some mental impairment.

Laboratory diagnosis by virus isolation was carried out with three brain materials obtained at post-mortem. The brain suspension was inoculated intracerebrally into suckling mice. The virus was isolated from one brain of which the autopsy was performed two hours after death. This isolated virus was further tested and proved to be a strain of JE virus which resembled the Jagar strain. Laboratory diagnosis by direct fluorescent antibody technique was also applied with these brain materials and JE virus could be detected in two brains.

Paired sera specimens from 22 cases were examined by HI test against JE-Jagar, JE-Nakayama, and Dengue 1-4 virus antigens. Positive JE infection based on four fold or greater increase of HI antibody against JE antigen was confirmed in 18 cases, and the reaction titer to JE-Jagar was definitely greater than that to JE-Nakayama. Although in some cases cross reactions to dengue viruses were demonstrated, such antibody titers were rather low

when comparing with those against JE virus.

Sera obtained from 20 slaughtered pigs in Chiang Mai Province were tested for HI antibody against JE and Dengue 1 - 4 viruses. All of them showed high HI antibody to JE virus, ranging from 1:160 to 1:5120; and in the sera of 3 pigs the decrease of HI titers 4 to 8 times after being treated with 2 mercaptoethanol was demonstrated. These observations support that pigs might be an amplifier host of JE virus in this area.

The isolation of JE virus from mosquitoes collected from Chiang Mai has not yet been successful.

(Prakorb Tuchinda)

REPORT FROM THE VIRUS DIVISION INSTITUTE FOR MEDICAL RESEARCH KUALA LUMPUR, MALAYSIA

The arbovirus section of the Virus Division started to function once again in May 1968. The first few months were spent preparing stock virus suspensions, hyperimmune sera and antigens of those arboviruses which are known to occur in West Malaysia. The seed viruses were kindly supplied by Dr. A. Rudnick of the Arbovirus Research Laboratory, Bacteriology Dept., University of Malaya.

In October 1968 a combined project with Dr. C. E. Gordon Smith of the MRC of Britain and this Institute was initiated to study the ecology of JE in Sarawak, East Malaysia. This was to last for 18 months. It was based on their hypothesis, as a result of previous study in the region, that JE was maintained throughout the year by pigs and the mosquito C. gelidus and transferred to man by Culex tritaeniorhynchus. We have selected as a study area a land dyak village called Tijarak on the 20th mile Serian road which consists of wet and dry padi fields, pepper gardens, secondary jungle and an old rubber estate. There are 3 full time entomologists from the U.K. working in the study area. Frequent visits are made by visiting entomologists, zoologists, virologists and epidemiologists from the U.K. and the IMR for short periods.

In order to clarify the epidemiology of JE, quantitative knowledge is required about the available mosquito and vertebrate populations and their relationships, about the time required for an infected mosquito to become infective, about what proportion of mosquitoes survive long enough for this and how frequently they bite thereafter. A bleeding programme among the villagers and other vertebrates in the study area have been carried out. Sera obtained will be tested for antibodies.

The mosquitoes are collected, identified and pooled according to species, site and date of collection and air-freighted from Sarawak to Kuala Lumpur in sealed bottles on dry ice. They are immediately stored at -70°C until they are processed. We have made 48 isolations in suckling mice out of a total of 620 pools processed so far. They have been sent to the Microbiological Research Establishment, Porton, England for identification.

Virus Isolation from Mosquito Pools

Mosquitoes	Virus isolation	No. of Pools Processed
Anopheles spp.	Unidentified (7)	74
A. curtipes	J.E.V. (1)	2
C. gelidus	Unidentified (5)	50
C. pseudovishnui	Unidentified (3)	14
C. tritaeniorhynchus	J.E.V. (1) Tembusu (1) Unidentified (25)	303
Culex spp.	Unidentified (1)	19
Mansonia bonneae dives	Unidentified (1)	55
Mansonia spp.	Unidentified (2)	25
Mansonia uniformis	Unidentified (1)	21

REPORT FROM THE MYSORE VIRUS DIAGNOSTIC LABORATORY SHIMOGA, MYSORE, INDIA

As usual, the Surveillance for the detection and follow-up of Kyasanur Forest Disease (K. F. D.) was continued during the year 1968-1969.

The Human incidence of K. F. D. during the current epidemic season was restricted to the period from December 1968 to June 1969. Blood specimens from 240 cases and autopsy materials of 15 monkeys yielded the KFD Virus during this period.

It may be noted here that the number of Virus positive human cases is the highest during the current epidemic season when compared with the number of similar cases in any of the corresponding previous epidemic seasons. Though no specific reasons could be attributed to this highest incidence, streamlining the surveillance machinery has played a positive role in detecting more number of Virus Positive cases during the current epidemic season.

The epidemic occurred not only in the known old theatres but also in the new theatres involving 34 new villages adjoining the old theatres.

Of the above Virus Positive cases, 14 cases ended fatally giving a case fatality rate of 5.83. These cases were either untreated or hospitalised in advanced states of the Disease.

An attempt was made to find out whether Control and Preventive measures could be contemplated against the incidence of this disease which is occurring regularly in the dry months of every year. A series of field experiments were designed and conducted from January 1968 to May 1968 as a joint project of the Mysore Department of Health and Family Planning Services and the Virus Research Centre, Poona, using Lindane 20% E. C. as an ixodicide sprayed on the Forest floor. These studies had shown promising results in this regard.

In order to further assess the value of the method of "area spraying" of BHC (Lindane quality) with a view to devise comprehensive methods for the control of ticks as a means for the control of K. F. D. infections in man,

further large-scale experiments are proposed during the next KFD season commencing from January 1970.

(R. Rama Rao)

REPORT FROM THE DEPARTMENT OF PREVENTIVE MEDICINE UNIVERSITY OF QUEENSLAND MOGGILL, AUSTRALIA

Allantoic Fluid as a Source of Arbovirus Haemagglutinin

Some arboviruses will multiply in developing chicken embryos, and virus is present in high titre in the allantoic fluid. The following method was used to prepare haemagglutinin for Sindbis virus and Murray Valley encephalitis virus from allantoic fluid from infected eggs.

Allantoic fluid from dead embryos was harvested and treated with both Tween-ether and arcton-heptane. The order of treatments was not important, but untreated allantoic fluid contained no detectable haemagglutinin, and allantoic fluid subjected to only one of the procedures contained very little haemagglutinin. Treated allantoic fluids contained haemagglutinins at titres of up to 1/128 for Sindbis virus, and 1/256 for Murray Valley encephalitis virus. The haemagglutinins were not infective. In the haemagglutination-inhibition test, antigen of egg origin reacted similarly to antigens prepared by sucrose-acetone extraction of mouse brain, and homologous antisera inhibited both preparations to the same titre.

Allantoic fluid may prove to be a convenient source of non-infectious antigen for arboviruses that multiply in the chick embryo. Allantoic fluid is more convenient to harvest than suckling mouse brain, and treatment with Tween-ether and arcton-heptane is more rapid than sucrose-acetone extraction.

(Y. S. Chung, and P. B. Spradbrow)

REPORT FROM THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH BRISBANE, AUSTRALIA

The following extracts are from the Annual Report of the Institute for 1968-1969. A limited number of copies of the full report may be obtained on request from Dr. R. L. Doherty.

"Several long term studies of arbovirus epidemiology continued this year, with most activity directed to the role of mammals in arbovirus survival at Mitchell River, further investigation of arbovirus activity in south-west Queensland, and to completion of a collaborative study of an epizootic of ephemeral fever of cattle. Perhaps the most important progress came from the development, in association with CSIRO workers, of techniques for handling the small arthropods Culicoides and Phlebotomus. This led to the isolation of three previously undiscovered viruses from Culicoides (two shown to be members of the Simbu group) and one from Phlebotomus. Other findings described in the body of this report concern isolation of another 'new' virus from mosquitoes, antibody surveys to various arboviruses including several of the 'new' agents, antibody and virus isolation evidence of the importance of wallabies as hosts of Ross River virus (the suspected cause of epidemic polyarthritis), and evidence of Japanese B encephalitis virus causing febrile illness in Australian servicemen in Vietnam.

Fifteen strains of viruses probably not arboviruses have also been isolated in weaned mice from ectoparasites or organs of animals taken at Mitchell River. Studies so far suggest some unusual properties and attempts are proceeding to identify these agents.

The entomology section carried out collections of arthropods at Brisbane, Charleville and Mitchell River. Large populations of Culicoides were encountered in all areas sampled, and were the dominant group of haematophagous arthropods at both Charleville and Mitchell River. This hitherto neglected group contains species feeding on birds and mammals and must be considered in studies of arbovirus ecology. Arthropods of the genus Phlebotomus, erroneously regarded as rare by many Australian entomologists, were collected in all areas, especially at Charleville. The large numbers of Culicoides and mosquitoes collected in the Charleville district in spite of drought indicated the importance of stream flow as well as local rainfall in studies of arbovirus ecology in this area.

The start is also reported of a study of experimental infection of mosquitoes by Ross River virus. Mechanical transmission by Aedes aegypti and biological transmission by both Aedes vigilax and Aedes aegypti were demonstrated. Further techniques were developed for infecting and manipulating infected mosquitoes in the laboratory.

Biochemical investigations of arboviruses were greatly facilitated by the development of a rapid method to concentrate and purify infectious virus particles. From purified Kunjin virus labelled with isotopes in cell cultures, four proteins were separated, and three additional virus-specific proteins were separated from the contents of infected cells. Previous observations on the greater specificity of 19S (large) antibodies than 7S (small) antibodies were extended by challenge experiments in rabbits using related group A or group B arboviruses.

Four selected strains of each of Murray Valley encephalitis and Kunjin viruses were investigated by standard and more sensitive serological techniques to determine the extent of intratypic antigenic variation. Although variations in reactivity of individual strains of each virus were detected, it was concluded that MVE virus strains comprised a homogeneous group whereas Kunjin virus strains exhibited some antigenic variation. These two closely related members of the group B MVE virus subgroup (MVE and Kunjin viruses) could be clearly differentiated by all selected sensitive techniques.

In other laboratory studies of arboviruses, an investigation of lipid inhibitors of arbovirus haemagglutination was completed, a cell line from Vipera russelli obtained from U.S.A. was found susceptible to a Queensland arbovirus isolated from a skink, and material for electron microscopy was provided for a collaborating scientist at the University of Melbourne.

(R.L. Doherty, J.G. Carley, E.G. Westaway, H.A. Standfast, E.J. Wetters, B.M. Reedman)."

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

Rift Valley fever

After an apparent absence of 13 years Rift Valley fever virus appeared in 1969 in Southern African with epizootics in Rhodesia, Mocambique and South Africa. The Rhodesian outbreak was very extensive and involved mainly cattle (sheep are not common) on some 200 farms, largely in the environs of Salisbury at altitudes of 4-5000 feet. A few human cases occurred, most of them apparently following autopsies on dead bovines. The outbreaks in Mocambique and South Africa were limited in extent. The present occurrence showed similarity to the outbreak in South Africa in 1951 in that the virus made its appearance more or less simultaneously at widely separated localities, probably as a result of dispersal of the vector over considerable distances.

We had the opportunity to carry out field observations near Salisbury during May as the epizootic was ending. Frosts had already occurred and mosquito densities were generally rather low. The most prevalent species was Aedes lineatopennis which made up 74% of the total catch of 4, 933. Anopheles coustani was the next most prevalent with 8% of the total. Both species feed readily on cattle and seemed to be the most likely vectors. Only one specimen of Aedes caballus and 44 of Culex theileri, both implicated in previous outbreaks, were collected. Six hundred-and-fifty-two Culicoides were also collected.

All the mosquitoes and Culicoides were tested for virus and the results are shown in table 1. The isolation of Rift Valley fever virus from lineatopennis and coustani is in agreement with our field observations and their involvement seems possible. The isolations of Wesselsbron virus were unexpected as the presence of this virus had not been suspected. All 17 isolations of this virus came from the same farm and for various reasons it is unlikely that it was involved to a significant extent. Blooded females of lineatopennis and coustani were collected and from them adults were bred out for transmission experiments with Rift Valley fever virus. Transmission experiments were also done with Aedes circumluteolus which had been implicated in the outbreak in Mocambique.

Table 1.

Number of isolations from mosquitoes from Rhodesia, 1969

	R.V.F.	W'bron	Middelburg	Unidentified
Aedes lineatopennis	2	17	2	1
Aedes dentatus	1			
Anopheles coustani	1			
Culex theileri	1			

Table 2.

Attempts to transmit Rift Valley fever virus

Mosquito	Virus Dose	Infectivity Rate	Transmission on day shown Positive	Negative
A. lineatopennis	> 6.5	4/6	NF	NF
"	> 7.5	2/3	NF	NF
"	6.4	3/7	NF	NF
A. circumluteolus	6.4	4/4	NF	NF
"	4.8	12/56	Nil	14, 19
An. coustani	> 7.5	8/8	NF	NF
"	> 7.5	10/10	Nil	16
"	5.5	4/8	NF	NF
"	4.3	4/11	NF	NF

NF = No mosquitoes fed in transmission attempt.

The results of the transmission experiments are shown in table 2. Unfortunately feeding rates in transmission attempts were low so this aspect of the experiments was inconclusive. Nevertheless, evidence is accumulating from our own experiments and the work of others that leads us to doubt whether Rift Valley fever is in fact transmitted by mosquitoes to the extent necessary to explain the explosive outbreaks in cattle and sheep encountered in Southern Africa. By various workers the virus has been transmitted by Aedes aegypti, Aedes triseriatus, Aedes caballus and Eretmapodites chrysogaster but there is also an impressive number of failures from among these species as well as others which cannot be ignored. Since 1955 this Unit has carried out 40 attempts to transmit Rift Valley fever virus with 13 species of mosquito. A number of mosquitoes of several species was infected but only one transmission was successful; by A. caballus on the 8th day. There were 36 failures, including 17 by A. caballus, by individual mosquitoes from lots of mosquitoes known to have been infected.

Culicoides are common where these outbreaks have occurred and it seems that consideration should be given to them as possible vectors of this virus.

REPORT FROM THE EAST AFRICAN VIRUS RESEARCH INSTITUTE ENTEBBE, UGANDA

In March 1969, cases were reported from Lango District, Uganda, of a dengue-like fever with history of fever, joint pains, rash and enlarged lymphnodes. Attempted virus isolation from blood samples collected from acute cases was unsuccessful. Serological studies on convalescent samples and from people staying together with the cases gave a high percentage of HI o'nyong-nyong and chikungunya antibody positives. The plaque inhibition results available so far, kindly provided by Dr. J. S. Porterfield, Medical Research Council, National Institute for Medical Research, Mill Hill, London N. W. 7., show most of the samples more positive to o'nyong-nyong than CHIK antibodies. It is very likely that the outbreak was caused by o'nyong-nyong virus. No cases were further reported after the month of May.

The mosquito collection at Zika forest and the virus isolation studies have yielded 8 strains of group B viruses from A. africanus and one strain of RVF virus from Culex annulioris between the period of August and October

1969. Three of these strains MP 9501, MP 9521 and MP 9602 have been identified as Zika virus by neutralization test (NT) in mice. The rest are not yet identified. While trying to get information on the role of forest monkeys during this epizootic, monkeys from nearby forest (Kisubi) were collected by shooting and immediately bled by cardiac puncture for attempted virus isolation and antibody studies. A strain of RVF virus was isolated from one of the monkeys. No Zika virus was isolated. A later sample of monkey blood will be collected when the virus has passed through the area so as to compare the antibody pattern.

At the Zika forest and around Entebbe area Zika and chikungunya epizootics appear to occur at 6 to 8 year intervals. On the basis of the evident periodic nature of Zika virus recurrence and the immune status of monkeys in the Entebbe area which was found to be low (seven C. ascanius schmidti were collected from the area near Zika forest and none of these had Zika antibody) it was predicted, as far back as February 1969, that there would be another epizootic of Zika virus in the Entebbe area soon. The isolation of Zika virus at this time is, therefore, of particular importance.

The potential role of Aedes apicoargenteus as a vector of arboviruses has been tested in several transmission experiments in the laboratory using CHIK and Zika viruses. This has necessitated feeding adult mosquitoes reared from eggs obtained from wild-caught adults on viraemic vertebrate hosts and then processing the engorged mosquitoes in groups of five or less from day zero for inoculation into mice. Some of the mosquitoes were kept for a period between 6 and 10 days and were fed on litters of normal newborn mice. It was found that the mosquito maintains both viruses between 10 to 14 days and in case of CHIK virus, the mosquito is able to transmit the virus to the mice by bite. Work continues.

Further studies on the natural hosts of A. simpsoni have confirmed that where A. simpsoni is non-anthropophilic, it feeds on rodents. Monkeys and man appear not to be among the major hosts. It is suggested, therefore, that the apparent absence of yellow fever in most parts of Uganda is due to A. simpsoni being not only non-anthropophilic but also non-primatophilic.

(Dr. B.G. Kirya and Mr. L.G. Mukwaya)

REPORT FROM ARBOVIRUS RESEARCH UNIT
INSTITUT PASTEUR AND ORSTOM
BANGUI - REPUBLIC OF CENTRAL AFRICA

During the year 1968, human sera and mosquitoes collections have been pursued on many foci selected under previous serological surveys.

I. Material collected:

I.1 Human samples

I.1.1 102 sera from patients, 30 spinal fluids and 3 fragments from Burkitt Tumors were inoculated for Arbovirus investigation.

I.1.2 2092 human sera were inoculated in different places of the Republic of Central Africa and Chad. They were examined by HI test.

I.2 Culicidae (table 1)

I.2.1 Trapping of culicidae: 53, 341 adults mosquitoes were trapped amongst 114 collections. 25, 831 were inoculated by means of 813 pools.

I.2.2 Biology and Ecology of Arboviruses vectors
Research deals with biology and Ecology of Aedes simpsoni and Aedes africanus.

II. Results

II.1 Isolation of virus

II.1.1 Human samples
two viruses were isolated : 1 strain CHIK and 1 strain WN.

II.1.2 Culicidae (table 2)
18 strains were isolated - 4 are new ones.

- A 994 belongs to Simbu group.
  it gives cross reaction in C'F with INGWAVUMA, but is different in NT.

TABLE 1

Inoculated Species

Inoculated Species	Number of Pools	Number of Inoculated Mosquitoes
Anophèles moucheti	4	20
" paludis	8	45
" ziemanni	1	20
" gambiae	10	326
" nili	3	93
" coustani	5	308
" funestus	6	296
" obscurus	1	1
" marshalli	2	28
" squamosus	2	200
" (coustani+ziemanni)	3	78
Culiseta fraseri	2	2
Eretmapodites groupe chrysogaster	20	116
Eretmapodites dracaenae	2	21
Mansonia uniformis	7	79
" africana	27	1.095
" (mansonioides)	33	3.086
" pseudoconopas	39	355
" maculipennis	24	259
" groupe chrysosoma	188	101
" metallica	21	133
" cristata	2	5

Inoculated Species	Number of Pools	Number of Inoculated Mosquitoes
Aedes argenteopunctatus	26	302
" africanus	33	1.423
" circumluteolus	27	518
" aegypti	3	3
" groupe palpalis	30	702
" Kummi	11	31
" ingrami	8	37
" groupe domesticus	20	66
" groupe abnormalis	21	988
" cumminsii	16	75
" pubescens	1	2
" groupe tarsalis	7	17
" apicoargenteus	1	1
" fraseri	4	6
Culex guiarti	64	3.245
" pruina	59	2.701
" weschei	43	1.212
" tigripes	38	152
" poïcilipes	24	502
" fatigans	2	78
" perfuscus	59	4.577
" groupe rima	18	242
" nebulosus	4	10
" annulioris	6	19
" telesilla	3	70
" cinereus	9	179
" groupe decens	3	41
" divers	33	1.965

TABLE 2

Strains of Viruses Isolated in 1968

Number of Lots	Species and Number	Origin	Strains
A. 637	24 Culex divers	NGOUPE II	Ntaya
A. 651	37 Aedes africanus	BOBIA	A 490 Bouboui
A. 656	83 Culex divers	BOTEMBI	A 365 M'Poko
A. 659	100 Culex divers	BOTEMBI	West Nile
A. 839	100 Anophèles funestus	POTOPOTE (Bangui)	Nyando
A. 937	27 Culex guiarti	BOTEMBI	Souche nouvelle non groupée Botembe
A. 994	17 Culex guiarti	BOUBOUI sources	Souche nouvelle Groupe Simbu
A. 1024	100 Culex perfuscus	NGOUPE II	BA. 209 sous-type de Ntaya
A. 1070	100 Culex perfuscus	BOTEMBI	Sindbis
A. 1077	6 Mansonia maculipennis	BOTEMBI	Souche nouvelle non groupée
A. 1111	66 Culex perfuscus	OUANGO	A 365 M'Poko
A. 1140	100 Culex pruina	BOUINIMO	Mossuril
A. 1211	7 Culex tigripes	OUANGO	Sindbis
A. 1291	21 Culex Weschei	ILE DAVID	West Nile
A. 1327	35 Aedes palpalis	PATA	Souche nouvelle non groupée
A. 1344	100 Aedes africanus	LA GOMOKA	CHikungunya
A. 1346	100 Aedes africanus	LA GOMOKA	Zika
A. 1351	29 Aedes circumluteolus	LA GOMOKA	Simbu

- A 937. A 1077. A 1327 are all different and don't have any antigenic relationship with 80 Arboviruses to which they were compared in the WHO Regional Center.

II.2 Human Immunological surveys

II.2.1 Republic of Central Africa

In 1968, the area prospected was the small portion of Oubangui the climate of which is Sahelo-Soudanian. This area is very rich in wild animals, has features different from Silvatic Savannah.

Small rate of CHIK antibodies, but high percentage of antibodies for group B with single reactions for ZIK, WN, UGS.

II.2.2 CHAD

The investigation begun in 1967 was pursued in 10 different foci from the southern border of lake Chad - CHIK antibodies decrease from South to north, antibodies for group B are secondary type reactions.

II.3 Identification of isolated Strains

- Among the strains isolated during the past few years two are new ones

- A 490 BOUBOUI. Isolated from Anopheles paludis, belongs to group B, Related but different from UGS.

- A 365 M'POKO. Isolated from a pool of different Culex belongs to group Turlock related to Yaba I in FC, but different in NT.

(JP. Digoutte Institut Pasteur, F.X. Pajot ORSTOM)

REPORT FROM THE ARBOVIRUS LABORATORY
UNIVERSITY OF IBADAN, NIGERIA

Investigation of Yellow Fever in the Jos Area

On Thursday, 23rd October, 1969, at 2.00 p.m., the Virus Research Laboratory of the University of Ibadan, in its capacity as the World Health Organization Yellow Fever Diagnostic Centre for Nigeria, was notified by Dr. V. Radojcic, WHO Lagos, of the occurrence of possible cases of Yellow Fever in the Jos area, Benue Plateau State. Arrangements were immediately made to depart for Jos, and on Friday, 24th October, a team consisting of an entomologist, a veterinarian and a physician departed for Jos. We arrived on Saturday evening, 25th October. Our information was that cases compatible with yellow fever had been seen at the Evangel Hospital Jos, and at the Benue Plateau Hospital. Material from some of the patients at the Evangel Hospital had been forwarded to Ibadan, but had not arrived by the time of our departure. These specimens arrived in fact about 2 hours after the departure of the team on Friday morning. The 12 serum samples from 9 patients were immediately inoculated into mice and set up for CF testing.

During our four-day stay in Jos, a number of cases of illness entirely compatible with yellow fever were seen, both at Evangel Hospital Jos, and at the Vom Christian Hospital, Vom. Acute phase serum specimens were obtained from febrile patients for preservation in liquid nitrogen until inoculation into mice for virus isolation at Ibadan could be done. It was also possible to obtain two post-mortem liver specimens for virus isolation attempt and also microscopic pathology.

On Monday afternoon, 27th October, word was received that the Ibadan laboratory had obtained the sera previously sent by the Evangel Hospital and in complement-fixation tests, one of these sera had shown a definite antibody rise for yellow fever. Two other single sera had low levels of antibody.

Examination of Hospital records revealed nine cases of death compatible with yellow fever at the Evangel Hospital and 29 cases at the Vom Christian Hospital. The earliest cases were seen at the end of August at the Vom Christian Hospital, but all but six of the cases occurred during October at both hospitals.

After returning to Ibadan on Friday evening, 31st October, over 50 acute-

phase specimens from patients were inoculated into suckling mice in virus isolation attempts.

On Saturday, 1st November, mice inoculated with material previously sent from the Evangel Hospital showed signs of illness. The brain of a sick mouse was shown by complement-fixation test to contain yellow fever virus. This identification was confirmed in a neutralization test. In addition, paired sera from one other patient which were brought by the investigating team to Ibadan from Jos were shown in complement-fixation to have developed antibodies against yellow fever virus.

Patients dying came from many widely scattered towns on the Jos plateau. No definite information was available regarding the occurrence of cases off the plateau.

By 15th November, a total of 9 yellow fever isolates had been identified with another 8 awaiting testing. Entomological investigations on the plateau have thus far shown the presence of A. simpson, A. africanus, and A. aegypti.

REPORT FROM THE ARBOVIRUS LABORATORY PASTEUR INSTITUTE AND ORSTOM DAKAR (SENEGAL)

Surveillance for arboviruses was continued at Bandia and Saboya field stations. (see AVIE, 1968, n° 18, 48-52).

1. BANDIA

1.1. Human studies

From January through September 1969, 204 human samples have been collected for virus studies. These specimens come from febrile children seen at the Bandia dispensary.

Eight isolations were made from which six have been identified as Tataguine

virus. Three available paired sera show sero conversion for Tataguine virus in CF test. The prototype strain was isolated from mixed Culex sp. and Anopheles sp. pool in 1962 (CAV, 1967, n° 190). Several isolates of the same virus were obtained from exanthematic fever cases in Yaounde (Cameroon) and Bangui (RCA) and from Anopheles gambiae mosquito pools in Yaounde.

1.2 Arthropods studies

Mosquitoes were collected on human bait around the dispensary. They have not yet been tested for virus in suckling mice : Anopheles gambiae was by far the most prevalent species.

1.3 Wild animal studies

One virus was isolated from Roussettus but it has not yet been typed. An other isolate from Tatera valida collected in Thiès area has been identified as Bandia virus.

2. SABOYA

Two isolations have been obtained from blood of Tatera valida. They both belong to Group B but they have not yet been typed.

Tatera valida isolate (RV 3150) stated as a new virus in the eighteen issue of the Arthropod-borne Virus Information Exchange, has been shown to be related to (if not identical with) Fika virus previously isolated in Ibadan. (AVIE, 1968, n° 18, 52-54).

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

REPORT FROM MEDICAL ZOOLOGY DEPARTMENT
UNITED STATES NAVAL MEDICAL RESEARCH UNIT NUMBER THREE
CAIRO, EGYPT, U. A. R.

Studies as background for tick-virus research

Ornithodoros (Alectorobius) muesebecki from a marine bird colony off the Arabian coast (described in Proc. Entomol. Soc. Wash. 71 (3):368-374, 1969) is closely related to O. (A.) amblus, from which Puentas Salinas virus has been isolated and which causes illness among guano laborers on islands off Peru. An effort is being made to follow up clues that muesebecki or a related species is responsible for fevers among tick-bitten oil rig workers in the Persian Gulf not far from the type locality of muesebecki.

Despite many published reports of Argas (Persicargas) persicus from southern Africa, all chicken-parasitizing argasid samples that we have seen consist only of A. (P.) walkerae (described in Ann. Entomol. Soc. Amer. 62 (4):885-890, 1969), a species whose arboviruses relationships should be compared with those of other Persicargas species. A. (P.) robertsi, described in 1968 from domestic fowl in Queensland, Australia, has more recently been found in Indonesia and Thailand. Nyamanini virus was isolated from the Thai sample. In a review of the American Persicargas species (in press in Ann. Entomol. Soc. America), the species persicus is shown to be rare and localized in the Americas, where it is replaced by several other bird-infesting species. Especially important candidates for arbovirus research are the species sanchezi and miniatus which parasitize a variety of small and medium size birds in SW USA and Mexico, and in South America, respectively. In other reports now in press in the same journal, the vulture-infesting Persicargas species of the world are shown to be more closely related to each other than to other species in this subgenus. Should vulture-related tickborne viruses be isolated, a comparative study of their tick-bird interrelationships should be scientifically rewarding. A new Persicargas species is now being described from doves in Africa and Cyprus; the ability of this tick to maintain and transmit a new Quaranfil group virus from doves (YARU:Q3255; see below) is being studied here. In order to provide information for tissue culture research at RML and for investigations on virus transmission by bite and transovarially, several papers dealing with the biochemistry and physiology and with gonad development and gametogenesis in A. (P.) arboreus (and Hyalomma species) have recently been published and are in press in the Journal of Medical Entomology and Journal of Parasitology.

Descriptions and reviews of numerous known or potential virus vectors in the genus Haemaphysalis are regularly being published in the Journal of Parasitology. One of the most interesting of these concerns H. (K.) longicornis (JP, 54(6):1197-1213, 1968), a vector of RSSE and other viruses, which is shown to occur in NE China and USSR and in Korea and Japan, from where it has been transported to and is now widely established in Australia, New Zealand, and numerous Pacific islands.

Viruses from argasid ticks in Afghanistan

Nineteen virus strains have been recovered from Argas (A.) reflexus group samples collected in shops in the Kabul market place and in pigeon breeding farms in the outskirts of Kabul. Fourteen of the isolates are related by cross CF to Quaranfil virus. The others show no relationship to reference material maintained in this laboratory and are being sent to YARU for further study.

Absence of viruses in argasid ticks from Nepal

Several lots of Argas (A.) reflexus group and Ornithodoros (Alectorobius) coniceps collected in pigeon houses in Kabul were processed but failed to yield virus isolates.

Qalyub virus (Catalogue number 222)

This virus (closely related to Bandia virus) was originally isolated by Taylor and Dressler in 1952 from a pool of Ornithodoros (P.) erraticus collected by Hoogstraal from an Arvicanthis (grass rat) burrow in Qalyub (Lower Egypt). No other information on the natural history of this virus was available. We have now collected 1545 adults and nymphs of O. erraticus from Arvicanthis burrows in Upper Egypt. These were grouped in 45 pools, four of which yielded isolates of Qalyub virus. Further studies on the epidemiology and transmission of this virus are in progress.

Characterization of Egyptian tickborne viruses

For study of host cell-virus relationships, Chenuda, Quaranfil, Qalyub, Wad Medani, and Nyamanini viruses were inoculated into stationary primary cultures of rabbit kidney cells. The degree of CPE produced varied from complete destruction of the cells within 48 hours by Chenuda (Ar 1170), titer $10^{9.1}$/ml, to negligible effect by the remaining viruses except for a late but distinct CPE by Nyamanini (Ar 1304), titer $10^{4.3}$/ml. Using inactivated immune homologous serum to which fresh normal mouse serum

had been added, the NI showed 4 logs of protection against Chenuda and Nyamanini viruses, respectively, in primary RK cultures.

Search for a suitable vertebrate host has begun for infection/transmission studies using Wad Medani virus as the agent and Rhipicephalus sanguineus as the vector. In attempts to induce viremia, doses varying from 300 to 10,000 SMIC LD_{50} have been inoculated IP into adult guinea pigs and white rats. No viremia, symptoms of illness, or antibodies (by CF and NT) could be detected in guinea pigs. Viremia and symptoms of illness have not appeared in white rats; tests for antibodies are yet to be performed.

Viruses recently isolated during Smithsonian Palearctic Migratory Bird Survey (guest investigators) and characterized at YARU

Forty agents in a group related to Eg An 1047-61 (from a lesser whitethroat warbler at NAMRU 3 in 1961) and to Sa An 3518 (from a spotted-backed weaver in South Africa in 1959) were recovered from 3300 migratory bird-blood samples from Egypt and Cyprus. About 75% of these isolates were from warblers (Sylviinae). One of the 40 isolates, B90 from a golden oriole, Oriolus oriolus, was found by Dr. Shope to pertain to the Bunyamwera supergroup and not to cross-react by HI with 64 other arboviruses outside this supergroup. This strain was called Bahig virus (catalogue number 237) after the Egyptian village from which it was isolated.

Other isolates include EgB 890-3, a new Simbu group virus from a southward migrating whitethroat warbler (fall, Egypt), Eg B 241 (probably not an arbovirus) from a red-backed shrike, Lanius collurio (fall, Egypt); C40, EgB 3505, and EgB 3127, related agents from a variety of species (spring and fall, Cyprus and Egypt), Ingwavuma from northward migrating flycatchers, Muscicapa striata (spring, Cyprus); Q3255, a Quaranfil group virus from the turtle dove, Streptopelia turtur (spring, Cyprus), and West Nile virus from the barred warbler, Sylvia nisoria (spring, Cyprus).

These data were presented at the 5th Symposium on Role of Migrating Birds in Distribution of Arboviruses, Novosibirsk, USSR (July 1969), and attended by Drs. G. Watson (USNM) and M.N. Kaiser (NAMRU-3).

(H. Hoogstraal, M.N. Kaiser, R.E. Williams, K.S.E. Abdel Wahab, and M.A.M. Attia)

REPORT FROM THE INSTITUTE OF MICROBIOLOGY
UNIVERSITY OF PARMA
OSPEDALE MAGGIORE
43100 PARMA, ITALY

A rapid procedure for the purification of arboviruses

Magnesium pyrophosphate gel chromatography has been used for the purification of arboviruses.

It is possible, through this method and by elution with 0.2M Na_2HPO_4, to obtain a quantitative recovery of the haemagglutinating activity and infectivity of the tested viruses (Sindbis and West Nile). The degree of purity has been controlled by analytical centrifugation. The electronmicroscopy and the cesium chloride density gradient centrifugation of the purified virus preparations have shown the presence of empty particles along with full viral particles. Giornale di Microbiologia, 16, 169-175, 1968.

Hemolytic activity of Sindbis virus

Carrying out the hemagglutination tests with Sindbis virus, it has been noticed that this virus has, in particular conditions, a hemolytic activity. The hemolytic activity is inhibited by the specific antisera, rises after some cycles of freezing and thawing, takes place at a temperature of 22° - 37°C and at an optimal pH of 5.75 - 6.0. The hemolytic activity is not present at a temperature of 4°C, is destroyed by heat (56°C), ether or $CaCl_2$, while it is not substantially influenced by a treatment with $MgCl_2$, trypsine or RDE.

Density gradient centrifugation of the virus in CsCl shows two peaks of hemolytic activity (full and empty particles) thus revealing the localization of the hemolysin in the viral capsid. Igiene Moderna, 61, 823-833, 1968.

The lipid content of Sindbis virus

The lipid composition of Sindbis virus purified on magnesium pyro-phosphate gel chromatography has been studied.

A qualitative and quantitative analysis of lipids demonstrated phospholipids (61%), sterols (23.1%), sterol-esters (9.3%) and triglycerides (6.6%).

The phospholipids present are sphingomyelin (44.5%), phosphatidyl-coline (42.6%), phosphatidyl-inositol (11.5%) and phosphatidyl-ethanolamine (1.4%). Gas-chromatography has demonstrated the presence of eight fatty acids from C_{12} to $C_{18:1}$. The highest concentrations were palmitic acid (40.3%), stearic acid (20.3%) and oleic acid (19.8%).

The virus lipid composition is substantially similar to that of the uninfected host cells (chick embryo fibroblasts). Nevertheless marked quantitative differences have been detected in the phospholipid composition: phosphatidyl-ethanolamine and phosphatidyl-coline are present in a higher quantity in the cells (14.5% and 63.5% respectively) than in the virus (1.4% and 42.6%), while spingomyelin and phosphatidyl-inositol are present in a lower quantity in the cells (16.3% and 5.7%) in comparison to the virus (44.5% and 11.5%). Giornale di Microbiologia, 16, 155-162, 1968.

Activity of Arboviruses in some Italian regions

As previously communicated, a program of researches to study Arboviruses activity in some Italian regions was planned.

This program concerns a large serological survey and virus isolation attempts from arthropods collected on the Mediterranean coast (Imperia, La Spezia, Livorno), in the Po Valley (Parma, Piacenza, Mantova), on the Adriatic coast (Ravenna, Po delta) and in Sardinia.

The researches are now in progress. Igiene Moderna, 60, 739-749, 1967.

(A. Sanna)

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

As referred in the eighteenth issue of the Arthropod-borne virus information exchange, Bhanja virus was isolated from Haemaphisalis punctata ticks in Italy.

Serologic examinations. The study of the distribution of HI antibodies to Bhanja virus in a total of 1,832 human and animal sera gave serologic evidence of its circulation in different Italian regions. The percentage of positive sera was higher in ovine species, mainly in goats (table 1).

Growth in tissue culture. BHK-21 cells were found to be the most suitable for studying this virus in tissue culture. In these cells Bhanja virus produces a marked cytopathic effect characterized by bubbling of the cell periphery with vacuolization of the cytoplasm and pyknosis of the nucleus. Some informations were obtained on the correlations between Bhanja virus multiplication in BHK-21 cell monolayers and the sequence of morphological changes leading to CPE (Fig. 1).

Structure of the virus. The Bhanja virions propagated in BHK-21 cells, are roughly spherical and consist of a dense core about 700 Å in diameter, surrounded by spiky surface subunits. The maximum diameter of the whole virus particle is about 900 Å.

Experimental pathogenicity in white mouse and monkey (M. mulatta). Bhanja virus infection in suckling mice is of neurotropic nature regardless of the route of inoculation. Viremia was detected after intracerebral (i.c.) inoculation, but not after intra-peritoneal (i.p.) inoculation. Histologically, lesions were limited to CNS and consisted of regressive alterations of neurons without marked inflammatory lesions, after i.c. inoculations; on the contrary, a clear picture of encephalitis was present after i.p. inoculation.

Intrathalamic inoculation of monkey (M. mulatta) caused temperature increase and clinical signs of involvment of the CNS, followed in some cases by the death of the animals. Virus was isolated from various parts of CNS. Histologically, lesions were limited to single neuronal cells and were of the regressive type affecting mainly the cytoplasm. Purkinje cells

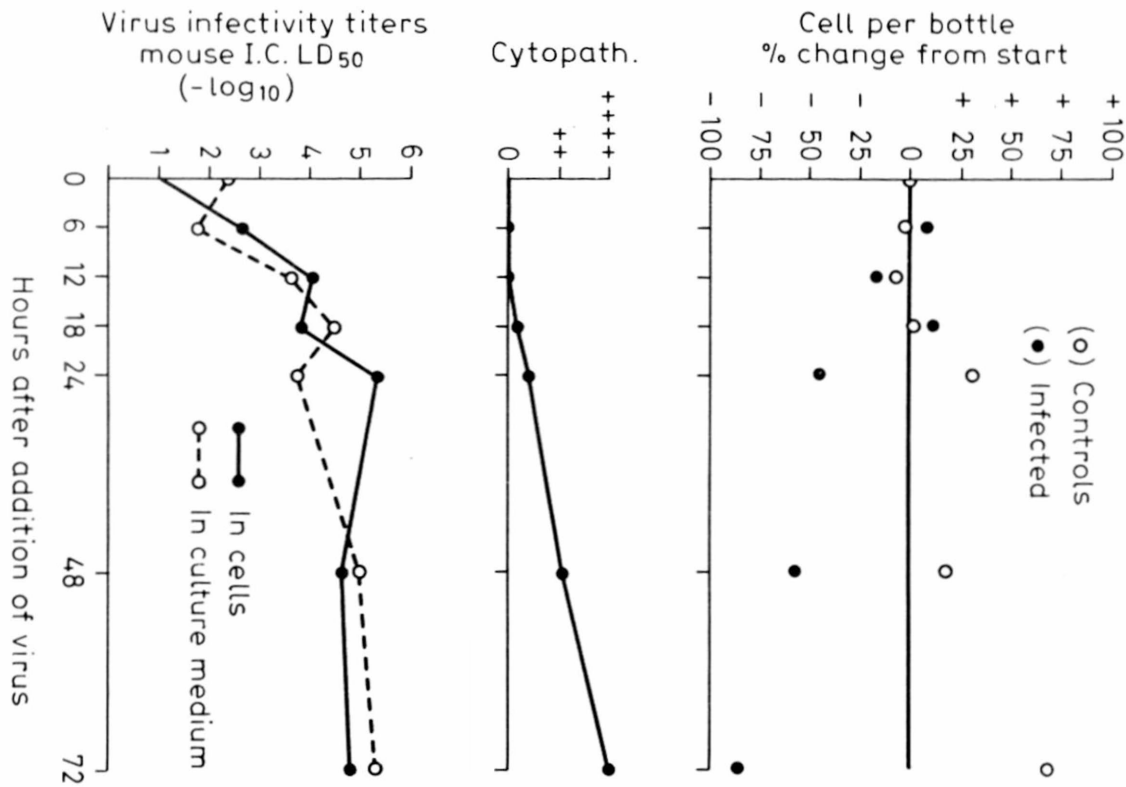
Table 1

HI ANTIBODIES TO BHANJA VIRUS IN HUMAN AND ANIMAL SERA FROM DIFFERENT ITALIAN REGIONS

Serum	Total no. of samples	NORTHERN REGION (Friuli - Venezia G.)		CENTRAL REGION (Lazio)		SOUTHERN REGIONS (Campania)		(Calabria)		Total % of positive sera
		No. of sera	No. positive and titre	No. of sera	No. positive and titre	No. of sera	No. positive and titre	No. of sera	No. positive and titre	
Human	542	40	0	40	1 x 1: 40	92	3 x 1: 20 1 x 1: 40	370	1 x 1: 20 4 x 1: 40	1.8
Goat	358	2	0	61	4 x 1: 20 16 x 1: 40 14 x 1: 80 7 x 1: 160 2 x 1: 640 1 x 1:1280	85	1 x 1: 80 1 x 1: 320	210	23 x 1: 40 25 x 1: 80 36 x 1: 160 25 x 1: 320 16 x 1: 640 6 x 1:1280	49.4
Sheep	80	48	2 x 1: 40 2 x 1: 80 3 x 1: 160 1 x 1: 320 11 x 1: 640 4 x 1:1280	32	2 x 1: 40 2 x 1: 80 3 x 1: 640	0	0	0	0	37.5
Bovine	105	29	0	56	2 x 1: 40 1 x 1: 160	20	0	0	0	2.8
Wild rodents	87	15	0	72	2 x 1: 20 2 x 1: 40	0	0	0	0	4.6
Migratory* birds	635	635	7 x 1: 20 3 x 1: 40 1 x 1: 80 1 x 1: 320	0	0	0	0	0	0	1.9
Domestic avian species	25	25	2 x 1: 160	0	0	0	0	0	0	8.0

* *Winter visitors*

Fig. 1



were particularly damaged.

(M. Balducci, P. Verani and M. C. Lopes)

REPORT FROM THE DEPARTMENT OF ARBOVIRUSES
D. I. IVANOVSKY INSTITUTE OF VIROLOGY
ACADEMY OF MEDICAL SCIENCE
MOSCOW, USSR

Assay of Arbovirus in Mouse Serum by Precipitation Test

Since viremia is one of the characteristics of arboviral infections we attempted to demonstrate viruses in the blood by means of an agar gel-precipitation test. Such a technique might allow rapid diagnosis of arboviral infections during the acute stage of disease. Twelve different arboviruses were studied in suckling mice, ages not exceeding 4-5 days.

Seven strains belonged to antigenic group A: Sindbis/EgAr-339, Semliki, Pixuna/BEAr 35645, Mucambo/BEAn 8, Chikungunya/, Ross, Venezuelan (VEE) and Western equine encephalomyelitis (WEE); four strains of group B: Japanese encephalitis (JE)/, P-1, West Nile (WN)/ Eg101, Powassan and St. Louis. Tribeć and Uukuniemi/S-23 strains were also used.

Ascitic fluids of mice immunized with each virus served as sources of antibodies; ascitic fluids containing A and B polytypic antibodies were used as well.

Mice were immunized with suspensions of infected suckling mouse brain plus Freund adjuvant. Sarcoma 180/TG cells were used for induction of ascites.

Blood sera of infected mice taken at the beginning of the disease were used as antigen without further treatment.

Infectivity titrations of those sera were performed in chick embryo cell cultures (VEE, Sindbis, Tribeć), in 2-4 day old mice (Mucambo, Pixuna,

Chikungunya, Uukuniemi) and in 6-7 g mice (Japanese encephalitis, St. Louis, West Nile and WEE) infected intracerebrally. The titres in the Table are expressed as logs of lethal or CPE $dose_{50}$.

Precipitation tests were performed according to Ouchterlony. Difco agar was washed free of polysaccharide sulphate and then used for preparations of 1% gel in borate buffer solution pH 9. The gel was layered on 9 x 6 cm glass plates and wells were cut. The diameters of the central wells were 5 mm of radial wells - 4mm. The distance between the centers of central and radial wells was 10 mm. Several wells were made near the periphery of the plate and filled with saline to prevent drying of the agar.

Two-four day old mice were infected intracerebrally with 10^3 - 10^4 LD_{50}/0.02 ml of each virus. The animals were bled immediately after appearance of the first signs of illness.

A part of each serum was used for titration of infectivity.

A 0.04 ml sample of serum (antigen) was put into the central well, and into each of the 4-6 radial wells were added 0.03 ml of ascitic fluids immune to the tested virus, to some viruses of the same antigenic group, polytypic to this group and also to viruses of other groups for control. Immunodiffusion was allowed to proceed for 18-20 hours at 37°C in humid chamber.

Positive results were obtained with Semliki, Pixuna, VEE, WN and Uukuniemi viruses. In these experiments the titres of virus in blood of mice not lower than 7.5 logs. The lines of precipitation were observed not only with antibodies to homologous virus but also with polytypic antibodies, and in one case with the antibodies to a virus of the same antigenic group (VEE and Mucambo).

The negative results with other viruses studied cannot be considered as definitive. Probably the levels of precipitating antibodies to these viruses in the ascitic fluids used were not high enough. The technique of detection of precipitating antigen in sera gives promise as a tool for rapid diagnosis of arboviral infections during the acute stage of disease.

(S. Ya. Gaidamovich, N.A. Krechetova, A.I. Lvova, E.E. Melnikova, and V.R. Obukhova)

Virus	Infectious titre in lg LD_{50}	Immune ascitic fluids with indication of titres to homologous virus in HI test or CF* test													
		Sindbis	Semliki	Pixuna	VEE	Mucambo	Chikungunya	WEE	JBE	WN	S-Louis	Tribec	Poly A	Poly B	Uukuniemi
		1280	2560	2560	1280	640	320	320	320	320	80	40*			80*
Sindbis	7,5	0	0	0	0	•	0	0	•	•	•	•	0	0	•
Semliki	8,75	0	P	•	0	•	0	0	•	•	•	•	0	•	•
Pixuna	7,75	0	0	0	0	0	0	•	•	•	•	•	0	•	•
	8,00	0	0	P	0	0,	0						0		
VEE	7,75	0	0	0	P	0	0	•	•	•	•	•	0	0	•
	8,25	0	0	0	P	P	0						P	0	
Mucambo	7,75	0	0	0	0	0	•	•	•	•	•	•	0	•	•
Chikungunya	•	0	0	0	0	•	0	0	•	•	•	•	0	•	•
WEE	6,75	0	0	•	0	•	0	0	•	•	•	•	0	•	•
JBE	5,0	•	•	•	•	•	•	•	0	0	0	•	•	0	•
WN	7,5	•	•	•	•	•	•	•	0	P	0	•	•	P	•
S-Louis	•	•	•	•	•	•	•	•	0	0	0	•	•	0	•
Tribec	4,0	0	0	•	0	•	•	•	•	•	•	0	0	0	•
Uukuniemi	•	•	•	•	•	•	•	•	0	•	0	•	•	0	P

P-positive; O-negative; • - not tested;

REPORT FROM THE INSTITUTE OF MEDICAL MICROBIOLOGY
UNIVERSITY OF AARHUS
DENMARK

Early circulating interferon in mice infected intraperitoneally with West Nile virus and exhibiting autointerference

Autointerference was observed in non-inbread albino mice on intraperitoneal inoculation of a laboratory strain of West Nile virus.

The phenomenon was most pronounced in older animals, which exhibit a natural resistance against the virus. In mice providing evidence of autointerference, circulating interferon was found 6 hours after virus inoculation, at a time when viremia had not yet developed. In mice receiving a smaller virus dose, interferon first appeared 1-2 days later and after viremia had appeared. Treatment with steroids prior to virus inoculation was associated with an inhibition of interferon induction and autointerference was not observed. No significant differences were found in the development of HI antibodies in the two groups of mice receiving different doses of virus.

The induction of early interferon in the mice exhibiting autointerference is considered of importance in the reduction of mortality.

Reduction of circulating interferon in anti lymphocyte serum treated mice injected intraperitoneally with West Nile virus

Mice treated with Rabbit (anti-mouse-lymphocyte) serum (A. L. S.), which depressed the lymphocyte count to 1/4-1/5 of that found in mice treated in the same way with normal rabbit serum (N. R. S.), were given different doses of West Nile virus intraperitoneally.

In the A. L. S. - treated mice given 10^6 LD_{50} doses of virus the circulating interferon 18 hours after virus-inoculation was appreciably reduced compared to that of the groups of mice given N. R. S. In A. L. S. - treated mice given 10^3 LD_{50} doses of virus, no, or only traces of, circulating interferon was found, while the N. R. S. - treated mice gave a normal response of circulating interferon 48-120 hours after virus inoculation.

The results obtained seem to suggest that circulating interferon in mice

inoculated with West Nile virus to a large degree is produced by circulating lymphocytes and/or lymphoid tissue.

(S. Haahr)

REPORT FROM THE WHO REGIONAL REFERENCE LABORATORY FOR ARBOVIRUSES INSTITUTE OF VIROLOGY SLOVAK ACADEMY OF SCIENCES BRATISLAVA, CZECHOSLOVAKIA

Sensitivity of Tribeč arbovirus to the temperature and pH changes.

Tribeč virus originally isolated from Ixodes ricinus ticks was inoculated intracerebrally in amounts of 0.01 ml. to suckling mice. After an incubation period of 3-4 days, when the animals became ill, they were killed, their brains dissected and this material stored at -60° C. A 20% brain suspension was prepared by homogenization in phosphate buffered saline pH 7.2. An additional treatment in sonicator (three times for 15 seconds) resulted in a homogeneous suspension which was then clarified at 13,000 g for 30 min. The supernatant fluid was used for further experiments.

Chick embryo cell (CEC) monolayers were infected at a multiplicity of infection of 1,000 LD_{50} per cell with the Tribeč virus suspension. Virus adsorption lasted for two hours at 37° C; the remaining fluid was then discarded and substituted with medium 199. After an incubation for 24 hours at 37° C, a distinct cytopathic effect was visible. Tissue culture medium containing the extracellular virus was clarified at 2,000 r.p.m. for 30 min. and the resulting supernatant fluid was used throughout.

Virus in all samples was titrated by inoculating tenfold dilutions of the materials intracerebrally into white mice; for comparison the plaque titration (Dulbecco, 1952) was used. The LD_{50} values were counted according to the Reed and Muench formula.

Influence of heat

Influence of heat was followed in the range from 40 to 70° C in the Wobser type ultrathermostat with a water bath. The virus material was incubated for 30 min. in sealed glass ampoules and after cooling it was titrated both intracerebrally in suckling mice (using the inoculum of 0.01 ml) and by the plaque method.

The infectivity of virus in 10% brain suspension dropped between 50-55° C and the virus was completely inactivated at 60° C. Similar results were obtained with tissue culture materials. Studies on the influence of heat on Tribeč virus showed that its thermoresistance was similar to that of A and B group arboviruses.

Influence of pH

The influence of pH in the range from 3 to 11 was studied. For pH 3 and pH 4 sodium citrate buffers according to Sörensen was used and from pH 9 to pH 11 alkaline glycine buffers. Buffered solutions were autoclaved at 1.5 atm for 30 min. and their pH values were checked with a pH meter using a calomel electrode as reference. The viral materials from mouse brains or tissue cultures were mixed with the given buffer in a 1:10 /v/v/ ratio and incubated at 4° C for 24 hours. After this period, the pH was adjusted to pH 7.0 and the resulting preparations were assayed for virus by intracerebral injection of 0.01 ml volume into suckling white mice.

The maximal infectivity titres for 10% infectious mouse brain suspension were found in the range of pH 7 to 8. The infectivity dropped to both the acid or alkaline region. The material from tissue cultures had its maximum of infectivity at pH 7.0; it was lower on either side of this point. The Tribeč virus showed certain differences in pH sensitivity as compared to that of A and B group arboviruses.

Purification and morphology of Tribeč arbovirus

The morphology of Tribeč virus was studied in the electron microscope by use of the negative staining technique. The virus was purified from brains of suckling mice by differential and sucrose density gradient centrifugation. All steps of the purification procedure were controlled under the electron microscope.

In the preparations made from virus-containing mouse brain suspension, clarified at 10,000 r.p.m. for 60 min., a moderate number of viral

particles were found. Further investigations revealed their regular hexagonal shape.

Proteins present in brain homogenates were partially removed by addition of streptomycin sulphate or polymyxin sulphate. No significant loss of infectivity after this treatment was observed but the virion structure was altered as indicated in the electron microscope. It was shown that the viral particles might have been damaged.

Since artificial disruption of viral particles after differential centrifugation was observed, clarified suspension or concentrated tissue culture fluid was laid on the top of sucrose gradient column. Fractions were examined for viral infectivity and CF activity. At the same time, the O.D. at 260 nm was determined.

The infectious particles were found in the first peak with the density of 1.16 g/cm^3. The CF antigen activity was evenly distributed, with some tendency to accumulate in the fraction of highest infectivity. No viral particles could be demonstrated under the electron microscope. In the material obtained from infected suckling mouse brains after homogenization, clarification and sucrose density gradient centrifugation, viral particles were observed, but serious differences in virion structure as compared with original material were found. The average particle size was 64$\pm\mu$m; on the surface of the particles, spiky projections were visible. The tendency of the viral particles to accumulate in clumps was observed under the electron microscope. A slight pleomorphism was seen, but the majority of the particles were roughly spherical.

According to our results we consider that the method of differential and sucrose density gradient centrifugation was not adequate for morphological studies of this arbovirus, probably due to the lability of virus against mechanical forces. Optimal results were obtained after clarification of mouse brain suspension, when no artificial disruption of viral particles was observed.

It may be concluded that virions of Tribeč virus, like other arboviruses, are extremely sensitive to physico-chemical treatment. Its morphology could not be studied in treated preparations. The virions in untreated preparations were hexagonal, measuring 70 nm between the opposite sides of hexagon and 75 nm between its corners.

Pathogenicity of Tribeč arbovirus for mice

By means of infectivity titration, immunofluorescence and histological examination, the pathogenicity of the Tribeč virus for suckling and adult mice was studied.

Virus. The strain of Tribeč virus was maintained in the laboratory by intracerebral (ic) inoculation into 2-day-old Swiss albino mice. A 10% brain suspension was prepared in medium 199 containing 5% fetal calf serum and lyophilized.

Experimental animals. Suckling mice 4-5 days old were infected ic with 0.01 ml or subcutaneously with 0.1 ml of undiluted stock virus solution. Adult mice weighing 6-8 g were infected ic with 0.03 ml of the same material.

Virus infectivity determination. The brains, livers, spleens and other tissues were homogenized in medium 199 containing 5% calf serum, 1000 units of penicillin and 1000 ug of streptomycin per ml. After centrifugation, the supernatants were collected and serial 10-fold dilutions were inoculated ic into 2 - to 5 - day-old Swiss albino mice.

Fluorescent antibody (FA) method. The brains of suckling and adult mice were fixed in chilled Carnoy's solution (at 4° C for 3-4 hours), passed through chloroform overnight at 4° C and embedded in paraffin. The sections cut at room temperature were quickly dried (30 min at 37° C), and after the removal of paraffin by xylene and passage through an alcohol series stained by the indirect method.

Hyperimmune sera against the Tribeč virus were prepared in mice and rabbits. The carcasses of the suckling mice were quickly frozen in liquid propane-butane, cut in cryostat in horizontal planes to obtain representative sections through the organs of the thoracic and abdominal cavities.

Histological methods. The bodies of suckling mice and brains of the adult ones were fixed in Bouin's solution and embedded into paraffin.

A. Intracerebral inoculation

Virus multiplication. The signs of encephalitis and death occured in suckling mice on the 4th day after inoculation.

The virus, however, was detectable in their brains beginning from the first postinoculation day and reaches the highest titres on the 2nd, 3rd and 4th postinoculation days (Table 1). On the 2nd day the virus was also found in blood, spleen and liver.

After ic inoculation of Tribeč virus into adult mice, no signs of illness were observed. The virus appeared at first in the brain, persisting there for a 10-day period (Table 2). It should be mentioned that the highest virus titre in the brain of adult mice was detected on the first postinoculation day; then the titre subsequently decreased and the virus could be detected irregularly.

The meninges were infiltrated by polymorphonuclear leucocytes and mononuclear cells. The inflammatory changes in the cortex striatum, thalamic and hypothalamic nuclei and in the brain stem consisted of hyperaemia, oedema, exudation and polymorphonuclear leucocytes and neuronal necrosis. Widespread neuronal damage and abundant polymorphonuclear infiltration were found especially in the cornu ammonis.

Bright specific fluorescence of the virus antigen was found in the neurons of the same parts of CNS, where the histological changes developed. In the cornu ammonis nearly all neurons contained virus and the majority of them showed severe damage or necrosis. The majority of the Purkynje cells showed neither specific fluorescence nor cytological changes after staining with haematoxylin and erythrosin.

No fluorescence was detected in the visceral organs of ic infected suckling mice.

In adult mice, lymphocytic meningitis from the first through the 6th postinoculation day was found. The meningeal infiltration consisted predominantly of lymphocytes and macrophages. Near the site of inoculation and below the ependymal layer in the walls of the lateral ventricles, a few mononuclear and glial cells contained viral antigen. No fluorescence was demonstrated in the neurons.

B. Subcutaneous inoculation

The virus could be detected first in the skin at the site of inoculation, 15 hours after inoculation. Soon after its appearance here, the virus was found in the blood; in the brain it appeared at first 48 hours after inoculation (Table 3). The infectivity titres were lower than those after ic infection.

Table 1

Intracerebral Tribeč Virus Infection in Suckling Mice

Organs	Days after infection			
	1st	2nd	3rd	4th
	log 10 LD_{50} per 0.01 ml			
Blood	0	1.5	0	0
Spleen	0	4.0	0	0
Liver	0	3.0	0	0
Brain	2.6§	6.5x	7.0x	6.5x

§ = specific fluorescence in some glial cells and mononuclears

x = specific fluorescence in numerous neurons and glial cells

0 = no virus was detected

Table 2

Intracerebral Tribeč Virus Infection in Adult Mice

Organs	Days after infection						
	1	2	3	5	6	7	10
	log 10 LD_{50} per 0.01 ml						
Blood	0	0	0	0	0	0	0
Spleen	0	0	0	0	0	0	0
Liver	0	0	0	0	0	0	0
Brain	5.5§	2.5§	3.4§	4.3§	2.7§	0	3.8§

§ = Positive immunofluorescence in some cells (glial, mononuclear)

0 = no virus was detected

Table 3

Subcutaneous Tribeč Virus Infection in Suckling Mice

Organs	15 hours	Days after infection			
		1	2	3	4
		log 10 LD_{50} per 0.01 ml			
Skin	1	0	0	0	0
Blood	0	2.6	0	0	0
Spleen	0	0	0	0	0
Liver	0	0	0	0	0
Brain	0	0	3.0	0	0

0 = no virus was detected

Table 4

The Comparison of the Pathogenicity of Tribeč Virus for Suckling and Adult Mice after Intracerebral Inoculation

	Kind of animals	
	suckling	adult
1. Clinical signs	death in 4 days	survival
2. Histological diagnosis	encephalomeningitis	meningitis
a. cells forming the infiltrate	neutrophils	mononuclears
b. neuronal damage	abundant	none
3. Immunofluorescence	positive in numerous neurons and glial cells	in some glial and mononuclear cells
4. Virus titer in the brain	increases up to high values	slowly decreases and persists at low values

The immunofluorescent studies in this group of animals did not yield any positive results. The differences between the reaction of suckling and adult mice after ic inoculation with Tribeč virus are given in table 4.

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REPORT FROM THE FEDERAL RESEARCH INSTITUTE FOR ANIMAL VIRUS DISEASES TÜBINGEN, GERMANY

Demonstration of nucleocapsids in togaviruses

The desoxycholate method for isolation of nucleocapsids from Sindbis virus preparations (1) could not be applied to other agents of presumably similar structure, as the detergent seems to degrade the core, too. Consequently other methods were tested, of which pronase (500 µg/ml), urea (6.2M) and saponin (0.1%) proved useful. Treatment with urea apparently renders the envelope penetrable for uranyl acetate, thus contrasting the core particle; pronase and saponin reacts with components of the viral envelope stripping off part of it. The methods have been applied to Sindbis, rubella, equine arteriitis and bovine viral diarrhea/mucosal disease viruses and isometric particles could be demonstrated, most probably representing the viral nucleocapsids; no such structures were seen when equine influenza virus was treated in the same ways. Work is in progress to show, whether these agents can be classified as togaviruses.

Structure of the surface projections of Sindbis virus

In a previous study on the structure of VEE virus (2) it was suggested that its surface projections are forming loops rather than spikes as in myxoviruses. Electron microscopy of Sindbis virus preparations pretreated with digitonin (0.1%), urea (6.2 M) or EDTA (0.15 M) revealed structures on the viral surface that could be interpreted when assuming their loop configuration. The electron density distribution at the particle periphery could be simulated by superimposition diagrams of model loops.

(1) Horzinek, M. & Mussgay, M. (1969) J. Virology 4:514-520

(2) Horzinek, M. & Munz, K. (1969) Arch. ges. Virusforsch. 27:94-108

(M. Horzinek, J. Maess, and M. Mussgay)

REPORT FROM PRINS LEOPOLD INSTITUUT VOOR TROPISCHE GENEESKUNDE ANTWERP, BELGIUM

Studies on the enhancing effect of DEAE-dextran on arboviral infections in mosquitoes have been continued.

In summary: the enhancement has been found for 3 group A viruses (Sindbis s and 1 plaque virus, Semliki Forest) and one group B virus (West-Nile).

It was not observed when Aedes aegypti was fed with Central European encephalitis virus, neurotropic or 17 D yellow fever viruses, nor in the system Anopheles stephensi - Sindbis virus. Thus the DEAE-dextran, which has to be given in toxic doses for the mosquitoes to become enhancing, influences in some way the mosquito "gut barrier" without altering the specificity of the sensitivity of a mosquito for a particular virus: viruses that normally multiply in A. aegypti are enhanced, viruses that normally do not multiply in A. aegypti are not enhanced, nor is Sindbis enhanced in Anopheles stephensi (which is normally insensitive for Sindbis virus).

As far as the mechanism of action is concerned Chamberlain and Sudia (Am. Rev. Entomol. 1961, 6, 371-390) proposed the following possible mechanisms for the stomach barrier:

1. A differential inactivation of viruses by digestive enzymes.

2. Virus inhibitory substances on the gut surface.

3. Limited number of receptors on the cell surface.

4. Impermeability of the peritrophic membrane.

Factors 1 and 2 could be ruled out in our observations. Factor 3 was very improbable.

Consequently, action of DEAE-dextran in A. aegypti is probably analogous with that observed in chick embryo tissue culture, this means on the adsorption of virus on the susceptible mosquito cells in which the first multiplication has to take place.

(Dr. S. R. Pattyn)

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

We recently described a micro-culture method for the titration of Group B arboviruses using the PS or PK 2a line of pig kidney cells. These cells had previously been used for Group B arbovirus studies by Dr. E. G. Westaway, who reported that there was some evidence that this line was contaminated with a virus of the mucosal disease-swine fever complex of viruses. More recently, Shimizu et al. have established that the PS or PK 2a cells are chronically infected with an attenuated form of swine fever virus. If an attempt to assess the possible significance of the swine fever virus in arbovirus assays, titrations of Group B viruses have been carried out in parallel in PS cells and in PK 15 cells, a clonal line of pig kidney cells originally derived from PK 2a cells and known to be free from infection with swine

fever virus. The more rapidly growing Group B arboviruses, including Ilheus, Kyasanur Forest Disease, Negishi, Tembusu, European tick borne encephalitis, Uganda S. Usutu, West Nile and Zika, give almost identical titres in the two cell lines, although plaques are generally larger in PS cells. The great majority of the remaining Group B viruses give titres ten-fold to one thousand-fold lower in PK 15 cells, and with Alfuy, Cowbone Ridge, Dengue 1 and Dengue 4, and Stratford viruses, no plaques developed in PK 15 cells. With one virus only was the use of PK 15 cells advantageous. Bukalasa Bat virus produces a very striking prozone effect when titrated in PS cells. Wells containing 10^{-3}, 10^{-4} and 10^{-5} dilutions of virus are almost indistinguishable from uninfected, control wells, but plaques develop at 10^{-6} and 10^{-7} dilutions, which, while not completely lytic, are nevertheless clearly visible and measure 2-3 mm in diamter. The same virus gives a lower titre in PK 15 cells, but no prozone effect occurs. Wells containing 10^{-3} and 10^{-4} dilutions of virus show confluent or almost confluent lysis of cells, but the plaques which develop with the further dilutions of virus are smaller and less numerous than those occurring beyond the prozone effect in PS cells.

Attempts to make PK 15 cells more susceptible to Group B arboviruses by infecting them with the attenuated swine fever virus present in PS cells have not been successful. On the contrary, swine fever virus appears to produce interference in PK 15 cells, rendering the cells even less sensitive to Group B arbovirus infection. Further studies on the mechanisms involved in this phenomenon are under study.

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

Mosquito populations associated with rice fields, Kano Plain, Kenya

During May and June 1969, the distribution and abundance of mosquito populations were studied on a 2,000 acre irrigation scheme assigned for rice production in Nyanza Province, Kenya. Identical studies were also made in an unmodified ecosystem 12 km. to the south west.

The Ahero Irrigation Pilot Scheme consists of 2,000 acres fed from the Nyando River and about 2/3 of this area is now producing two crops of rice per year.

On the scheme, 4 species of rodents were trapped and 415 sera obtained. Previous studies in Kenya indicated that these rodents can act as hosts of Rickettsiae, plague and Leptospira spp.

Ten species of large birds were found associated with the rice fields, at least two of which, the cattle egret and sacred ibis, are known to act as vertebrate hosts of arboviruses in southern Africa.

Twenty-six schools were visited and 4,106 serum samples of 5 ml. obtained. These were processed and flown back to England for subsequent study. Samples can be made available for other studies by interested workers. Sera were grouped by yearly age-groups from 5 to 15 years and 15+ years. Of the first 301 sera examined from 12 year olds, 197 (66%) had HI antibodies to o'nyong'nyong, 150 (50%) antibodies to chikungunya, 32 (11%) antibodies to Nyando and 17 (6%) antibodies to Bunyamwera viruses.

Adult mosquitoes were caught on human bait and most of these, over 60,000 were made up into pools for virus isolation attempts. A reference collection was also made for subsequent workers. Although the overall numbers caught in a village in the rice fields and in a village in an unchanged area were very similar (23,641 and 22,966) the species composition was different for the two sites. The most abundant species biting man on the scheme was Anopheles gambiae which made up 65% of the adult catch while Mansonia uniformis constituted 28%. In the unchanged area, M. uniformis made up 86% of the catch while A. gambiae made up less than 1% of the total. On the scheme, Culex fatigans made up 5% of the catch but was

virtually absent in the other study site.

Anopheles gambiae larvae were very abundant in young padi on the irrigation scheme while Culex species were dominant among older plants. Anopheles were relatively less abundant in swampy areas in the unchanged area.

Twenty-one species of mosquito were recorded, 6 of which are known to carry arboviruses in East Africa. So far there have been seven primary virus isolations from M. uniformis and one from A. gambiae.

Semliki Forest Virus

Virulence of original Strains of Semliki Forest Virus

Studies of virulence, as an intrinsic property of a virus strain, in relation to susceptibility to infection, as a characteristic of the host, have been extended from those reported in Exchange # 19 of July, 1969.

Four distinct low-passage strains of Semliki forest virus (A7, VR(67)13, LS10, HP6) have been inoculated by intraperitoneal (i.p.) and intracerebral (i.c.) routes into Porton mice of closely defined ages (± 4 days). The A7 strain has an LD50 (i.c. or i.p.) of essentially 1 plaque forming unit (PFU) for mice up to 13 days old but of over 10^5 PFU for mice over 20 days old. This very sharp change from lethal to benign infection with subsequent immunity defines a critical age which is characteristic of the strain, host and route of administration. If a datum response level of, say, 100 PFU/LD50 is selected for comparison then strain A7(i.c. & i.p.) gives such a response in mice of about 18 days old, strain VR(67)13(i.p.) at 25 days old, LS10(i.p.) at 55 days old, VR(67)13(i.c.) at 65 days old, HP6(i.p.) at 90 days old and HP6 or LS10(i.c.) at over 140 days old. Thus the SFV-Porton mouse system demonstrates the related gradients of virulence and susceptibility (refer Exchange # 19) for a single host with age as the controlling factor. This system is thus ideal for examination of those properties of the virus which control virulence and of response mechanisms which control susceptibility.

When the same strains of Semliki Forest Virus were administered to rabbits by the respiratory route the same gradient of virulence was demonstrated. For strain A7 the infection was benign and indicated a 50% protective dose (PD50) of 2,000 PFU. With strain HP6 the infection was lethal with an LD50 of 200 PFU; all survivors were susceptible to subsequent challenge. The intermediate strains in the gradient of virulence (VR(67)13 and LS10) demonstrated an intermediate condition with strain LS10 showing 200

PFU/LD50 and a high incidence of protection in survivors, and strain VR (67)13 showing a PD50 of 100 PFU and lethality at higher doses. A similar gradient of virulence was confirmed by the respiratory infection of 12-week old guinea pigs with these strains of Semliki Forest Virus. Strains A7 and VR(67)13 caused benign infections in guinea-pigs and indicated a PD50 of less than 10 PFU. Strains LS10 and HP6 caused lethal infections with an LD50 of about 1,000 PFU and a high incidence of protection in survivors: this response in guinea pigs appears to be irregular because the probabilities of benign (protective) and lethal infection are similar.

Antigenic Properties of Fragments of Semliki Forest Virus

The treatment of purified suspensions of Semliki Forest Virus with the detergent Nonidet P40 splits virus particles into intact cores and envelope fragments which can be separated by zonal centrifugation. The haemagglutinating, complement fixing and neutralizing-antibody blocking (NAB) activities of the envelope are not altered by this procedure. Further treatment of envelope fragments with dilute trypsin liberates at least three antigens which are distinct in immunodiffusion analyses and only one of these antigens appears to have haemagglutinating and NAB activities. The immunization of mice with Nonidet disrupted virus or with isolated envelope fragments protects against infection by Semliki Forest Virus, but is less effective than immunization with formalized whole virus.

Quaranfil Virus

Further studies revealed that Quaranfil virus is completely non-pathogenic to rhesus monkeys (Macacca mulatta). Even intracerebral inoculations did not produce any signs of illness or histological brain lesions.

Subcutaneous inoculations of Quaranfil virus into sheep gave rise to neutralizing antibody formation, but without any symptoms or lesions in the CNS.

In spite of its high virulence for mice and hamsters after intracerebral inoculation, Quaranfil virus did not give rise to symptoms or death when given by the respiratory route. Lesions were, however, invariably found in selected areas of the brain both in hamsters and in mice.

EDITORIAL NOTE

Contributions for Issue Number 21 of the Arthropod-borne Virus Information Exchange will be due 1 November 1970. They should be addressed to:

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