ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

Number Twelve September 1965

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REPORT OF THE CHAIRMAN OF THE SUBCOMMITTEE ON ARBOVIRUS INFORMATION EXCHANGE

Following the first revision of the Catalogue of the Arthropod-borne Viruses of the World in 1963, a Special Report was issued as a supplement to Infoexchange Number 8. Now that the second revision of the Catalogue has been assembled and distributed, it seemed appropriate to issue another Special Report on the Catalogue. In keeping with the previous one, this report consists mainly of the tabulation of data extractable from the Catalogue by the marginal key-sort system. Also as previously, it is issued as a supplement to the Infoexchange.

Opinion on the value and interest of the accompanying Special Report on the Catalogue and suggestions for improvement will be appreciated. If these reports prove to be of sufficient interest to the readers of the Infoexchange, consideration will be given to issuing them annually to incorporate newly registered viruses and additional information received during the year on viruses previously registered.

Several typographical errors in arranging and labeling some of the Figures, Maps, and Tables have resulted because the secretary, Mrs. Erma Wellington, has been ill during a part of the time this report was being assembled. It is gratifying to report that she has now returned to duty.

EDITORIAL NOTES

Since 1960 Mrs. Jane Sweat, Secretary to Chief of the Virology Section of CDC has been responsible for the mechanics of transcribing, reproducing, and distributing the complex of communications involved in the Information Exchange activity. In June she resigned to accompany her husband who is a dentist in the Navy. In September they had a baby, named Jane.

Mrs. Sweat's devoted and careful attention to excellence of information transmittal marked an era of transition from experimental initiation of a global exchange of timely information on arboviruses to an established and recognized system for such communication among widely distributed scientific investigators. For her contribution to this evolution and accomplishment, which was largely voluntary and extracurricular, as are most efforts of the American Committee on Arthropod-borne Viruses, we collectively owe a special note of appreciation.

Responsibility for this increasingly complex effort has been assumed by Mrs. Betty Foster, who has cheerfully accepted the challenge and has produced her first issue, Number 12. Some minor shifts in editorial policy are noted here as necessary to minimize the problems of an increasing number of participants from a wider spectrum of scientific activity associated with arbovirus investigators.

The number and variety of names included as participants in the work or as contributors of information has become so chaotic - as many as seven or eight names per paragraph sometimes - that a more uniform policy has been established. Henceforth, the contributing institution will be presented in the title of the report. Where names are associated with contributions, they will be included parenthetically at the end of the contribution.

Newer contributors, unacquainted with the original objective of brief and timely contributions, have submitted entries ranging from complete manuscripts to lists of references of published work. These cannot be accepted. Editorial attempts are made to lift out the essence of the message for concise presentation of a new idea or data.

Vague carbon copies are received as contributions, the originals having obviously been sent somewhere else for other purposes. These are very difficult to decipher and transcribe. Many tables are still not submitted in a clear or orderly enough condition for direct reproduction. Recasting and retyping take a great deal of time.

Please remember the voluntary nature of this effort and volunteer a little more care in preparation of contributions. It will expedite earlier preparation and distribution of the Infoexchange and will facilitate the efforts of Mrs. Foster and her associates in producing as neat and readable a preparation as possible.

The Rockefeller Foundation continues to provide the colored cover sheets which are so useful in color coding the different issues that come under retrospective consideration.

Special attention is directed to the Special Notice attached to the end of this issue.

Deadline for contributions to Issue Number 13 of the Information Exchange will be January 3, 1966.

Telford H. Work, M.D.
Editor

REPORT FROM DR. ARTURO C. SAENZ, VIRUS DISEASES UNIT
WORLD HEALTH ORGANIZATION, GENEVA, SWITZERLAND

Development of the WHO Arbovirus Reference Centres' Network.

The Department of Epidemiology and Public Health, Yale University School of Medicine, has been designated WHO International Reference Centre for Arthropod-Borne Viruses. Dr. Wilbur G. Downs is the Director of this Centre.

The functions of the International Reference Centre have been defined as:

1) The identification of strains of arboviruses of clinical or epidemiological importance.

2) The provision of prototype strains of viruses to reference laboratories.

3) The preparation of limited quantities of specific sera for reference purposes.

4) The control of the specificity of reagents produced elsewhere on behalf of WHO.

5) The collection and dissemination of epidemiological and laboratory information on arthropod-borne viruses in cooperation with WHO Headquarters and other institutions or organizations working in this field.

The Virus Research Centre in Poona, India, has been designated WHO Collaborating Laboratory for Arboviruses. Dr. T. R. Rao is the Director of this laboratory.

The Service de la Fievre jaune et des Arbovirus, Institut Pasteur, Paris under Dr. P. Panthier, has been designated WHO Regional Reference Centre for Arboviruses.

The designation of the Virus Laboratory of the National Institute for Medical Research, London under Dr. J. S. Porterfield has been changed from 'WHO Regional Reference Centre' to 'WHO Collaborating Virus Laboratory'. This laboratory will carry out research on the use of cell and tissue culture systems for the study of arboviruses, including the possible use of invertebrates' cells.

REPORT FROM DR. WILLIAM C. REEVES
SCHOOL OF PUBLIC HEALTH, UNIVERSITY OF CALIFORNIA

COOPERATIVE RESEARCH PROJECT WITH DISEASE ECOLOGY SECTION, C.D.C. AND CALIFORNIA STATE DEPARTMENT OF HEALTH

This report reviews field and laboratory studies on arboviruses during the period May 1, 1964 through April 30, 1965.

The final year of evaluation was completed on the effectiveness of intensive Culex tarsalis control as a means of suppressing Western equine (WEE) and St. Louis (SLE) encephalitis virus transmission in Kern County. The population of adult and immature C. tarsalis quickly returned to relatively large numbers with the cessation of intensive control in 1964. No isolation of WEE or SLE viruses were made in tests of 40,609 C. tarsalis, and there was no evidence of WEE or SLE virus transmission to chicks that were exposed to vector attack for one night. There was no evidence of SLE virus transmission to sentinel flocks of chickens exposed the entire season; however, there was a low incidence of WEE virus transmission in the late summer and early fall. The sentinel birds also developed antibodies to California, Buttonwillow, Turlock, and Bunyamwera group viruses. It is concluded that intensive C. tarsalis control in 18 to 60 square mile areas from 1960 through 1963 did not accomplish a reduction in the population of females that would stop transmission of WEE or SLE viruses. Female C. tarsalis that infiltrated into the controlled areas posed a major problem in further reduction of the vector population.

General epidemiologic observations in 1964 revealed very low levels of WEE and SLE virus activity in Kern County. There were no recognized clinical cases in man or horse. Serologic conversions to WEE virus in sentinel chicken flocks were very low, particularly in urban areas. St. Louis encephalitis antibody conversions were limited to one sentinel flock in a foothill area. Culex tarsalis populations were held to a low level over most of the county.

Tests have now been run on 155 paired acute and convalescent phase sera that were collected from undiagnosable cases of central nervous system disease with fever. These cases had onsets in the summer and most resided in the Central Valley of California. Serologic tests on these sera with WEE, SLE, Powassan, Modoc, Rio Bravo Bat, California, Trivittatus, Bunyamwera group, Buttonwillow, Turlock, Kern Canyon, and Hart Park antigens have not clarified the diagnosis. Many sera had

low HAI titers to Group B, California, and Buttonwillow antigens but no rises in titer could be detected.

An intensive two year study has been made of the population of mammals that can be captured on a 40 acre grid in a desert area on the margin of agricultural land in Kern County. Over 2,000 animals have been captured and marked with numbered tags. The mammalian population was quite stable in the first year. In the second year, there was a continual decline in the numbers of most species, only Ammospermophilus nelsoni increased in numbers. The monthly success in capturing individual marked specimens continued at a high frequency. We do not believe the decrease in population was a result of trapping and bleeding activities, but was due to other factors that caused a decline in population over most of the region.

Studies of fleas and ticks that are ectoparasites on the grid mammal population have continued to yield information on the prevalence of various species in different months but have not led to virus isolations.

Blood samples have been collected from 1,852 wild mammals from the grid; as many were resampled on recapture, there were 3,392 blood samples. Virus tests are completed on 1,817 samples and have resulted in six virus isolations: one WEE virus, one Bunyamwera group virus, one Buttonwillow virus, and three unidentified viruses. All isolations were from rabbits or ground squirrels. HAI tests are completed on 3,392 plasma samples from these animals. Western equine encephalitis antibodies occurred in low frequency in Lepus californicus, Sylvilagus audubonii, A. nelsoni, Citellus beecheyi, and Spilogales gracilis. The seasonal prevalence of antibody indicates that A. nelsoni may become infected with WEE virus in the winter but L. californicus primarily in the summer. HAI reactors to one or more Group B arboviruses were found in all the common species of lagomorphs, rodents, and carnivores. Fifty-six per cent of the positive plasmas reacted monotypically to one virus. Monotypic reactions to Modoc virus were most frequent in Peromyscus maniculatus and to Powassan virus in A. nelsoni. Analysis of the seasonal distribution of monotypic and polytypic reactions in sera did not clarify the continuing problem of which Group B virus or viruses are responsible for the Group B seroreactors. HAI reactions to California virus were found in 12.5 per cent of lagomorphs and 3.5 per cent of rodents. Reactors to a Bunyamwera group and Buttonwillow

antigens were almost all in L. californicus or S. audubonii. Only one animal, a Dipodomys heermanni, had HAI antibodies to Turlock virus.

Supplementary samplings of 532 wild mammals from nongrid areas revealed the same basic distribution of HAI reactors to arboviruses, but a lower proportion of animals were immune.

An intensive study was begun of Culicoides as vectors of arboviruses in Kern County, CO_2 bait traps and battery-powered portable light traps were effective in collecting large numbers of female and male Culicoides. Eight species of Culicoides were collected, and over 99 per cent were in the Culicoides variipennis complex. Tests of 25,000 Culicoides led to 17 virus isolations. Eleven of 17 isolations were from collections made in May. These viruses are unidentified but their characteristics in tissue cultures and suckling mice indicate there probably are at least two distinct agents.

Studies of the blood-feeding habits of mosquitoes were continued with the identification of blood meals from mosquitoes collected in Colorado, the Sacramento Valley of California, Utah, Arizona, and Illinois. Tests of 3,629 C. tarsalis from Colorado revealed they had the same basic feeding patterns as were observed earlier in California. Most of the feedings were on passeriforme or columbiforme birds and cattle. There was a possible a biphasic shift in feedings between mammals and birds with a spring and late summer increase in the proportion of feedings on mammals. Culex pipiens was more aviophilic than C. tarsalis. Culiseta inornata and five species of Aedes had fed almost solely on domestic mammals.

Tests of 803 blood-engorged C. tarsalis from the Sacramento Valley revealed a pattern of host feedings very similar to those observed in Kern County and Colorado, feeding predominantly on birds and with an increase in feedings on mammals in late summer. Other Culex were aviophilic. Culiseta inornata and Culiseta incidens and three species of Aedes fed principally on cattle. Anopheles freeborni had fed predominantly on cattle and rabbits with a few feedings on birds. Anopheles franciscanus and Anopheles punctipennis had fed solely on domestic mammals.

Mosquitoes collected in Arizona and Utah had fed on the expected host. Culex pipiens collected in an epidemic area in southern Illinois had fed on a surprisingly wide range of avian and mammalian hosts.

Tests on 164 Stomoxys calcitrans collected on chicken farms in California indicated they had all fed on large mammals.

A study was completed on the pathogenicity of three strains of WEE virus in five species of mammals native to Kern County. Dipodomys nitratoides and D. heermanni developed high level viremias and high mortality rates with two of the three virus strains. Ammosperomphilus nelsoni and S. audubonii developed high titer viremias but rarely became ill. Peromyscus maniculatus was very resistant to infection and only an occasional animal became infected. Except for P. maniculatus, all species developed high titer HAI antibodies that persisted for 29 to 58 weeks. Virus could not be recovered from organ cultures of 9 animals infected 58 weeks earlier. It is concluded that these five species of animals do not become infected frequently in Kern County or serologic evidence of infection would be found.

Studies on the pathogenicity of WEE virus in small passerine birds were extended by studies of the course of infection in seven species. As expected from earlier studies, there were high mortality rates in Red-winged Blackbirds. Tricolored Blackbirds, White-crowned Sparrows, and Golden-crowned Sparrows. Only one of three House Finches and no Cowbirds or English Sparrows died. All species had high titer viremias varying in duration from one to six days in the various species. Viremias tended to be highest in the species that experienced mortality.

Culex tarsalis were infected with the Clone 15 attenuated WEE virus strain, and they transmitted this virus when fed on chicks. The attenuated character of the virus did not change after multiplication in mosquitoes or chicks.

A study was begun on the pathogenicity of Group B arboviruses in small mammals. Five species of wild rodents and two species of lagomorphs were inoculated subcutaneously with 1,000 sm LD_{50} of strain BFS-1750 second passage level in hamster kidney cell culture. None of the animals became ill. The C. beecheyi, D. heermanni, and P. maniculatus did not develop HAI antibodies. Ammospermophilus nelsoni, D. nitratoides, and S. audubonii developed HAI antibodies to SLE virus, and a number of these animals developed lower titers of HAI antibodies to Powassan, Modoc and/or Rio Bravo viruses.

Characterization of recently isolated viruses indicates that Buttonwillow virus is a member of the Simbu group. This

virus will infect a wide range of rodents, lagomorphs, and birds; but only an occasional rabbit or ground squirrel had detectable levels of viremia. Virus was maintained in Culicoides for 21 days. Further studies on the Trivittatus-like agent, reported as isolated from C. inornata, revealed major differences from other members of the California virus complex. A virus isolated from the blood of a jackrabbit seems to be closely related to a Bunyamwera group agent that was isolated earlier from C. tarsalis. A number of virus strains that were isolated from mammalian bloods or pools of Culicoides are under study.

A comparative study was completed on the sensitivity of three procedures for detecting WEE and/or SLE viruses in field collected mosquito pools. The three methods were: observation of cytopathogenesis (CPE) in hamster kidney cell cultures (HKCC), followed by neutralization of CPE with specific immune sera; staining of inoculated HKCC cells with fluorescent conjugates to WEE and SLE viruses; and plaquing on duck embryo cell cultures with agar overlay followed by plaque inhibition with specific antisera. All three methods were effective in the detection of WEE and/or SLE virus, although the system that depended on CPE inhibition in HKCC tended to miss mixed WEE and SLE infections. There was a 95 per cent correlation between the other two methods.

A single broadly reacting fluorescent conjugate has been prepared for application to suspected Group B viruses that are isolated from field specimens.

New procedures have been developed for electrophoretic diffusion of antigens in agar and evaluation of precipitating antisera prepared in chickens. This procedure should assist in identifying species specific antigens and the component fractions of antisera.

A collaborative study was begun on the value of the capillary tube and micro-double diffusion in agar procedures for identification of arthropod blood meals.

The new edition of the "Catalogue of Arthropod-borne Viruses" was completed and distributed.

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

REPORT FROM DR. ALBERT RUDNICK
UNIVERSITY OF CALIFORNIA INTERNATIONAL CENTER
FOR MEDICAL RESEARCH AND TRAINING
G. W. HOOPER FOUNDATION, SAN FRANCISCO MEDICAL CENTER

Singapore Virus Isolations.

The identification of 6 strains of dengue virus, 5 from Aedes aegypti and one from A. albopictus collected in 1960, all of which had been tentatively identified as type 2 by neutralization tests, has been confirmed by results of the micro-precipitation agar gel diffusion technique performed by Dr. Y. C. Chan. Precipitation occurred only with dengue type 2 and not with dengue types 1, 3, 4, or Japanese encephalitis hyperimmune mouse serum. No nonspecific precipitation occurred with normal mouse serum.

The desoxycholate-sensitive virus isolated from Boophilus ticks, collected from cattle in Singapore in 1961, has been referred to Dr. Jordi Casals for further identification attempts.

Mosquito-borne Hemorrhagic Fever in Malaya.

Dengue viruses have been isolated from the acute-phase sera of 14 of 61 laboratory - confirmed cases, which occurred in Penang from November 1962 to April 1964. Three of these have been tentatively identified as type 2 on the basis of results of neutralization tests. Chikungunya virus did not appear to be involved in Penang as evidenced by negative serology and failure to isolate the virus.

Three relatively mild cases, more similar to classical dengue disease, were diagnosed in adults in Kuala Lumpur from December 1963 to February 1964. Two viruses, tentatively identified as type 2 dengue strains, were isolated from the acute-phase sera.

It was interesting to observe that dengue virus could be isolated from some acute-phase sera that had high levels of dengue neutralizing antibody.

Dengue Ecology Studies

The preliminary studies of dengue ecology, based on the hypothesis that dengue is a zoonosis, were conducted in

Malaya from August 1962, to June 1964. Blood and tissue specimens were collected from over 2300 wild and domestic animals representing over 55 species of 28 genera. Over 25,000 live adult mosquitoes were taken from urban and forest areas. Attempts to isolate dengue virus from the mosquitoes, sera and tissues collected failed, although 18 strains of other viruses, as yet unidentified, were isolated. Serial blind passages of infant mouse brain and dengue challenge were employed for isolation. A few mice inoculated with monkey sera subsequently resisted lethal dengue challenge, but continued serial passage of that material failed to demonstrate the presence of dengue virus.

The serology is far from complete, but the results of serological tests performed with the sera of wild monkeys, collected in areas away from normal human activity, suggest that strains of dengue virus are active in forest areas of Malaya. Of 223 monkeys, 62.8% had dengue 2 neutralizing antibody of 2 logs or greater, while only 2 of 46 tested had Japanese encephalitis neutralizing antibody (4.3%). While 68.7% of 154 Macaca irus monkey sera tested neutralized 2.0 logs or more of dengue 2 virus, the majority (57.1%) neutralized 3.0 logs or more, and 12.3% neutralized 4.0 logs or more.

Table 1 presents a list of the unidentified virus isolates.

On the basis of the results to date of the preliminnary survey, a new and expanded field program will begin in July 1965. For the first two years, Dr. Nyven J. Marchette will be in charge of the Malaysian unit, which will be based in the Department of Bacteriology of the new University of Malaya Faculty of Medicine in Kuala Lumpur. Some of the principal objectives of the program will be:

1) To delineate a focus of dengue virus activity away from man, not only on the basis of serological evidence, but by isolation of dengue virus.

2) To conduct intensive ecological studies of urban and possible jungle dengue foci to determine the vertebrate and arthropod hosts involved.

3) To conduct studies of comparative experimental transmission of strains of dengue virus types by A. aegypti and A. albopictus mosquitoes in an attempt to evaluate relationship of vector to human disease.

4) To evaluate the potential importance as zoonoses of other little - known arboviruses of Malaysia.

Table 1

Unidentified Viruses Isolated in Suckling Mice

Virus Number	Species	Specimen	Locality	Date Collected (Month, Year)
R-876	Man (PUO)	Serum	Penang	XI-63
R-751	Pig	Serum	Penang	X-63
V-294	Chicken	Tissues*	Penang	IX-63
V-323	Suncus murinus	Tissues	Penang	II-64
P-378	Suncus murinus	Tissues	Kuala Lumpur	XII-63
R-340	Rattus norvegicus	Serum	Penang	V-63
P-223	Rattus jalorensis	Tissues	Rantau Panjang	IV-63
P-225	Rattus jalorensis	Tissues	Rantau Panjang	IV-63
SF-37	Rattus jalorensis	Tissues	Rantau Panjang	IV-63
N-316	Rattus jalorensis	Tissues	Bt. Pakoh, Temerloh	V-64
P-73	Rattus jalorensis	Tissues	Sungei Buloh	XI-62
P-106	Rattus rattus diardi	Tissues	Paroi	XII-62
P-303	Rattus rattus roa	Tissues	Pulau Aur	VII-63
P-132	R. bowersi, R. whiteheadi, and T. glis	Tissues	Bukit Lanjan	IX, X-63
E-24	Aedes albopictus	Mosquitoes	Kuala Lumpur	I-63
E-140	Culex sinensis	Mosquitoes	Ulu Mandul	II-63
E-145	Culex sinensis	Mosquitoes	Ulu Bendol	XII-62 to V-63
E-210	Culex near nilgiricus	Mosquitoes	Pacific Tin	III, IV-64

* Tissues usually included spleen, liver, heart, and kidney.

REPORT FROM THE ARBOVIRUS UNIT, DEPARTMENT OF MICROBIOLOGY UNIVERSITY OF ARIZONA, TUCSON, ARIZONA

Virus Isolation Attempts.

In the last Information Exchange, we reported that we had not isolated any viruses from arthropods, mammals or birds during the hot season of 1964 in Southern Arizona. Subsequently, three suspected isolations were made from three separate pools of Culex quinquefasciatus all collected on September 17, 1964 at the City Jail Farm near Tucson. Six pools of 50 mosquitoes each were collected at the same location on this day and three yielded possible virus isolates. One virus (A652) was isolated in primary hamster kidney tissue culture and subsequently passaged in suckling mice. This has been shown to be related to SLE on the basis of HI and NT results. The other two isolations were made in suckling mice. One is currently being tested and also appears to be SLE-like while the third as yet has not been identified.

Sentinel Flocks.

During 1964 we maintained five flocks of sentinel chickens. These were bled at four to six week intervals between April and November of 1964, and the sera samples obtained, following acetone extraction, were tested by the HI test using WE and an SLE-like (A169, local isolate) antigens. The results using the A169 antigen were clear cut with only four sera, all collected from chickens at the City Jail Farm on November 11, 1964, showing positive HI titers. It is of interest to note that it was from this chicken house that the mosquitoes yielding the virus isolates described above were obtained.

The results obtained with the WE HI tests were not as easy to interpret as were those of the A169 test. Many of the sera tested inhibited the hemagglutination of our WE antigen to high titers. We suspected that this was non-specific in nature as we had observed this phenomenon before, and also several other investigators have reported this in both the Information Exchange and in various journals.

Several methods have been recommended to destroy this inhibitor. The one we tried was recommended to us by Dr. Peter Allen and Mr. Orville Brand of Fort Detrick and consisted of Kaolin extraction of the serum followed by

acteone extraction. A comparison of the results of HI tests on sera following Kaolin-acetone compared to acetone extraction alone is seen in the following table:

Date serum obtained	Acetone alone	Kaolin-acetone extraction
4/14/64	51/64*	1/64
8/11/64	10/40	0/40
11/ 5/64	15/38	1/38

*Number of WE HI titers 20> / Total Number of sera tested

As can be seen, the Kaolin-acteone extraction destroyed the HI inhibiting substance in all but two sera. Although the above results looked very promising, we had at this time no proof that the Kaolin-acetone extraction procedure, although effective in destroying inhibitor, might be equally effective in destroying antibody, at least that present in low titer. Dr. Preston Holden at Greeley, Colorado, was kind enough to send us 10 chicken sera with WE positive HI and NT titers. We tested acetone extracted, Kaolin extracted and Kaolin-acetone extracted samples of these sera against WE antigen and found no significant difference in the HI titers. Nine were high titered sera, but the tenth, which showed a scant 20 titer at Greeley titered 10 in our tests using all three extraction procedures. No decrease in HI titer was noted in SLE-positive sera treated in the same manner. Although it appears that this method is effective in destroying inhibitor without destroying specific HI antibody, more studies must be performed on low titered sera to confirm these results. These studies are currently in progress.

Other Serological Studies.

In addition to the sentinel chickens described above, serological testing is continuing using human, bird, horse, rodent, snake, deer, and pig sera. All samples are routinely tested against WE and A169 (local SLE strain) antigens. We are also starting to screen several of the sera against Powassan, California Encephalitis virus, Buttonwillow, Colorado tick fever, and Cache Valley. Results of these studies will be reported in a later Information Exchange.

Preliminary screening of 102 pig sera with WE and A169 antigens has yielded no positive HI titers against either virus.

Serum from a diamond back rattlesnake (Crotalus atrox) inhibited four units of WE HA antigen to a titer of 640, but a subsequent NT test was negative. The serum was retested following Kaolin-acetone extraction and the WE HI titer was reduced to <10.

Snake Cell Culture Studies.

The studies described in the last Information Exchange are continuing. Several primary snake kidney and lung cultures have been tested for susceptibility to WE and SLE virus. CPE has been noted with WE virus but not SLE, and studies are in progress to determine the specificity of the CPE observed. Further studies will be reported at a later date.

REPORT FROM DR. JOHN G. BOWNE
ANIMAL DISEASE RESEARCH LABORATORY, DENVER, COLORADO

Bluetongue virus was proven to have multiplied in Culicoides variipennis following the intrathoracic inoculation of the virus into the insect. Virus increased as much as 1,000 to 10,000 fold during the first 7 days following infection of the insect and remained at a high level in the insect for over three weeks. Experiments were conducted to determine the influence of the amount of virus inoculated and the duration of incubation of the fly in influencing the amount of virus recovered during the experiment. Statistical analysis showed that the amount of virus inoculated was less important than the duration of incubation of the insect in determining the titer. Preliminary results, using wild-caught culicoid flies, indicate that they are as susceptible to the intrathoracic inoculation of BT virus as colony flies have been shown to be.

Bluetongue virus was isolated from the blood of cattle in the area of a severe BT outbreak in sheep during the 1963 season in Colorado. The cattle were asymptomatic for bluetongue disease. The sheep were imported into the area for winter feeding on refuse from irrigated beet fields. The first symptoms were seen in the sheep ten days after their arrival in the area. This would indicate that the insect vectors,

C. variipennis, were infective at the time the sheep arrived, since it takes approximately fourteen days to experimentally incubate the virus in the vector.

Bluetongue virus has been photographed and studied in the salivary glands of Culicoides variipennis. The cytopathology of BT virus in insect cells is unique and is not recorded in the literature.

REPORT FROM THE DISEASE ECOLOGY SECTION, USPHS COMMUNICABLE DISEASE CENTER, GREELEY, COLORADO

Serologic Response of Horses to Vaccination.

A study was made of the HI antibody response of 48 young horses residing in Iowa following their vaccination during a non-epidemic season with bivalent encephalomyelitis vaccine. None of the pre-vaccination sera had HI antibodies against EE antigen, six (12%) had HI antibodies against WE antigen. Approximately 70% of the animals developed serum inhibitor in titers of 1/20 to 1/80 in tests against EE antigen. Most of the horses without pre-vaccination HI titers against WE antigen developed post-vaccination titers comparable with the EE titers. However, post-vaccination titers increased markedly in animals with pre-vaccination antibodies. One post-vaccination titer among these six animals was 1/160; a second, 1/320; the other four were 1/1280 or greater. Although all six animals developed EE inhibitor, the titers were not appreciably greater than among horses without pre-existing WEE antibodies.

All of the sera from these animals will be tested for CF antibodies. Selected ones will be tested for neutralizing antibodies.

Hale County, Texas, Studies.

In cooperation with the State and local health departments, an intensive investigation of WE, SLE, and other arboviruses has been initiated in Hale County, Texas, where outbreaks of WE and SLE have occurred during the past two years. This will include: (1) epidemiological investigation of suspect human cases of encephalitis; (2) interval bleeding of avian sentinel flocks and testing for HAI antibodies; (3) population indices,

age composition, virus infection rates, and host preferences of mosquitoes; and (4) population indices, virus infections, and antibody rates in wild birds, mammals and reptiles. Experimental control of virus transmission through outdoor residual spraying will also be continued.

Virus Activity in Colorado.

The pattern of weather conditions in eastern Colorado during the spring of 1965 is similar to those which have favored high arbovirus activity in previous years. In view of this and the disastrous floods which occurred in this area during June, field observations on arbovirus activity are being intensified.

Attempts to Isolate Virus from Mammals and Birds.

Three hundred thirty-two mammals and 113 birds were collected from Colorado study sites in 1962. Various tissues were screened in duck embryo tissue culture. In most cases the tissues taken from birds were blood, brain, heart, liver, and kidney; while the mammal tissues included blood, brain, spleen, kidney, and liver. One thousand three hundred eighty-one mammal tissues and 487 bird tissues were tested for virus. All bird and mammal tissues were negative in duck embryo tissue culture.

(Drs. A.D. Hess, L.C. LaMotte, Preston Holden, Richard O. Hayes, and George W. Sciple)

REPORT FROM DR. S. EDWARD SULKIN
THE UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL SCHOOL
DALLAS, TEXAS

Although rabies virus was demonstrated in bats in South America early in the 20th century, it was not until this agent was isolated from insectivorous bats in the United States in 1953 that investigators began to focus their attention on this flying mammal as a possible natural reservoir host for rabies and other viruses. Since that time several experimental studies on viral infections in bats have been reported which seems to have stimulated the inclusion of Chiroptera in recent surveys of animal

populations for evidence of natural viral infection. In view of the current interest in the bat as a carrier of viruses, it seemed pertinent to gather together the documented evidence for considering these animals in this light. Since a large portion of these experimental and field studies have been concerned with the potential role of bats in the epidemiology of the arboviruses, it seemed likely that those receiving the Information Exchange would be interested in seeing the data accumulated to date and in having an opportunity to add to and/or correct the information presented in the accompanying table. Such a cooperative effort would exercise to the fullest the aims of the Exchange with regard to the interchange of information among investigators with similar interests as well as make good use of the international scope of this service. Since significant observations may have been overlooked, it would be appreciated if experiences not recorded in the table would be called to our attention in order that they could be included in a final tabulation being prepared for publication.

FIELD AND EXPERIMENTAL EVIDENCE FOR ROLE OF BATS AS RESERVOIRS OF VIRUSES IN NATURE

VIRUS	BAT SPECIES	GEOGRAPHIC AREA	VIRUS * ISOLATION	ANTIBODY STUDIES † (Test)	EXPERIMENTAL INFECTIONS *	INVESTIGATORS
Rabies	Numerous species	Western Hemisphere, Europe, Far East, Middle East	Numerous isolations		Several studies	1, 2
GROUP A ARBOVIRUSES Venezuelan equine encephalitis	*Corynorhinus rafinesquii* *Eptesicus fuscus fuscus* *Myotis lucifugus lucifugus* *Pipistrellus subflavus*				No symptoms, viremia	3
Eastern equine encephalitis	*Eptesicus fuscus fuscus* *Myotis lucifugus lucifugus*	U.S.A. (Massachusetts)		N	No symptoms, viremia	4, 5
Chikungunya	*Tadarida*, sp (Several unidentified bat species)	East Africa (Entebbe, Uganda)		HI		6, 7
Semliki Forest	*Myotis lucifugus lucifugus*				CNS symptoms, Br	8
Dakar 288,301	*Scotophilus*, sp	Senegal (Guinguindo, St. Louis)	SG			9
GROUP B ARBOVIRUSES Yellow fever	*Eidolon helvum* *Epomphorus wahlbergi haldemani* *Eptesicus fuscus fuscus* *Glossophaga soricina* *Myotis lucifugus lucifugus*	Brazil (Almada)		N	No symptoms, viremia, Br, BF, Sp	10, 11, 12
Dengue, type 1	*Pteropus gouldi*	Australia (North Queensland)		N		13
Bat Salivary Gland (Rio Bravo)	*Eptesicus fuscus fuscus* *Tadarida brasiliensis mexicana*	U.S.A. (California, Texas)	SG, BI			11, 14, 15
Entebbe Bat Salivary Gland	*Tadarida (Chaerephon) hindei*	East Africa (Entebbe, Uganda)	SG			16
Japanese B encephalitis	*Corynorhinus rafinesquii* *Eptesicus fuscus fuscus* *Hipposideros*, sp *Miniopterus*, sp *Miniopterus schreibersii fuliginosus* *Myotis lucifugus lucifugus* *Myotis nattereri bombinus* *Myotis macrodactylus* *Murina leucogaster hilgendorfi* *Pippistrellus abramus* *Pippistrellus subflavus* *Rhinolophus cornutus cornutus* *Rhinolophus ferrum-equinum nippon* *Tadarida brasiliensis mexicana*	Taiwan (northern and eastern) Japan (Kanto Plain, Kyushu, Tokyo)		N	No symptoms, viremia, Br, BF, Ki, antibody production, transplacental passage	2, 3, 4, 11, 17-20
St. Louis encephalitis	*Eptesicus fuscus fuscus* *Myotis lucifugus lucifugus* *Tadarida brasiliensis mexicana*	Minnesota		N	No symptoms, viremia, Br, BF, Ki, transplacental passage	18, 21
Murray Valley	*Eptesicus pumilus*	Australia (Meekatharra)		N, HI		22

Zika	*Rousettus, sp*				Viremia	7
Untyped viruses	*Scotophilus, sp* *Tadarida, sp*	East Africa (Entebbe, Uganda) Senegal (Thies)	SG	HI		6, 7, 9
Tick-borne encephalitis	*Barbastella barbastellus* *Myotis myotis* *Plecotus auritus* *Rhinolophus hipposideros*	Central Bohemia		N	CNS symptoms and pathology, viremia, BF, St	23, 24, 25
Kyasanur Forest Disease	*Rousettus leschenaulti*	India (Manjri near Podna)		N, HI		26
BUNYAMWERA GROUP Bunyamwera	*Myotis lucifugus lucifugus* *Tadarida, sp*	East Africa (Entebbe, Uganda)		HI	CNS symptoms, viremia, Br	7,8
TACARIBE GROUP Tacaribe	*Artibeus jamaicensis trinitatis* *Artibeus lituratus palmarum* *Desmodus rotundus*	Trinidad (Port of Spain and environs)	Br, SG, Sp, Li		No symptoms, viremia, SG, Sp, Li, neutralizing antibody	27
MISCELLANEOUS VIRUSES Poliovirus (Lansing)	*Myotis lucifugus lucifugus*				CNS symptoms, Br	28
Coxsackie virus, B-3	*Eptesicus fuscus fuscus*				Pathology, viremia, BF, Br, He	29
Reovirus, type I	*Eptesicus pumilus*	Australia (Meekatharra)		HI		30
Montana Myotis leuko-encephalitis (MML)	*Myotis lucifugus lucifugus*	U.S.A. (Montana)	Sa, Br, BF, Biting suckling mice		Disease in Myotis l. lucifugus and Eptesicus f. fuscus	31
Lagos bat	*Eidolon helvum*	Nigeria (Lagos)	Br			32
Kern Canyon	*Myotis yumanensis*	U.S.A. (California)	Sp-He			33
UNCHARACTERIZED VIRUSES	*Desmodus rotundus* *Miniopterus schreibersii fuliginosus* *Myotis lucifigus lucifugus* *Myotis velifer velifer* *Tadarida brasiliensis*	Brazil (Sao Paulo) U.S.A. (Texas, New York) Japan (Kyusha) Mexico (Morelos)	Br, SG, Li Bl			11, 34, 35, 36

* Tissues involved - SG, salivary gland; BF, brown fat; Br, brain; Bl, blood; Sp, spleen; Li, liver; Sa, saliva; Ki, kidney; St, stool; He, heart.

† N - neutralization test; HI - hemagglutination-inhibition test.

References

1. Enright: Ann. Rev. Microbiol. *10*:369 (1956)
2. Sulkin: Progr. Med. Virol. 4:157 (1962)
3. Corristan, *et al*: Fed. Proc. *15*:584 (1956)
4. LaMotte: Am. J. Hyg. 67:101 (1958)
5. Daniels, *et al*: New Eng. J. Med. 263:516 (1960)
6. Bres and Chambon: Ann. Inst. Pasteur *106*:34 (1964)
7. Williams, *et al*: Nature 203:670 (1964)
8. Reagan, *et al*: Cornell Vet. 44:298 (1954)
9. Bres and Chambon: Ann. Inst. Pasteur *104*:705 (1963)
10. Rodhain: C. R. Soc. Biol. *123*:1007 (1936)
11. Sulkin, *et al*: Unpublished observations.
12. Dick: Cited by Williams, *et al* (7)
13. O'Connor and Rowan: Nature *176*:472 (1955)
14. Burns, *et al*: Am. J. Public Health 46:1089 (1956)
15. Johnson: 9th Pacific Science Cong., Pacific Assoc., p. 185 (1957)
16. Lumsden, *et al*: Ann. Trop. Med. and Parasit. 55:389 (1961)
17. Ito and Saito: Jap. J. Bact. 7:617 (1952)
18. Sulkin, *et al*: Am. J. Trop. Med. Hyg. *12*:800 (1963); *13*:475 (1964)
19. Miura, *et al*: Unpublished observations.
20. Grayston, *et al*: Am. J. Trop. Med. Hyg. *11*:126 (1962)
21. Olson: Personal communication
22. Stanley and Choo: Bull. Wld. Hlth. Org. 30:221 (1964)
23. Havlik and Kolman: Csl. Epidemiol:, Mikrobiol., Immunol. (Praha) 6:241 (1957)
24. Kolman, *et al*: Acta Univ. Carol., Med., Praha 6:147 (1960)
25. Nosek, *et al*: Acta Virol. 5:112 (1961)
26. Virus Research Centre, Poona, India, (1965)
27. Downs, *et al*: Am. J. Trop. Med. Hyg. *12*:640 (1963)
28. Reagan: Cornell Vet. 44:449 (1954)
29. Dempster, *et al*: Canad. J. Microbiol. 7:587 (1961)
30. Stanley, *et al*: Aust. J. Exp. Biol. Med. Sci. 42:373 (1964)
31. Bell and Thomas: Am. J. Trop. Med. Hyg. *13*:607 (1964)
32. Boulger and Porterfield: Trans. Roy. Soc. Trop. Med. Hyg. 52:421 (1958)
33. Johnson: Info. Exch. No. 2, p. 21 (1960)
34. Dean, *et al*: N. Y. State Dept. Health Annual Report, p. 51 (1963)
35. Irons: Personal communication
36. Trapp: Personal communication

REPORT FROM TEXAS STATE DEPARTMENT OF HEALTH LABORATORIES AUSTIN, TEXAS

In an investigation of possible persistence and overwintering of the SLE virus in Houston following the 1964 outbreak, more than 4,000 culicine mosquitoes were collected January - May 1965. SLE virus has not been found in 85 pools tested in suckling mice.

Similarly neither WEE or SLE virus was found in tests of more than 150 pools of more than 8,000 culicine mosquitoes from Cameron County in the Lower Rio Grande Valley. These mosquitoes were collected from January until May, 1965, after WEE virus was found in a small pool of C. tarsalis collected November 13, 1964.

The Turlock and Hart Park (Flanders) viruses were found during the winter in pools of culicines, and a few isolates are yet to be identified.

Of 42 pigeon sera obtained in Lubbock County in February, 1965, three were reactive and three were equivocal in the HI tests with WEE, but none were reactive with SLE antigen.

Some additional tests on bird sera are in progress, including those on current sentinel flocks of chickens at Houston.

(Drs. J.V. Irons, J.S. Wiseman, and Julian Feild)

REPORT FROM DR. S.S. KALTER, DEPARTMENT OF MICROBIOLOGY SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION SAN ANTONIO, TEXAS

Since our previous communication concerning the inability to detect yellow fever antibodies in vaccinated baboons, we have assayed a number of additional primate serums for this antibody. As indicated in the enclosed table, serums from chimpanzees, orangutans, gibbons, cebus monkeys and humans have now been surveyed. The human serums are from laboratory personnel vaccinated in the United States prior to travelling in endemic yellow fever areas. As can be seen, the number of serums considered as positive is significantly low with the exception of the human group.

In an attempt to continue our baboon survey for antibodies to various arboviruses present in the U.S., approximately 60 baboon serums were tested for CF antibodies to Eastern, Western, and St. Louis Encephalitis virus antigens. This was considered pertinent as these animals live in open, outdoor cages in possible contact with numerous vectors. To date, one animal was found to have antibodies to Western Encephalitis and another had antibodies to St. Louis Encephalitis. We are now in the process of determining the time of appearance of these antibodies as indicated by collection of serum samples.

TABLE NO. 1

YELLOW FEVER SERUM ANTIBODY SURVEY *

ANIMAL	NUMBER TESTED	Results		
		Positive	Partial**	Negative
Chimpanzees	50	3	24	23
Orangutans	23	2	15	6
Gibbons	9	0	2	7
Cebus Monkeys	4	0	1	3
Humans	3	3	0	0

* 1:10 Dilution serum vs. 100 LD_{50} (3-4 week old mice).

** 1-4 of 5 inoculated animals survived.

REPORT FROM THE UNIVERSITY OF ILLINOIS CENTER FOR ZOONOSES RESEARCH, URBANA, ILLINOIS

A study of the susceptibility of a range of small mammals to St. Louis and Powassan viruses was undertaken this spring. The animals infected with St. Louis virus included 4 skunks, 4 raccoons, 1 gray fox, 3 opossums and 5 gray squirrels.

The strain of virus used was isolated from a pool of Culex pipiens mosquitoes collected September 1964 at McLeansboro, Illinois. The fox circulated virus at a low level on the second and third post-inoculation days. All of the opossums and 3 of the squirrels circulated virus. Viremia was detected in 2 of the opossums for the first 6 post-inoculation days and in 2 of the squirrels for the first 5 days. The level of circulating St. Louis virus on each of these days has not been determined.

The animals infected with Powassan virus included 4 raccoons, 2 gray foxes, 2 red foxes, 4 skunks, 2 opossums, 7 gray squirrels, and 4 woodchucks. All of the foxes showed evidence of virus circulation up to the third post-inoculation day. Similarly, all of the squirrels developed a viremia which persisted, in one case, up to the eighth post-inoculation day. All of the opossums and woodchucks circulated virus up to the sixth post-inoculation day. In the case of the foxes and squirrels, the maximum titer of circulating virus was 1.83 logs. The maximum titer of the opossum and woodchuck sera has yet to be determined; however, it does exceed 3 logs. No overt signs of illness were manifest by either the St. Louis- or Powassan-infected animals. Subsequent bleedings will be carried out to ascertain the pattern of the antibody response in them to infection with the respective viruses.

REPORT FROM DR. DONALD M. McLEAN
THE HOSPITAL FOR SICK CHILDREN, TORONTO, ONTARIO, CANADA

Between 7 May and 7 June 1965, 81 forest rodents have been collected by shooting or trapping in the Powassan-North Bay area of northern Ontario, Canada. Of 42 animals examined by both neutralization (NT) and hemagglutination inhibition (HI) tests, 4 of 10 juveniles and 10 of 26 adult groundhogs (Marmota monax) contained both NT and HI antibodies to Powassan virus, a further 4 adult groundhogs neutralized this agent, and 2 animals showed antihemagglutinin only. No antibody was detected in 4 red squirrels (Tamiasciurus hudsonicus) or two other animals. Of another 35 groundhogs, 2 squirrels and 2 other animals examined by HI tests only, 4 of 11 juvenile and 13 of 24 adult groundhogs showed antihemagglutinin. Ixodes cookei nymphal or adult ticks were removed from 15 juvenile and 24 adult groundhogs. Preliminary results strongly suggest the isolation of three strains of Powassan virus from

pools of 1, 6, and 9 nymphal or adult ticks removed from groundhogs captured in Bonfield Township between 24 and 29 May. These preliminary results provide further evidence of a natural cycle of infection involving I. cookei ticks and groundhogs in the vicinity of Powassan during spring 1965.

Sera collected from rodents, principally cotton rats, in the Tampa Bay area, Florida, were made available for testing for hemagglutination inhibition reactions to Powassan antigen by Dr. J.O. Bond and Dr. W.L. Jennings. Antibody was detected by HI in 5 of 127 sera collected during late 1963, 2 of 111 sera collected during early 1964 and 1 of 135 sera collected during late 1964. HI activity against MVE virus was detected only in one serum. Serum from a cotton rat taken during December 1963 neutralized Powassan virus. These findings provide suggestive, but inconclusive, evidence of a focus of Powassan virus infection in the Tampa Bay area.

REPORT FROM DR. ROBERT J. TONN, ENCEPHALITIS FIELD STATION AND JOAN DANIELS, VIROLOGY SECTION MASSACHUSETTS DEPARTMENT OF PUBLIC HEALTH

The Field Station has moved from the Paul A. Dever State School in Taunton to the Lakeville Hospital. The new address is: Encephalitis Field Station, Lakeville Hospital, Middleboro, Massachusetts.

Testing of the 1964 pools has been completed. WE virus was isolated from one pool of Culiseta melanura collected in September at site III. From one pool of Culex pipiens collected in September at site I, a Hart Park like virus was isolated by the Arbovirus Unit of the Communicable Disease Center, Atlanta, Georgia.

The survey of mammals in southeastern Massachusetts has been expanded. None of the 1964 mammals had either EE or WE virus or antibody. To date, EE antibody was found in one cottontail rabbit collected at Lakeville. This rabbit was trapped a block from the site of some cases of EE in horses during the 1955-1956 epidemic.

Very little EE or WE antibody activity was detected in chicken flocks during 1964. Sites I and IV each had one chicken with exhibited WE antibodies. Site II and the

aviary each had one chicken which showed EE antibodies. This was the first indication of EE in the Transmission Rate Chickens since 1962.

The Duxbury Beach Survey of immature birds migrating from the north of the established netting sites to the south yielded no EE or WE antibodies.

Five of 11 blue jays (45 percent) bled during a survey of winter resident birds west of Taunton had EE antibodies. This finding is of interest as none of the 23 blue jays bled during the summer of 1964 at our regular sites had EE antibodies. This summer more birds are being captured at this site.

The results of the overwintering study in reptiles have not been completed to date. Three of the surviving turtles had EE virus and two of the surviving turtles had WE virus present in their blood.

REPORT SUBMITTED BY DR. J. CASALS
YALE ARBOVIRUS RESEARCH UNIT
ROCKEFELLER FOUNDATION VIRUS LABORATORIES
NEW HAVEN, CONNECTICUT

In March, 1964, Dr. A.A. Smorodintsev sent to the RFVL 15 strains isolated in different areas of the Soviet Union, for assistance in serological identification. Extensive studies have been conducted with 8 of the strains; the results reported here are considered to be of particular interest.

Strain Y 62-33. According to Dr. Smorodintsev, this strain was isolated from mosquitoes, Aedes cinereus and A. cantans, wild caught in July, 1962 near Izewsk, Udmurt.

By means of the hemagglutination-inhibition test using group polyvalent immune sera, it was easily established that this agent belonged in group A. Within this group, strain Y 62-33 appeared to be closer to WEE (McMillan) than to any of the remaining viruses of the group; the strain was, however, easily distinguishable from WEE (Tables 1 and 2).

Since antigenic differences are known to exist among strains of arboviruses, including WEE and Sindbis, additional comparisons of Y 62-33 with several strains of WEE and Sindbis were carried out. This was done in order to decide whether

or not Y 62-33 differed sufficiently from these 2 agents to consider it a distinct agent. Three strains of WEE virus, differing widely in antigenic make-up were used: McMillan, TR 25717 and Highlands J. Also several strains of Sindbis virus, and a virus, New Zealand M 78, sent to this laboratory by Prof. Miles; M 78 while related closely to Sindbis is, however distinct. The result of the test is shown in Table 3; there is no doubt that strain Y 62-33 differed from all the WEE strains with which it was compared and even more so from the Sindbis strains.

In conclusion, Y 62-33 is an agent of group A antigenically closer to WEE virus than to any of the remaining viruses of the group; studies now in progress such as comparison with additional strains of WEE virus and the use of complement-fixation and neutralization test will decide whether Y 62-33 should be considered as a distinct virus or as a strain of WEE. At any rate, the strain belongs in the WEE-Sindbis complex; and from all available serological evidence it appears to be a genuine isolate from nature.

Strains K 62-196, K63-35, Y 62-13 and AJ 62-57. These strains were isolated under the following circumstances, as reported by Dr. Smorodintsev: K 62-13, in the Kaliningrad area from a bird, Carduelis spinus (siskin), in October 1962; K 62-35, in the Kaliningrad area from a bird, Fringilla coelebs (chaffinch), in April 1963; Y 62-13 in Udmurt from mosquitoes, Aedes cantans, in July 1962; and AJ 62-57 in Azerbaijan from ticks, Hyaloma detritum, in May 1962. None of these strains has yielded a hemagglutinating antigen for goose erythrocytes, under the usual procedures, i.e., aqueous suspension or sucrose-acetone methods and pH between 6.0 and 7.0; the strains have, however, yielded good complement-fixing antigens with titers between 1:16 and 1:64. By complement-fixation these 4 strains were closely allied, if not identical. This similarity among strains originating in such separate places and from such diversity of host is extremely perplexing; the possibility of a murine virus contaminant cannot be completely dismissed.

The strains are inactivated by 1 per cent sodium deoxycholate; they are highly pathogenic for adult mice or intraperitoneal inoculation.

Serological studies by complement-fixation test have failed to show any relationship between these strains and a number

of immune type sera, as follows. Type immune sera whose known homologous titers varied from 1:64 to 1:512, were used in increasing two-fold dilutions, from 1:4 to 1:64; the antigens, in dilutions 1:4 and 1:16.

In addition to "specific" sera for reference viruses, polyvalent sera for arboviruses groups A and B were used, the latter known to react with all the known agents in the respective groups. The result of the complement-fixation test showed no positive reaction between antigens K 62-196, K 63-35 and Y 62-13 and any of the following immune sera: polyvalent group A, polyvalent group B, Caraparu, Marituba, Bunyamwera, Germiston, Ilesha, Kairi, Wyeomyia, California encephalitis, Guaroa, Catu, Bwamba, Ketapang, Anopheles A, Junin, Umbre, Chenuda, Colorado Tick Fever, Eretmapodites #147, Hughes, IG 619, IG 673, IG 700, Kemerovo, M 1056, Malaya TP 94, Nyamanini, Quaranfil, Rift Valley Fever (Lunyo), Semunya, Silverwater, Thogoto, Tacaiuma, Wad Medani, encephalomyocarditis of mice, GD 7, herpes, lymphocytic choriomeningitis, rabies, and Vilyuisk encephalitis. Finally, immune sera for two of the strains, K 62-196 and Y 62-13, failed to react with an antigen prepared with a strain of mouse hepatoencephalitis, TR 23421.

Table

Hemagglutination-inhibition Test

Identification of Strain Y 62-33

Serum	Antigen, 8 units		Titer with Y 62-33/ titer with homologous
	Y 62-33	Homologous	
Y 62-33	10240		
WEE, McMillan	80	320	1/4
Sindbis, Eg Ar 339	80	1280	1/16
Aura, Be Ar 10315	160	10240	1/64
Una, Be Ar 13136	20	640	1/32
EEE, prototype	40	10240	1/128<
VEE, Trinidad 43	80	20480	1/128<
Getah, MM2021	0	10240	1/128<
Bebaru, MM2354	20	5120	1/128<
Chikungunya, Ross	80	20480	1/128<
Mayaro, Tr 4675	0	1280	1/128<
Middleburg, SAAR 749	40	20480	1/128<
Semliki, prototype	40	10240	1/128<

Reciprocal of serum titers.

First dilution of serum 1:40, except with Una, 1:20.

Table

Hemagglutination-inhibition Test

Identication of Strain Y 62-33

Antigen, 8 units	Serum, Y 62-33	
	#1	#2
Y 62-33	2560	10240
WEE, McMillan	640	2560
Sindbis, Eg Ar 339	80	320
Aura, Be Ar 10315	0	80
Una, Be Ar 13136	0	0
EEE, prototype	-	-
VEE, Trinidad 43	-	-
Getah, MM2021	0	0
Chikungunya, Ross	0	0
Mayaro, TR 4675	0	0
Middleburg, SAAR 749	0	0
Semliki, prototype	0	0

Reciprocal of serum titers.

First dilution of serum, 1:40.

Table

Hemagglutination-inhibition Test

Identification of Strain Y 62-33

Serum	Antigen, 8 units								
		WEE			Sindbis				
	Y 62-33	McM	Tr	HJ	339	2215	886	39	MZ
Y 62-33, #1	2560	640	640	320	80	80	80	80	80
" " #2	10240	2560	2560	1280	320	320	320	320	640
" " #3	2560	640	1280	160	40	80	160	40	160
WEE, McMillan, #1	80	320	160	-	0	0	20	20	0
" , #2	5120	20480	10240	-	640	640	640	640	1280
Tr 25717	640	1280	2560	-	80	80	80	80	160
Highlands J	80	-	-	640	-	-	-	-	-
Sindbis, Eg Ar 339	80	160	640	-	1280	640	1280	320	640
MM2215	80	160	640	-	640	2560	2560	640	320
P 886	80	160	320	-	320	2560	2560	160	160
MRM 39	0	0	0	-	20	40	80	320	20
NZ - M 78	80	40	160	-	80	80	80	160	2560

Reciprocal of serum titers.

O - negative at dilution 1:40.

REPORT FROM ELINOR WHITNEY, DIVISION OF LABORATORIES AND RESEARCH
New York State Department of Health, Albany, New York

The processing of specimens from live-trapped wild animals and arthropods collected in St. Lawrence County during the summer and fall of 1964 and of animal tissues collected during the winter and spring months of 1965 has been continued. The arthropod survey was completed: 202 pools of 2582 insects from the following species: 12 Aedes, 2 Anopheles, 1 Chrysops, 4 Culex, 2 Culiseta, 1 Mansonia, and 1 Tabanus. No infectious agents were recovered in suckling mice observed for 21 days.

A third strain of Powassan (POW) virus was isolated from the spleen of Marmota monax #134 which was trapped on Barnhart Island, August 4, 1964: it is the 4th strain isolated in New York State. (See Proc. Soc. Exp. Biol. & Med., 1965, 119, 432-435 for the final report of the others.)

Ten filterable infectious agents were recovered from venous blood, spleen, liver, kidney, and brain tissues from 3 Microtus pennsylvanicus, #'s 141, 256 and 257 collected at two sites on Barnhart Island, St. Lawrence County, August 4 (#141) and August 31, 1964 (#256 and #257). An 11th strain having the same properties was isolated from a suspension of mites taken from Microtus pennsylvanicus #603 trapped on Barnhart Island March 8, 1965. These agents were not neutralized by immune sera prepared from viruses of EE, WE, SLE, POW, Flanders, A. trivittatus, Modoc, CFT, LCM, Theiler's GD VII, MM, herpes simplex, Cache Valley, and psittacosis. The incubation perios is 8 to 9 days and LD_{50} in suckling mice is the range of $10^{6.5}$ per 0.03 ml. on intracerebral inoculation. In weanling mice death seldom occurred but the mice injected intracerebrally with brain suspensions of 10^{-1} and 10^{-6} when twirled by the tail went into LCM-like convulsions. These convulsions began on the 8th or 9th post-inoculation day and frequently continued until the 20th day. Identification of these agents is being attempted.

From the liver and spleen tissue of a Clethrionomys gapperi #138 collected August 4, 1964, on the Wilson Hill Road, Louisville, in northern St. Lawrence County, two infectious agents which have an incubation period of 5 days were recovered. Both agents were infectious for suckling mice when inoculated intracerebrally or intraperitoneally and for

weanling mice only when inoculated intracerebrally. The LD_{50} of the brain suspension was $10^{7.5}$ per 0.03 ml. The agents were not neutralized by serum prepared with the viruses of EE, WE, POW, SLE, Cache Valley, Theiler's GD VII, MM, herpes simplex, or LCM. Modoc antiserum, however, increased the average survival time by 3 days; 5 of 16 mice were still alive on the 12th day. Identification of these agents is being attempted.

REPORT FROM DR. WILLIAM L. SCHERER, DEPARTMENT OF MICROBIOLOGY, CORNELL UNIVERSITY MEDICAL COLLEGE, NEW YORK

Investigations of arthropod-borne viruses from the eastern coastal tropics of Mexico were continued in 1964 in collaboration with the Pan American Health Organization, the Mexican Government and Mexican scientists (Drs. Campillo, de Mucha and associates of the National Institute of Virology, and Dr. Diaz Najera, of the Institute of Health and Tropical Diseases).

The main region studied was the vicinity of Sontecomapan, a small village on a lagoon within a few miles of the Gulf of Mexico coast in southern Veracruz. Canopy rain forest, secondary forest and mangrove habitats bordering the lagoon have yielded Venezuelan encephalitis virus (Science 3629: 274-275, 1964) and at least two other as yet unidentified viruses, pathogenic for suckling mice. Recoveries of VE virus currently total 7 strains from Culex mosquitoes, 2 from wild terrestrial mammals, 1 from bats, 20 from sentinel hamsters and 21 from sentinel suckling mice.

During summer 1961, a Bunyamwera group virus, possibly Cache Valley, was recovered from three pools of mosquitoes (Mansonia and Anopheles) collected at Tlacotalpan, Veracruz (a river delta, savannah habitat 75 miles west of Sontecomapan). Mosquitoes collected during 1963 yielded 4 additional strains of this virus from Aedes taeniorhynchus. A neutralizing antibody survey of Mexican sera with the 61D240 strain of Bunyamwera group virus revealed antibody in some humans at Tlacotalpan but not in human sera collected at 4,000 feet altitude in 1961 from Coatetelco, Morelos, south of Mexico City. At Tlacotalpan, N antibodies were found only in persons over 41 years of age. Plasmas from a few cattle at Tlacotalpan were negative in 1961 and 1962, but during 1962 and 1963, plasmas and one serum from three other areas on the

tropical eastern coast in Veracruz and Tabasco, and one plasma from the tropical western coast contained N substances. Two suckling pig plasmas were positive at Tlacotalpan in 1961, and plasma from one mature pig was positive in 1962 at Minatitlan, a city southeast of Tlacotalpan in Veracruz. Sentinel chickens at Tlacotalpan and resident chickens at Minatitlan were negative in 1962 and 1963 for N antibody to this virus.

REPORT FROM DRS. R. WALTER SCHLESINGER AND THOMAS M. STEVENS
DEPARTMENT OF MICROBIOLOGY, RUTGERS MEDICAL SCHOOL
NEW BRUNSWICK, NEW JERSEY

Continuing Studies on the Nature of Dengue Virus.

Previous reports with (a) the nature of the inhibition of dengue-2 virus by purified agar extracts and (b) the characterization of the infectious and noninfectious HA fractions from CsCl gradients with respect to RNA content.

Since the last report work has proceeded along the following lines:

1) Improvement of Plaque Assay. In an adaptation of the method of De Maeyer and Schonne (1964), we are now using hydrolyzed starch in the overlay instead of methyl cellulose. 10 ml of the usual nutrient medium (Schulz and Schlesinger 1963) containing 8.5% hydrolyzed starch is followed after 3 days by 5 ml of nutrient agar plus neutral red. Plaques are apparent in 5 days and final counts can be made at 6-8 days. By this method plaques are larger and clearer than under methyl cellulose and the KB cell monolayer is more stable.

2) Virus Activity in High Salt Concentrations. Frequent reports in the literature on thermal stabilization of viruses by high salt concentrations have led to comparisons of dengue activity in the presence of salts such as MgCl, Mg_2SO_4, K citrate, and CsCl. Results presently available indicate that all of these in concentrations of 0.5 - 1.0 M result in an initial increase in HA by approximately 2 fold, presumably due to disaggregation. Mg_2SO_4 causes the same increase in infectivity initially, but greater infectivity losses than in controls are caused by high salt concentrations on storage at 4°, -20° and -70° C for 48 hours or more.

3) Gradient Studies. Previous studies on dengue-2 virus in CsCl gradients disclosed two major types of hemagglutinating particles having average densities of 1.19 and 1.24 (Stevens and Schlesinger 1965). The latter had PFU/HAU ratios at least 2 logs greater than the former. P^{32} labeling experiments indicated a direct correlation between particle density, RNA content, and infectivity.

Since CsCl inactivated 90-99% of viral infectivity, other gradient materials were tested. Cs_2SO_4 also inactivated 90% of the virus. Sucrose and K tartrate reduced titers by only 20% and 40% respectively. The latter two substances, unlike the cesium salts, must be used in preformed gradients, and the separation of particles depends on their sedimentation rates in the individual gradient materials rather than on their buoyant densities. Both types of preformed gradients separated HA particles into two groups (Fig. 1). The faster moving component in the tartrate gradient, like the dense component from a CsCl equilibrium gradient, has a PFU/HAU ratio two logs greater than the slow moving component. In contrast, the ratios for the two HA components in sucrose gradients varied little. The sucrose gradient has very high viscosity compared with the tartrate and CsCl gradients which means that size and shape of the particles assume greater relevancy as compared with density in this type of gradient.

4) Hemagglutinating Subcomponents. Arboviruses have been characterized as lipid-containing due to their ether and detergent sensitivity and to the fact that some members of the group have been demonstrated to be enclosed in host cell-derived membranes. We have extracted dengue-2 virus suspensions with Tween-80 and ether (Mussgay and Rott 1964) and have found an increase in HA titer accompanied by complete inactivation of infectivity. Fig. 2A shows HA curves from 3 sucrose gradients: #1 contained an unconcentrated Tween-80 - ether treated dengue preparation; #2, a concentrated normal virus preparation; and #3, a mixture of both. In sucrose the Tween-ether treated particles move with the second HA component of normal virus. Fig. 2B shows the same experiment in 3 CSCl gradients, instead of sucrose. Here the Tween-ether treated particles are clearly separated on a density basis and are shown to be denser (density 1.27) than either of the usual HA components. Work on the electron microscopic appearance of all three types of HA particles is in progress.

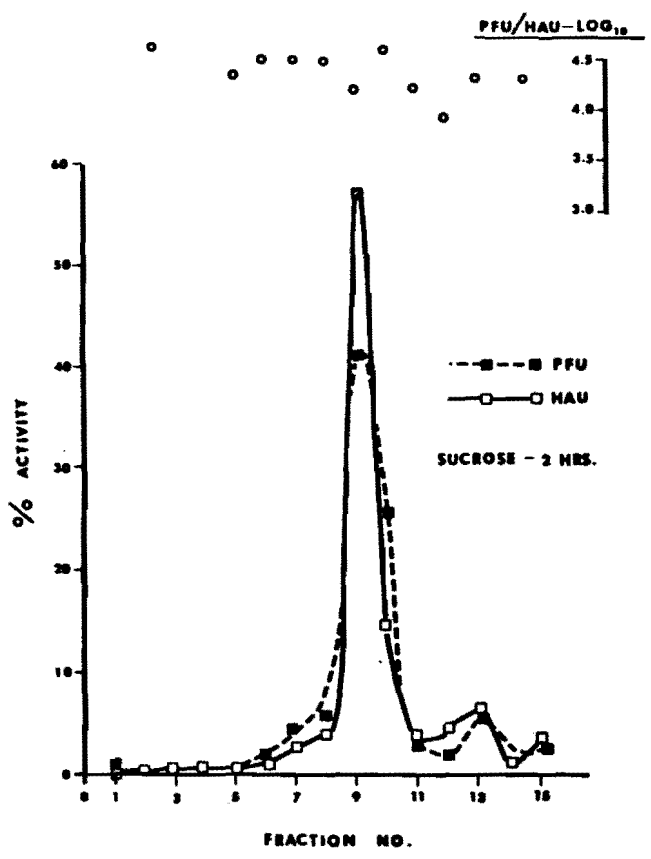
References:

DeMaeyer, E. and Schonne, E. (1964). Starch gel as an overlay for the plaque assay of animal viruses. Virology 24, 13-18.

Mussgay, M. and Rott, R. (1964). Studies on the structures of a hemagglutinating component of a group A arbovirus (Sindbis). Virology 23, 573-581.

Schulze, I.T. and Schlesinger, R.W. (1963). Plaque assay of dengue and other group B arthropod-borne viruses under methyl cellulose overlay media. Virology 19, 40-48.

Stevens, T.M. and Schlesinger, R.W. (1965). Studies on the nature of dengue viruses. I. Correlation of particle density, infectivity, and RNA content of Type 2 virus. Virology (In press).



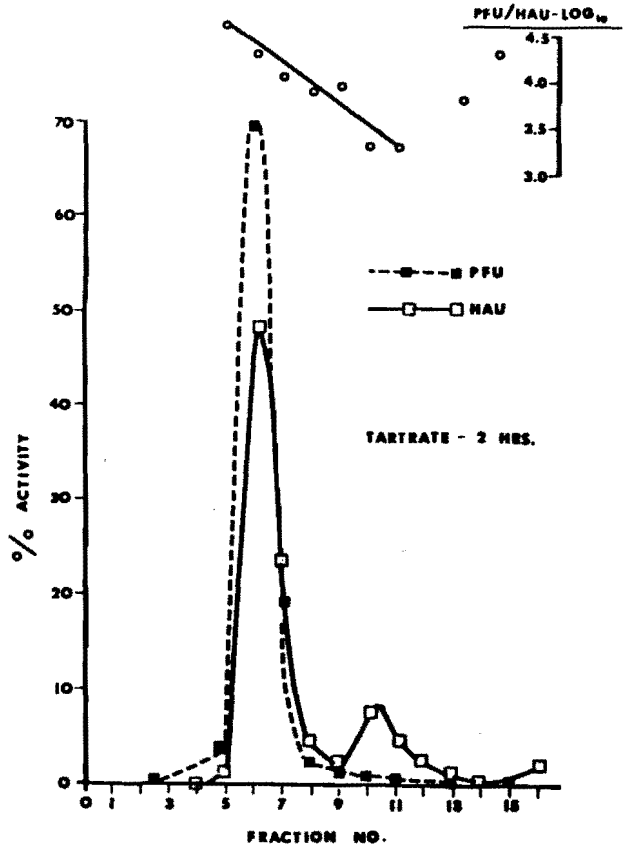
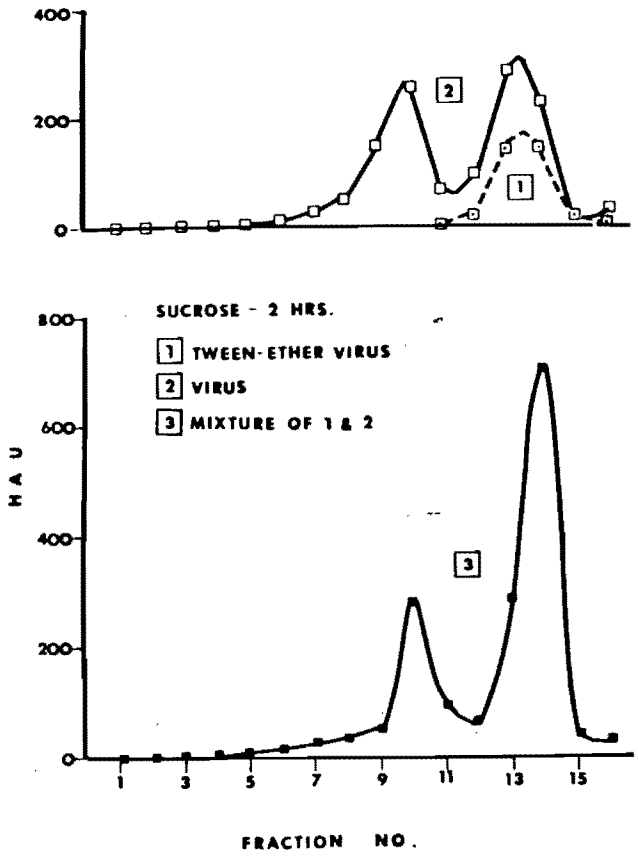
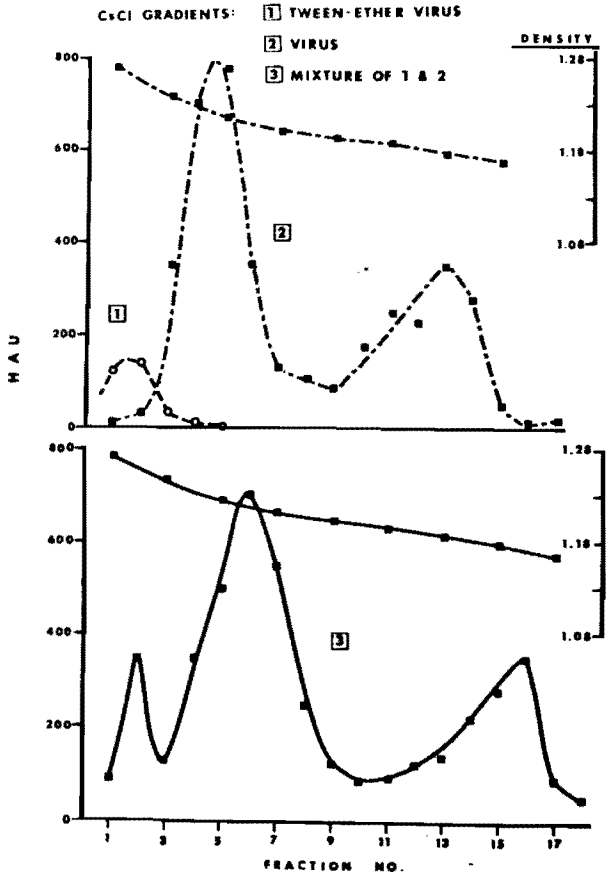


Fig. 1 - Centrifugation of dengue-2 virus layered on preformed sucrose and tartrate gradients.



A



B

Fig. 2 - Comparison of dengue-2 virus concentrate with Tween 80 - ether treated unconcentrated virus in sucrose and CsCl gradients.

REPORT FROM DAVID E. DAVIS AND RICHARD L. BEAUDOIN THE PENNSYLVANIA STATE UNIVERSITY, PENNSYLVANIA

The training of persons to conduct research or surveillance work on birds continues with four students during the entire year and four for the summer. A grid of 64 nets is being operated for the third year. From March 1 to May 31, 934 birds of 58 species were captured, banded and released. Sera were obtained from 412 and blood smears from 555. The data from previous years are being analyzed by the computer. Programs are being tested for analysis of seasonal changes, movement results, recapture estimates and other details.

REPORT FROM DR. HILARY KOPROWSKI, THE WISTAR INSTITUTE PHILADELPHIA, PENNSYLVANIA

Through a system of backcross and brother-sister mating based essentially on a design devised by Snell (9), a C3HRV strain of mice congenic with the virus-susceptible C3H/He strain has been developed. This C3HRV strain is homozygous for the gene of resistance to Arbo B viruses and can be used for exchange transplants of various organs and tissues. Susceptibility of the the C3HRV strain to the mammary tumor agent seemed to be equal to the parent C3H/He strain. Availability of this strain makes it possible to investigate more fully the nature of natural resistance to virus infection.

Congenicity of the C3HRV Line with the Parent C3H/He Line.

A. Incidence of spontaneous mammary adenocarcinoma. In studying the degree of resistance to mammary tumor virus (MTV) present in C3H/He mice, it has been shown that their resistance is dependent upon the number of genes necessary to establish the tumor providing that the virus is transmitted when the newborn is nursed by an infected female. These data provided background for the continued observation of the development of tumors in female progeny from backcross matings of resistant hybrids. According to the scheme of the backcross breeding, male progeny at each generation was mated to female C3H/He mice and all newborn litters nursed by the C3H/He mother in order to insure infection with MTV. Adult female hybrid mice from these litters were forcebred to

male stock mice and checked for the presence of mammay gland tumors. All the controls (C3H/He) developed carcinoma beginning at the age of seven months, while only 16 per cent of the F1 hybrids had tumors at the age of 24 months. However, in subsequent backcross generations the incidence of mammary carcinomas in forcebred females increased but the age at which the tumor was first observed decreased from 12 to 5 months. The observations indicate that as more genes were introduced, the oncogenic expression of MTV infection took place in a more compatible genetic environment.

B. Tests for Histocompatibility. Starting with the 8th generation of backcross breeding, skin was transplanted among mice of the same generation and also to C3H/He mice. The results indicate that only one of the reciprocal grafts was rejected, suggesting histocompatibility of hybrids with the parent C3H/He line.

These three sublines, A, B, and C, of the C3HRV line were tested by the exchange of skin grafts to determine whether they were isohistogenic. In most cases, reciprocal grafting with the B and C sublines resulted in graft rejection within a relatively short time. In contrast, reciprocal grafts exchanged among litters 1 and 5 of generation G6 of subline A remained intact during the 75 day observation period, but grafts exchanged between sibling litters 22 and 42 were rejected after 29 and 33 days, respectively. Although these results would indicate a residual heterozygosity within subline A, histocompatibility exists between subline A and the parent C3H/He strain as indicated by reciprocal acceptance of skin grafts. Also, mice of the one strain were injected with cells of the other and their serum tested for isohemagglutinins and cytotoxins. Both tests were negative. In contrast to the results obtained with subline A, the heterozygosity of sublines B and C parallels that observed after virus challenge.

Cellular Expression of Virus Resistance of the C3HRV Mice.

It has been demonstrated that macrophages obtained at various backcross levels from mice which resisted West Nile virus challenge did not support growth of the virus in tissue culture. Peritoneal macrophages obtained from C3HRV and C3H/He mice were infected in tissue culture with West Nile Virus. At various times after exposure, the cell extracts and the medium were check for infectivity in MK2 monolayers. The results of this experiment indicated that while C3H/He

macrophage cultures supported the growth of West Nile virus, no virus was isolated from C3HRV cultures two to three days after infection.

REPORT FROM DR. ARTHUR N. GORELICK, VIRUS AND RICKETTSIA SECTION
U. S. ARMY BIOLOGICAL LABORATORIES
FORT DETRICK, FREDERICK, MARYLAND

Dr. W.P. Allen continues his studies on the cross-protection among the arboviruses. The susceptibility of rhesus monkeys (Macaca mulatta) to 5 Group C arboviruses has been examined by subcutaneous inoculation of 1×10^3 to 5×10^6 mouse intracerebral LD_{50}. This species was very susceptible to Oriboca (AN 17), Murutucu (AN 974), Marituba (AN 15), and Apeu (AN 848) viruses. In each case clear evidence of viremia and neutralizing antibody was found, but no signs of neurologic involvement were seen in any of the monkeys and only a few had detectable, low-grade fevers. Rhesus monkeys appeared relatively resistant to Caraparu (AN 3994)virus in that they circulated only very low titers of virus for no more than 2 days. One of 6 monkeys tested and pre-existing neutralizing antibodies to Caraparu virus. These antibodies were not attributable to laboratory exposure of the monkey to this or any other Group C virus.

Uninoculated monkeys that were held in close proximity to monkeys that were in the viremic stage of infection with Group C viruses failed to contract infections with these viruses. Apparently these Group C viruses were not excreted in high titer or the monkeys were relatively insusceptible to infection by the oral or respiratory route.

Preliminary results have also indicated that monkeys recovering from infections with any of the 5 viruses mentioned above possess a high degree of immunity against challenge infections with heterotypic Group C viruses.

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

The Effect of Elevated Temperature on Dengue Virus Infection In Vivo.

Two strains of type 1 dengue currently under study have been shown to exhibit marked differences in their virulence for mice with passively induced hyperthermia. When doses ranging from 10 to 1000 LD_{50} of the MD-1 strain (having a history of 33 suckling mouse passages) are inoculated intracerebrally (IC) into 4-week-old mice housed at a constant temperature of 35° C, clinical disease is either completely aborted or significantly delayed over a 21 day observation period. Similarly inoculated control mice housed at 22° C all die within this period. If, after 21 days at 35° C, surviving mice are placed at 22° C, clinical disease and mortality ensue in patterns approximating those of the control group.

The complete protective effect of elevated temperature can be demonstrated when mice previously housed at 22° are inoculated IC with 1000 LD_{50} and then subsequently placed at 35° C up to 5 days post-inoculation. Although clinical disease may develop in these animals, approximately 50% survive.

In sharp contrast to the MD-1 strain, a "fixed" laboratory strain having a history of 125 passages in mice of various ages, is relatively insensitive to the effect of elevated temperature. Although the onset of disease elicited by graded doses of this strain is delayed by approximately 2-3 days in mice housed at 35° C, these animals display mortality patterns which are essentially identical to those similarly inoculated but kept at 22° C.

The $ICLD_{50}$ titers of both strains in mice housed for 21 days at 35° C and an additional 15 days at 22° C as compared with titers in animals housed only at 22° C are shown in Table 1.

Table 1. $ICLD_{50}$ titers of MD-1 and fixed strains of type 1 dengue in mice housed at 22° and 35°.

STRAIN		POST-INOC. 35°	DAY 21 22°	POST-INOC. 35°-22°	DAY 36 22°
MD-1	Exp. 1	<3.0	5.5	4.7	5.7
	Exp. 2	3.5	6.0	5.5	6.0
"Fixed"	Exp. 1	6.1	7.0	6.2	7.0
	Exp. 2	6.5	7.1	6.8	7.1

Although studies are still incomplete, the effect of passively induced hyperthermia on the course of MD-1 virus infection does not seem to be mediated by either humoral antibody or interferon but appears to be due to a reduced efficiency of this strain to multiply at a supraoptimal temperature. (Mr. Cole)

REPORT FROM DR. ALEXIS SHELOKOV
LABORATORY OF VIROLOGY AND RICKETTSIOLOGY
DIVISION OF BIOLOGICS STANDARDS
BETHESDA, MD.

At our last writing (Infoexchange issue No. 10) Drs. A. Shelokov and N.M. Tauraso just moved from Laboratory of Tropical Virology, National Institute of Allergy and Infectious Diseases to Division of Biologics Standards at NIH to assume new responsibilities, including the establishment of an arbovirus laboratory. By now a comprehensive collection of arboviruses, with standardized virus pools, CF and HA antigens and antibody reagents, has been organized.

We have been concerned with the immunological relationships among members of the new and important group of South American viruses; two of these are known to produce hemorrhagic fever in man. We attempted to examine what protection might be induced in guinea pigs against Junin virus by immunization with

live Tacaribe virus and to determine the antibody content of sera from these animals.

A group of immunized adult female guinea pigs received 3 x 10^5 PFU's of Tacaribe virus. This represented a 1:20 dilution of the stock 20% Tacaribe virus-infected SMB. A control group received an inoculation of 20% normal SMB at the same dilution. Groups of immunized and control animals were challenged with Junin virus on the 14th day. Complement-fixation and plaque neutralization tests were performed on unpooled samples of sera obtained from individual control and immunized guinea pigs which were bled out by cardiac puncture prior to immunization and on days 14, 21, 32, and 49.

Adult guinea pigs were protected against challenge with Junin virus 14 days after immunization with live Tacaribe virus administered intramuscularly. Titration of Junin virus performed simultaneously in control and immunized animals resulted in a LD_{50} of $10^{6.5}$ in the former and $<10^{1.0}$ in the latter group (protection index >5.5).

Sera from the virus-immunized animals had significant CF antibody titers on the 14th day in tests with both Junin and Tacaribe antigens. However, the sera reacted with the Junin antigen to a lower degree than with the homologous antigen. The titers continued to rise until the termination of the experiment on the 49th day.

Plaque neutralization tests were performed on bottle culture of MA-104, a continuous line of embryonic rhesus monkey kidney cells provided by Mr. M. Vincent of MBA, Inc. Significant homologous neutralizing antibody titers were demonstrated in sera obtained on the 14th day. A progressive increase in titer was demonstrated on days 21 and 32, at which time the titer was higher than the plaque neutralization test could detect with sera diluted 1:4.

Long term experiments are currently in progress to determine how long this protection exists (so far it has lasted for at least 9 months).

Dr. Shelokov spent 30 days in the Soviet Union as one of the six members of the U.S. Delegation on Hemorrhagic Fevers in the U.S.S.R. Other participants were Drs. J. Casals, RFVL, H. Hoogstraal, NAMRU-3, K.M. Johnson, MARU, N. Wiebenga, NIAID, and T.H. Work, CDC. A report on some of the results of the mission will be found elsewhere.

REPORT FROM FLORIDA STATE BOARD OF HEALTH LABORATORY JACKSONVILLE, FLORIDA

Following the epidemic of SLE in the Tampa Bay area of Florida in 1962, the need for an expansion of research, surveillance, and control of encephalitis was recognized and additional state and federal funds were obtained for this purpose. With the aid of an NIH Grant, the Florida State Board of Health Encephalitis Research Center (ERC) was established in Tampa under the direction of Dr. J.O.Bond, and an investigation of the factors influencing the transmission of SLE and other arboviruses in the four counties where the outbreak occurred was undertaken. These findings are reported separately. Observations in the other 63 counties are considered here.

An intensified state-wide arbovirus surveillance program was initiated at the SBH in Jacksonville involving the combined efforts of the Bureaus of Preventable Diseases, Entomology and Laboratories. In 1963, the Virology Section and animal facilities of the Bureau of Laboratories were expanded and additional personnel recruited to provide the capabilities for performing arboviral serology and isolation procedures on field specimens collected for these surveillance studies.

Since information on the distribution of arboviruses outside of the Tampa Bay region was fragmentary or non-existent, early efforts were directed toward identifying areas of activity of EE, WE, and SLE, viruses previously known to be present in Florida. In late 1963 and early 1964, personnel of the Division of Veterinary Public Health collected 491 blood specimens from small backyard chicken flocks located in counties scattered throughout the state but including those in which equine cases had occurred during the previous year. Birds one year of age or less were chosen when possible. Of the 491 sera examined by HI test 126 (39%) reacted at a 1:10 dilution or greater with EE antigen, 60 (25%) with WE and 21 (4%) with SLE. EE and WE reactors were found more commonly in the eastern panhandle and adjacent gulf coast counties, suggestive evidence of minimal SLE activity was restricted to the upper peninsular gulf coast and middle and lower east coast counties. (A more detailed summary of these findings was presented in the 10/64 issue of the Exchange.) As a result of the preliminary studies, sentinel chicken flocks were placed in three of the larger north and central Florida metropolitan areas and also in the panhandle region where evidence of a high degree of EE activity had been noted. Although blood samples drawn

at bi-weekly intervals throughout the mosquito season were tested with EE, WE, and SLE antigens, only one EE-HI converstion was noted in a bird exposed in the panhandle district. All of these surveillance observations, together with morbidity and mortality reporting of encephalitis in horses and game bird flocks by practicing veterinarians provided useful guides for the 1964 mosquito trapping program of the Bureau of Entomology.

During May through December, 772 pools of mosquitoes trapped in 20 panhandle, north and central Florida counties were submitted for virus isolation studies. Results of suckling mouse inoculation tests were as follows:

ARBOVIRUS TYPES ISOLATED FROM 772 MOSQUITO POOLS
COLLECTED IN FLORIDA DURING MAY - DECEMBER, 1964

Mosquito Species	Arbovirus Type					
	EE	WE	CALIFORNIA COMPLEX	BUNYAMWERA GROUP	HART PART LIKE	TOTAL
Aedes Atlanticius	1	0	8	1	0	10
Aedes infirmatus	0	0	2	0	0	2
Aedes taeniorhynchus	0	0	1	0	0	1
Aedes species	2*	0	4	0	0	6
Anopheles crucians	0	0	0	5	0	5
Culex nigripalpus	3	0	1	0	0	4
Culex (Melanoconion) Sp.	1	0	0	0	0	1
Culiseta melanura	6	1	0	0	5	12
Totals	13	1	16	6	5	41

*EE and California Complex isolated from one pool.

Viruses were isolated from collections made in 11 of the 20 counties sampled. EE, Hart Park like, and Bunyamwera group viruses were recovered from the panhandle and central Florida districts, California Complex viruses from virtually all areas, while the single WE strain was from the panhandle region. In three instances EE was isolated from collections made in the vicinity of human cases.

Four human cases of EE were diagnosed in Florida during 1964, two of which terminated fatally. Each was confirmed serologically and in one instance the virus was recovered from autopsy material at ERC. No other confirmed cases of encephalitis were reported from the 63 counties although specimens from 183 CNS disease cases were examined. Of these, 115 were diagnosed clinically as suspect encephalitis. Positive laboratory findings were obtained in 24 instances, viz, 2 EE, 16 mumps, 2 herpes simplex, 1 Coxsackie B4, 1 Coxsackie B5, 1 Coxsackie A9 and 1 ECHO 2. The remaining 68 cases were reported as suspect aseptic meningitis. Serological confirmation of mumps was established in 12 and of leptospirosis in 3 of these individuals.

In February 1965, evidence of SLE was noted in backyard chicken flocks located in several southwest counties of the state. The finding of HI and SN antibodies in the sera of birds presumably one year of age or less, constitutes the first indication of SLE in Florida since 1962. Intensive follow-up studies are currently underway in that area.

All concerned with state-wide study, surveillance and control of arbovirus infections in humans together with consultants, have evolved detailed plans of programs to cope with any of the varying degrees of arbovirus activity. Through a personal letter from the State Health Officer, practicing physicians, pathologists and hospital administrators, have been advised of the availability of expanded virus diagnostic services and invited to use them. They were also urged to report promptly any suspect case of CNS viral infection. The detailed operational plans were placed in the hands of each county health officer for their guidance. The hope is that through early recognition and promptly intensified control measures, epidemic arbovirus infection can be avoided.

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

Viral Isolations, 1964.

In contrast to 1963, when only 10 arboviruses were recovered from field specimens, 91 isolations of viral agents were accomplished during 1964. Table I gives the distribution of these isolations by viral type and source of specimen. All initial isolations from a given source were identified by both CF and SN tests at the ERC and then sent to reference laboratories in C.D.C. or the University of Pittsburgh for confirmation. Later isolations from the same source were identified by CF tests only, using crude mouse brain antigen. Reisolation was accomplished from over 90% of the original specimens.

Of particular interest in the Table are the following points. The two EE virus isolations from human specimens were from the same individual, one from brain, one from cerebrospinal fluid. The variety of mosquito species from which EE viruses were obtained was new and unusual in Florida experience. Whether this represents the peripheral involvement of a large number of bird-biting mosquitoes in an epizootic year or whether potential new vectors have been identified will be questions for future studies. The isolation of WE virus from a horse brain is the first known such observation on the eastern seaboard of the United States. Isolation of WE from Florida mosquitoes and wild birds has been easily accomplished in the past. Arboviruses belonging to the California complex are now the most commonly found type of arbovirus in Florida mosquitoes. The apparent significance of this will be discussed later in this report. Bunyamwera group and Hart Park-like viral agents are also apparently common throughout Florida as in the entire eastern and southeastern U.S.A. A limited search for tick-borne arboviruses resulted in 9 recoveries, 3 of which are being studied further at the University of Pittsburgh Laboratories under Dr. Hammon's direction. It is important to note the absence of any SLE virus isolations in the Table of Results for 1964. Despite a most intensive search, there has been no virologic or serologic evidence of activity of this virus in the Tampa Bay region since the epidemic of 1962.

TABLE I

Viral isolations by type of specimen collected and examined January 1 through December 31, 1964

Type of specimen	Viral agent isolated							Estimated number of specimens examined
	Eastern enceph.	Western enceph.	California complex	Bunyamwera group	Hart Park like	Unidentified	Total	
Human	2						2	99
Equine	5	1					6	14
Pheasant	1						1	1
Exposure chicks	2	2					4	4
Culex nigripalpus	4				1		5	2,205 pools
Aedes infirmatus	1	2	8	2			13	322 "
Aedes atlanticus			19				19	68 "
Culiseta melanura	8	4			5		17	142 "
Aedes species			2				2	33 "
Anopheles crucians	1			9			10	167 "
Aedes taeniorhynchus			1				1	85 "
Culex quinquefasciatus	1						1	379 "
Mansonia perturbans	1						1	150 "
Dermacentor variabilis						8	8	73 "
Haemaphysalis leporis palustris						1	1	
T o t a l	26	9	30	11	6	9	91	

June 10, 1965/ALL/iem

Equine Encephalitis Epizootic.

An epizootic of equine encephalitis occurred in Florida during 1964. In the Tampa Bay region this was studied intensively in Hillsborough County, where 22 equine cases were reported. Brains from fourteen of these horses were examined for virus at the ERC; five isolates of EE and one of WE were made. A survey of the equine population was then made for inapparent infection rates by probability sampling techniques. The total equine population in the county was estimated at 6,400, 40% of which was unvaccinated. A subsample of 107 horses (chosen to represent both vaccinated and unvaccinated animals) was then bled. The prevalence of HI antibodies to the respective antigens in vaccinated and unvaccinated animals was as follows: EE, 58% vs. 18%, WE, 78% vs. 26%; SLE, 52% vs. 34%; California, 0 vs. 2%. The clinical attack rate in susceptible animals (unvaccinated) was 22 per 1,747 for both viruses. Assuming only one clinical case was due to WE virus, the inapparent infection to disease ratio for EE virus was 1.5 to 1, and for WE, 45 to 1. Further serologic studies for CF and SN antibodies are in process on these sera.

During the epizootic there were repeated recoveries of EE and WE virus from mosquitoes. From Culiseta melanura, 8 EE and 4 WE isolates were made. From Aedes infirmatus, 1 EE and 2 WE viruses were recovered; also one recovery of EE virus was made from each of single pools of Culex quinquefasciatus, Culex nigripalpus and Mansonia perturbans mosquitoes. Isolates were made as early as March and as late as November with maximum recoveries in May. In February, 1965, the EE virus was recovered from yet another species of mosquitoes, Culex salinarius. All WE isolates from 1964 were sent to Dr. Henderson at Yale University for serotyping. All were found to belong to his antigenic group, phase II (Highland J).

California Virus Studies.

Two strains of California group viruses were first obtained in the Tampa Bay area of Florida during the SLE epidemic in the fall of 1962. Early in 1963, an HA antigen from the prototype strain (BFS-283) was provided to the ERC by Dr. Hammon of the University of Pittsburgh. Subsequent joint virologic and serologic studies carried out by these two laboratories are summarized as follows:

Two seemingly different arboviral agents, both members of the California complex, have been recovered from fresh water Aedes mosquitoes collected in the Tampa Bay area of Florida during the past two and a half years. From 20,017 Aedes infirmatus, 16 viral strains have been isolated. Early serologic studies reveal them to be similar to each other and to the trivittatus isolate of Eklund. From 3,430 Aedes atlanticus mosquitoes tested, 19 strains have been recovered; all with similar biologic properties. Preliminary studies suggest these isolates are more closely related antigenically to the prototype BFS-283 strain. Recoveries of virus were made in both years, at all seasons of the year, and from different areas of the Bay region.

Serologic evidence of infection with the California group of arboviruses in man and wild mammals has been obtained with HA antigens prepared from the BFS-283 prototype strain. As part of the routine surveillance for viral infections in man in the area (population around one million) 603 individuals have been tested for HI antibody to BFS-283. Two recent infections were identified by four-fold or greater HI antibody rises (Tampa Laboratory) and confirmed by CF and SN antibody rises (Pittsburgh Laboratory). Both cases occurred in children (a white female, age 11, and a white male, age 2) who had moderately severe encephalitic syndromes from which they recovered without apparent sequelae.

Serologic surveys have been carried out for inapparent infections in man. Of 1,005 individuals tested with California antigens through June of 1965, five demonstrated HI titers of 1:20 and twenty had titers of 1:10. A small group of six of these sera received SN antibody tests in Pittsburgh by standard mouse neutralization techniques. Three of the HI-positive sera had SN antibody at greater than 2.5 logs; three of the HI-negative failed to show protection. Approximately 300 persons from the larger survey group have been bled at annual intervals for two years. No serologic conversions to California antigen have been detected.

Serologic examinations have also been performed on bloods collected from 705 wild or domestic mammals representing 20 different species. The largest numbers have come from the cotton rat, horse, opposum, rice rat and house mouse. One of 21 rabbits tested demonstrated a titer of 1:40; one of 122 horses tested had a titer of 1:20; one of 221 cotton rats had a titer of 1:20. In eight sera representing the

squirrel, horse, opossum, and racoon, low titers of 1:10 were detected. None of these mammalian sera with HI titers have yet been processed for neutralizing antibody.

It is evident that California complex viruses are readily obtained from fresh water Aedes mosquitoes in the Tampa Bay region. Present evidence suggests there are at least two distinct serotypes, which appear to be species related. Despite this evidence of activity in mosquitoes, there is minimal evidence by HI tests with BFS-283 antigen of infection in man or other animals.

REPORT FROM DRS. WILLIAM L. POND AND N. JOEL EHRENKRANZ
INFECTIOUS DISEASES DIVISION, DEPARTMENT OF MEDICINE
UNIVERSITY OF MIAMI SCHOOL OF MEDICINE, MIAMI, FLORIDA

Detection of SLE Activity in South Florida in 1964 Using Sentinel Pigeons.

In conference with University Zoologists and Ornithologists, we established what we believe to be an effective sentinel program in the Miami area (using the ubiquitous pigeon) which was capable of revealing activity of SLE virus in the South Florida area in 1964, a year when there was no other observable proven activity of this virus in man here or elsewhere in Florida. In addition to our surveillance of the changing serological status of the free-flying pigeon population in the area we have been continually monitoring since July of 1963, groups of pigeons which we strategically placed at home sites of previous SLE victims. These experimental birds have been maintained in cages which allow free access of mosquitoes and all these pigeons have been bled at approximately six week intervals. Promptly after each collection of blood, these sera are included in HI tests for arbovirus antibodies. Over these two years of continuing observation, our convictions have been strengthened as to the effectiveness of our methods of using this species in indicating small amounts of SLE viral activity (without the appearance of serological activity due to non-specific factors). Our continuing studies to date (to May 1965) have indicated that the pigeon population in the Miami area has now reached a serologically negative condition as compared to the situation in 1963. However, as judged by conversion

of the serological status (HI and neutralization) of some of our sentinel pigeons there was SLE activity in this community in October of 1964. This serological change in our pigeons appears to be the only evidence of SLE viral activity in Florida this past year.

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY UNIVERSITY OF THE WEST INDIES, JAMAICA

There has been a severe drought in Jamaica from December 1964 to April 1965. This has affected our entomological programme very much. The accompanying table shows rainfall figures in inches for the months of January - March 1965, and also normal figures in areas where we do most of our collection of mosquitoes.

Areas	Rainfall in Inches					
	January		February		March	
	1965	Normal	1965	Normal	1965	Normal
Caymanas	1.08	0.97	0.11	1.16	Not reported	
Milk River	0.70	1.58	0.55	0.55	0.15	1.43
Four Paths	0.73	1.48	Nil	1.85	1.38	2.32
Duckenfield	2.91	2.79	0.91	4.13	0.51	2.34

Mosquito collection for the same periods, January-March 1965, has dropped considerably. Detailed figures would be available for the next quarterly report.

Isolation from Anopheles Grabhamii.

A viral agent has been isolated from a pool of 133 Anopheles grabhamii collected in the parish of St. Thomas in May 1965. This agent is sensitive to ether and to chloroform. We are now attempting to identify the agent.

REPORT FROM THE TRINIDAD REGIONAL VIRUS LABORATORY

The rodent population in Bush Bush Forest has remained at exceedingly low levels since the population crash in 1963. In Bush Bush, sentinel mouse exposure has continued as in previous years and the mosquitoes attracted to them have routinely been tested for virus. Human bait catches of mosquitoes were curtailed as new information from this activity was not likely to be forthcoming. No viruses were isolated from Bush Bush material since July 1964. As alternate study site the Arena Forest near Brazil Village in central Trinidad has been selected. This area has been a center of EEE and SLE activity in previous years and represents the only source of Triniti virus (TRVL 7994 - one strain). Human bait captures (day and early evening) and trap catches (chick and mouse bait) have been made in this area, and so far without virus recoveries.

Bird bleeding, banding and releasing has continued at four localities. No activity of EEE virus has been discovered in 1964. In the first half of 1965 some serological conversions were discovered.

A mosquito survey of the island of Dominica was initiated 3 June 1965.

Two agents which were isolated from dengue cases occurring in Antigua are being studied in tissue culture.

(L. Spence, T.H.G. Aitken, C. Brooke Worth and A.H. Jonkers).

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

At the beginning of 1965 studies of the local tick fauna were begun with a view to seeking possible tick-borne viruses. Preliminary probes in the high altitude "tierra fria" where the landscapes superficially resemble those of Northern Europe in which tick-borne viruses are prevalent, have revealed that no tick species appear to have become successfully adapted to domestic animals.

There were 19 virus isolations from mosquitoes captured at the Rio Raposo field station, from January through December 1963. The station is located in the Pacific lowland rain forest. The following table summarizes these isolations made by baby mouse inoculation.

Raposo River, 1963
Virus isolations from mosquitoes captured with human bait

Pool No.	Date capture	Mosquito identification	No. in the pool	Virus identity
919	Jan. 14-15	Wyeomyia complosa	47	Wyeomyia complex
931	Jan. 15-21	Trichoprosopon longipes	26	Wyeomyia complex
932	Jan.15-16-21	Anopheles sp.	9	Wyeomyia complex
1071	March 18-19	Wyeomyia aporonoma	18	?
1112	April 16	Wyeomyia spp.	300	Bunyamwera group (Wyeomyia complex?)
1184	May 6-7-8	Wyeomyia melanocephala	66	Wyeomyia complex
1263	May 28	Wyeomyia complosa	176	?
1268	May 28-29 June 3-4	Trichoprosopon digitatum	27	Wyeomyia complex
1270	May 28-29 June 3-4	Trichoprosopon leucopus	75	Wyeomyia complex
1271	May 28-29 June 3-4	Limatus spp.	56	Wyeomyia complex
1274	May 28-29 June 3-4	Psorophora ferox	27	Una
1292	June 5-10	Psorophora ferox	25	Una
1315	June 10-11	Wyeomyia aporonoma	50	Wyeomyia complex
1340	June 18	Wyeomyia sp.	45	Wyeomyia complex
1369	July 1-2	Mansonia arribalzagae	228	Wyeomyia complex
1377	July 1-2-3	Wyeomyia aporonoma	55	Wyeomyia complex
1459	July 17	Wyeomyia spp.	195	Wyeomyia complex
1492	Aug.12-13-20	Trichoprosopon leucopus	41	Bunyamwera group (Wyeomyia complex?)
1507	Aug. 12	Wyeomyia spp.	244	Wyeomyia complex

REPORT FROM DR. ENRIQUE PRIAS-LANDINEZ AND DR. CARLOS BERNAL-CUBIDES
ARBOVIRUS LABORATORY, INSTITUTO NACIONAL DE SALUD, BOGOTA, COLOMBIA

From our field work during 1964, we extracted the following results:

As shown in Table I, 105 bats (Carollia perspicillata) were captured at San Vicente de Chucuri. Of sera submitted to the NT, three were positive, nine inconclusive and all the rest negative. Ninety eight sera were tested by the HI test, and only one reacted up to the 1:20 dilution against the Neurotropic French strain of yellow fever antigen. Three sera reacted at dilutions 1:20, 1:40 and 1:40 respectively, with the Ilheus antigen. Five sera reacted up to dilutions 1:40 or 1:160, even up to 1:25600 in one case with the VEE antigen. Only one serum reacted with the Mayaro antigen (Dilution 1:80).

Only three Glossophaga longirostris were examined and all were negative.

One goat serum was positive at the 1:640 dilution in the HI test with the VEE antigen and one mule serum titered 1:40 with the Ilheus and VEE antigens. During 1964, one hundred bats were captured at "La Guajira" (98 Glossophaga longirostris and 2 Carollia perspicillata). Table 2 summarizes the serologic results with bats captured at the "Baja Guajira". Only one of the G. longirostris was positive in the NT with yellow fever virus. Many sera reacted with Group B antigens in the HI test. The titers ranged between dilutions 1:20 and 1:25600; arithmetic mean 1:1160 with the yellow fever antigen.

The titers ranged between dilutions 1:20 and 1:1280; arithmetic mean 1:330 when tested against the VEE antigen. All sera were nonreactive with the Mayaro antigen.

The HI titers are referred to 8 units of antigen. The NT was performed by the i.c. route in white albino-swiss mice, 25 to 45 days old and the virus-serum mixtures contained 50 to 200 MLD_{50} per inoculum.

TABLE I

Positive serologic results with mammal sera. San Vicente, 1.964.

Animal No.	Animal Species	N.T.			HI Test				
		Positive	Inconclus.	Total examined	Y.F.	Ilh.	VEE	May.	Total examined
A-9	Carollia perspicillata	-	+		0	0	0	0	
A-17		-	+		20	0	0	80	
A-18		-	+		0	0	0	0	
A-25		-	+		0	0	0	0	
A-29		-	+		0	0	0	0	
A-31		-	-		0	0	160	0	
A-41		+	-		0	0	0	0	
A-43		-	-		0	40	0	0	
A-57		-	+		0	0	0	0	
A-59		-	+		0	0	0	0	
A-60		+	-		0	0	0	0	
A-63		+	-		0	0	0	0	
A-67		-	-		0	0	25600	0	
A-71		-	-		0	0	40	0	
A-99		-	-		0	40	0	0	
A-101		-	+		0	20	0	0	
A-104		-	-		0	0	40	0	
A-105		-	-		0	0	40	0	
A-112		-	+		0	0	0	0	
Totales - - -		3	9	105	1	3	5	1	98
Glossophaga longirostris		-	-		0	0	0	0	3
Goat		-	-		0	0	640	10	1
Mule		-	-		0	40	40	0	1
Rattus r.		-	-		0	0	0	0	1

TABLE II

Serologic results with bat sera, Guajira, 1.964

Animal Species	N.T. Y.F.	HI Tests			
		Yellow fever	Ilheus	VEE	Mayaro
Glossophaga l.	1/98	81/96	85/96	5/96	0/96
Carollia p.	0/2	1/2	1/2	0/2	0/2

NOTE: positives indicated by the numerator; examined by the denominator.

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

Five strains of a Tacaribe group virus have been isolated from material collected in Amapa Territory, north of the mouth of the Amazon. The first strain came from the viscera of a small spiny rat (Neacomys guianae) taken in July 1964. Three additional strains have been isolated from pools of heart, kidney, liver, and spleen of Oryzomys. These rodents were taken during the Christmas holidays and shipped alive to Belem in January 1965, when they were sacrificed for virus studies. Blood inoculated at the same time as the organs was negative for virus. A pool of Gigantolaelaps taken from Oryzomys and including parasites from two of the organ-positive animals, also yielded the agent.

CF studies of 16 bats, 4 Neacomys, 18 Oryzomys, 23 Proechimys, and 26 marsupials of Amapa, yielded only 3 positives: 1 Neacomys and 2 Oryzomys. Of 24 sera of rodents from the Utinga recapture area near Belem, none were CF positive.

This Amapa project is being carried out by Dr. Francisco Pinheiro, with the collaboration of the ICOMI Mining Company. Dr. Robert Shope left Belem in July to join the Yale Arbovirus Unit. Dr. Jorge Boshell and Dr. Jack Woodall have replaced him in Belem.

REPORT FROM DR. OSCAR DE SOUZA LOPES, ARBOVIRUS LABORATORY INSTITUTO ADOLFO LUTZ AND DR. OSWALDO P. FORATTINI, DEPARTMENT OF PARASITOLOGY, SCHOOL OF HYGIENE AND PUBLIC HEALTH, S. PAULO, BRASIL

Our surveillance program, as described previously in the Infoexchange, followed the plans we have made. This report is conserned about the virological aspects of our work.

From Cotia Field Station, in addition to Cotia virus, published in the Am. J. Trop. Med. & Hyg. 14:156, 1965, a new agent was identified. It is an ungrouped virus (SP An 880), which we named as "Embu virus" from a small locality near Cotia. This agent was isolated in 1962 from sentinel mice. It was sent for registration in the Catalogue and will be submitted for publication.

We worked in 1962 during some months in a beach forest, previously described as "Bertioga Field Station". The project was discontinued but, two agents were isolated from sentinel mice. Both turned out identical and this virus, also an ungrouped one, was sent for registration in the Catalogue under the name of "Bertioga virus". The prototype strain has the number SP An 1098.

From Casa Grande two viruses have been identified by now. The first, SPAr 395 was identified as a new strain of Anopheles B virus. It was isolated from a pool of Anopheles (k) cruzii collected on 3-30-1962. This agent did not give an HA, but it grows readily in BHK cells. A serological survey is being done with sera collected in the region and some of the human sera are able to protect the cells. No vertebrate sera were tested up to now but, the work is in progress and the results will be described soon.

The second agent SP AR2317 was also isolated from a pool of A. (k) cruzii, collected on 2-17-1964. Preliminary results

from a serological survey in human sera from Casa Grande showed that about 7% of them have antibodies for this virus.

As we observed that some of the viruses isolated in Casa Grande are able to infect local people, we bled them again in order to have a more precise idea for our epidemiological studies. The population is very stable because they are employees from the Water Department. The first bleeding was made in March 1964 and during last May a second sample was obtained. The sera are being examined and the results will be described. We plan next season to make a house to house inquiry three times a week to observe febrile and other clinical cases in order to attempt isolation of virus from human beings.

During last year a number of immune sera and antigens were prepared in this laboratory. We have now, for identification of arboviruses the following systems: EEE, WEE, VEE, Pixuna, Aura, Mayaro, Una, Mucambo; YF (Asibi), Bussuquara, SLE, Powassan, Ilheus, Rio Bravo; Oriboca, Marituba, Caraparu; Cache Valley, Guaroa, Kairi, Wyeomia, Sororoca; California HR, Melao, Trivitattus; Guama, Catu, Moju; Capim, Guajara, Bush-Bush, Mirim; Icoaracy, Itaporanga, Anhanga; Turlock; Oropouche; Manzanilla; Lukuni, Anopheles A; Irituia; Cocal; Timbo; Chaco; Tacaribe, Junin; Tacaiuma, Candiru, Piry, Acara, Marco, Jurona, CTF, Anopheles B, Tembe, Pacui.

REPORT FROM THE INSTITUTO DE VIROLOGIA DE CORDOBA, ARGENTINA

Virus Isolation Attempts During the Summer 1964 and 1965: Manfredi area, Cordoba Province.

Virus isolation attempts from domestic animals during 1964 yielded two strains; one from the brain of a 6 day old colt which died suddenly, and the other from the blood of a febrile horse. These agents were identified by complement fixation and neutralization tests as members of group A, closely related to, or identical with Una virus, Be AR 13136 strain. Reisolation was obtained from the original specimens after 30 days in the freezer at - 70° C. The horse that yielded the blood strain showed a rise in neutralizing and HI homologous antibodies.

In the same area, during the last summer, virus isolation attempts from 5,453 Aedes (O.) albifasciatus and 28 Culex sp., in 114 pools, yielded 3 strains: AR 420, AR 426, and AR 439. All three from Aedes caught during January. These agents kill infant mice in 2 to 3 days by i.c. and i.p. route and adult mice in 5 to 8 days following i.c. inoculation, but did not kill adult mice by i.p. route. The AR 439 strain was inactivated by DCA and its infectious titer in 2 day old mice by i.c. route was 10^6 LD_{50}. The reisolation attempt was positive with AR 420 strain. Further testing will be done with these agents.

The mosquitoes collected from this area with a Shannon - light trap and human bait were identified as Aedes (O.) albifasciatus, Aedes (O.) scapularis, Culex (C.) maxi, Culex (C.) brethesi, Culex (C.) bidens, Haemagogus spegazzinii, Mansonia (M.) indubitans, Psorophora (J.) cyanescens, Psorophora (J.) discrucians, Psorophora (J.) ferox, Psorophora (J.) varipes and Psorophora (P.) pallescens. Aedes (O.) albifasciatus is caught throughout the summer and is the most abundant species.

No sentinel mice showed signs of infection of the 20 groups exposed during January of 1965; we also noticed that they have no signs of mosquito bites.

(M.S. Sabattini, N.R. Biachini, and L.E. Gonzalez).

REPORT FROM CATEDRA DE MICROBIOLOGIA Y PARASITOLOGIA UNIVERSIDAD DE BUENOS AIRES, ARGENTINA

Guinea Pigs Immunization Against Junin Virus By Vaccination With Inactivated Virus.

Two groups of 20 guinea pigs were vaccinated with two strains of Junin virus antigens (# XJ and # RC) respectively. Antigens were prepared from infected mice brain following Clarke and Casals technique. They were irradiated with fluorescent light in the presence of neutral red diluted 1:50,000 during 1 or 2 hours. The animals received three inoculations by i.m. route of 0.4 ml at intervals of one day. The three injections were repeated after 15 days, and after one month, 15 days after the last injection, the guinea pigs were inoculated with infectious Junin virus, strain XJ.

The animals immunized with XJ antigen received 0.2 ml of $10^{-4.5}$ dilution of virus stock; those immunized with RC antigen received a $10^{-4.0}$ dilution of the same stock (approximately 100 LD_{50}). Eighteen days after the active virus inoculation, two guinea pigs immunized with RC antigen were bled for complement fixation and the inguinal lymph nodes were used to try virus isolation.

Results: The guinea pigs inoculated with XJ antigen, had no significative protection against active Junin virus. This suspension of virus was from guinea pigs plasma stock diluted $10^{-4.5}$ and killed 9 of the 10 control animals inoculated. On the other hand 100% of the animals immunized with the RC strain resisted an active XJ dose of greater than 0.5 log units. They did not show signs of illness according to the weight curve during a month after the challenge inoculation. Isolation of virus from lymph nodes of RC immunized animals was negative, and their sera was positive by complement fixation against XJ until 32/16.

Briefly: 1) An RC antigen prepared following Clarke and Casals technique, and inactivated by irradiation with fluorescent light in the presence of neutral red protects guinea pigs against XJ infection.

2) An XJ antigen prepared in the same way does not protect the guinea pigs.

3) Eighteen days after XJ inoculation, RC immunized guinea pigs had a good titer of complement fixing antibodies. No active virus could be isolated from their lymph nodes. It is therefore possible to immunize guinea pigs against experimental hemorrhagic fever with an inactivated Junin virus strain.

TABLE I

Immunizing	Irradiation time	XJ Challenge	
		$10^{-4.5}$	$10^{-4.0}$
XJ	1 h.	5/10	
	2 h.	4/10	
RC	1 h.		0/10
	2 h.		0/10
XJ Controls	---	9/10	10/10

Antigenic relationships between 7 strains of Junin virus isolated in epidemics of different years.

The cross-relationship between 64 homologous and heterologous antigen-antibody systems, was studied through the analysis of 7 strains of Junin virus, isolated from patients' blood during the period 1958-1964. The Tr 11 573 strain of Tacaribe virus was included in the present comparative study.

Antigens were prepared by the sucrose-acetone method.

Specific antisera were obtained from 8-week old white mice, by injecting 3 doses of infectious virus in saline.

Antigen-antiserum systems were tested by the disposo-trays complement fixation technique.

The results were expressed as follows:

1) Cross-fixation ratio: $\frac{\text{titer of heterologous serum}}{\text{titer of homologous serum}}$

in presence of constant antigen dilutions. (Tables No. 1 and 2).

2) Cross-fixation product: product of cross-fixation ratios of each antigen-antibody pair, that is, serum A versus antigen B and serum B versus antigen A. (Table No. 3).

Strains of virus which exhibited cross-fixation products less than 0.5, have been classified as non-identical types. Every Junin/Tacaribe system showed cross-fixation ratios less than 0.5.

No one antigen-antibody system of the 7 strains of Junin virus exhibited cross-fixation ratios less than 0.5.

Some anomalous results in heterologous systems of Junin virus were found. Those systems showed a titer higher than the homologous one. The unpurified infectious virus used to obtain the antisera, could bring about in the animal a selection of antigenic particles, more related with the heterologous antigen. This would be manifested as a lower specificity in the systems.

The high degree of serologic-crossing suggests the existence of only one type of Junin virus, as far as it could be detected by the technique employed.

However, more sensitive methods of different immunization schedules, could prove the existence of sub-types of Junin virus.

* This research was in part supported by Grant E 4753, from the National Institutes of Health, U.S.A.

** Research Fellow of the Consejo Nacional de Investigaciones Cientificas y Technicas - Republica Argentina.

Table 1 - Serum titer of 8 homologous and heterologous antigen-antibody systems, at a constant antigen dilution (1/32)

Virus antigen	Antisera XJ	RP	RJ	CH	LO	MA	SA	Tr
XJ	128	64	128	32	128	128	128	32
RP	128	128	128	64	128	128	128	64
RJ	128	128	128	32	64	128	128	16
CH	64	128	128	32	128	64	128	64
LO	128	128	256	64	128	128	64	32
MA	256	256	256	64	128	128	128	64
SA	256	256	256	64	256	128	128	16
Tr	64	64	64	16	64	64	32	128

Table 2 - Cross-fixation ratios of 8 specific antisera against 8 antigens.

Virus antigen	Antisera XJ	RP	RJ	CH	LO	MA	SA	Tr
XJ	1	0.5	1	1	1	1	1	0.25
RP	1	1	1	2	1	1	1	0.5
RJ	1	1	1	1	0.5	1	1	0.125
CH	0.5	1	1	1	1	0.5	1	0.5
LO	1	1	2	2	1	1	0.5	0.25
MA	2	2	2	2	1	1	1	0.5
SA	2	2	2	2	2	1	1	0.125
Tr	0.5	0.5	0.5	0.5	0.5	0.5	0.25	1

Table 3 - Cross-fixation products showing the antigenic relationship between virus strains.

Strains	XJ	RP	RJ	CH	LO	MA	SA	Tr
XJ	1							
RP	0.5	1						
RJ	1	1	1					
CH	0.5	2	1	1				
LO	1	1	1	2	1			
MA	2	2	2	1	1	1		
SA	2	2	2	2	1	1	1	
Tr	0.125	0.25	0.063	0.25	0.125	0.25	0.031	1

Serologic study of the Argentine Hemorrhagic Fever, Year 1964 (Complement Fixation).

Complement fixation with paired serum from the epidemic area sent to this laboratory from Provincias de Buenos Aires y Cordoba Antigen-Acetone-Sucrose (Clarke-Casals).

Distribution by month

Month	Positive / total
January	5/11
February	12/16
March	16/17
April	54/73
May	78/106
June	47/74
July	20/30
August	5/5
September	0/1

Distribution of Cases by Locality

Place	Positive total	Place	Positive total
Junin	69/91	Pergamino	3/4
Rojas	36/46	Lujan	1/2
Laplacette	12/18	Los Toldos	1/2
Baigorrita	10/14	Las Flores	0/4
Irala	7/7	Tandil	2/3
Gral. Viamonte	17/24	Pehuajo	0/1
Morse	8/10	La Plata	1/1
Castilla	4/6	Castellano	1/2
Bayanca	3/6	Arenales	0/1
Rawson	7/10	Villa Rosa	1/1
Ameghino	4/7	Falucho	1/1
Agustin Roca	6/7	Warnes	1/1
Chacabuco	4/5	Verdun	1/1
Lincoln	5/5	12 de Octubre	0/1
Bragado	3/6	Trenque Launquen	0/1
O'Higgins	2/4	O'Brien	1/1
R. Obligado	4/5	25 de Mayo	0/1
Gral.Pinto	2/2	Pcia.de Cordoba	19/25
Chivilcoy	3/4	Pcia.de San Luis	0/1
9 de Julio	3/4		

(Armando S. Parodi, Lucia B. de Guerrere, Mercedes Weisenbacher, Edda Adler, and F. Kierszenbaum).

REPORT FROM ARBOVIRUS UNIT, MICROBIOLOGICAL RESEARCH ESTABLISHMENT, PORTON, SALISBURY, WILTS., ENGLAND

This unit, partly financed by a research grant from the Ministry of Overseas Development, is concerned mainly with epidemiological studies of mosquito-transmitted human encephalitis in Sarawak, and of tick-transmitted encephalomyelitis of sheep (louping ill) in Scotland, but also with studies of the pathogenesis of encephalitis and with vaccines against these infections.

The Sarawak study is based on a large amount of material collected during two 2-month visits to the area. So far about 70,000 mosquitoes have been tested for virus and 12 strains isolated: seven of these are Japanese encephalitis (or a closely related agent), the main cause of human encephalitis in Sarawak. These were isolated from Culex gelidus, a mosquito which bites pigs much more frequently than man. Two strains of Tembusu virus were isolated from Culex tritaeniorhynchus and a strain of Bunyamwera virus from Aedes (Cancraedes) curtipes.

In 229 pairs of sera from patients with febrile illnesses, the sera from 2 showed evidence of Bunyamwera infections. About 1,100 human sera from 7 ecologically different types of locality have so far been tested for antibody to 8 viruses. Human infections with Japanese encephalitis virus appear to be commonest in agricultural and forested areas (7-11% per annum) and less in urban and suburban areas (4-5% p.a.). Dengue infections are commonest in or near the coastal swamp (about 12% p.a.). Japanese encephalitis infections are much commoner in pigs than man and as the mosquito involved also bites pigs it is likely that pigs, which are very numerous in Sarawak, are the main source of infection for man. Studies of a further 13,000 mosquitoes and of further sera from man, pigs, fowls and a range of wild species should enable further elucidation of the epidemiology of human disease due to arboviruses in Sarawak.

The study of louping ill is a long-term one which started in 1961 following an epizootic in 1960 which killed about 50% of the lambs on farms in the area. Such epizootics occur at intervals of about 7 years but the reasons are obscure. The studies have been made on 4 farms in Ayrshire and have included sheep, ticks and wild life. Virus isolations have shown that shrews and voles are infected but the influence of population

fluctuations of these small animals on tick populations is perhaps more important. The 1960 epizootic was preceded by a "vole plague." The infection rate in sheep has fallen steadily from 1961 until 1964. The tick population dynamics and the now rapid accumulation of susceptible ewes suggest that a further epizootic may occur in 1966.

REPORT FROM NATIONAL INSTITUTE FOR MEDICAL RESEARCH, MILL HILL, LONDON N.W. 7. ENGLAND

When Tahyna virus was originally tested in 1960 against a battery of arbovirus antisera by the plaque inhibition test, transient zones of protection were observed around beads containing Bunyamwera antiserum. These observations were discounted in view of the recognition by Dr. Casals that Tahyna virus was a member of the California virus group. More recently, the relationship between Tahyna and Bunyamwera viruses has been re-examined using the more precise method of plaque reduction. Serial dilution of antisera were incubated with a constant dose of virus, and the antibody content was expressed as the dilution of serum which brought about a 50% reduction in plaque titre. Both homologous systems gave clear end-points with high levels of antibody. Bunyamwera antiserum was active against Tahyna virus, although more plaques appeared between day 3 and day 4, so that the 50% plaque reduction titre fell from 256 to 50. The results, which show a one-way relationship between the two viruses, are expressed in the following table:

	Antiserum	
Virus	Bunyamwera	Tahyna
Bunyamwera	16,000[1] (16,000)[2]	4 (4)
Tahyna	256 (50)	6,000 (6,000)

1 = 50% plaque reduction titre day 3.

2 = 50% plaque reduction titre day 4.

Attempts to initiate cell cultures of Aedes aegypti mosquito tissues have so far proved unsuccessful, although a variety of methods, media and tissue sources have been tried. The most satisfactory medium in our hands for obtaining survival of tissues has been one based on Tragers' medium for Glossina tissues, but having a modified salt balance to resemble more that of mosquito larval haemolymph. Adult tissues survive in this medium for periods varying from 2 days to over 2 months, depending upon the tissue. Multiplications of Semliki Forest virus of the order of 100 or 1000-fold increase have been demonstrated in surviving adult gut, ovary, malpighian tubes, flight muscle and salivary gland. Liberation of virus from gut has been shown to occur over 23 days. More recently cell suspensions in plasma clots from trypsin-treated whole pupae have been infected with Semliki forest and chikungunya viruses, and increases in virus titre of 10^4 and 10^5 have been observed. The pupal cells in these systems survive (as judged by the spontaneous contractions of single isolated muscle cells) for several days, but no cell division has been observed, nor will trypsinised pupal cells attach to glass or plastic substrates.

(J. S. Porterfield and J. Boorman)

REPORT FROM PROF. STEFAAN R. PATTYN, BACTERIOLOGY DEPARTMENT, PRINS LEOPOLD INSTITUUT VOOR TROPISCHE GENEESKUNDE, ANTWERPEN, BELGIUM

In Infoexchange No. 11, we reported on the frequent occurrence of arboviral haemagglutinin inhibitors in hedgehog sera. These were thought to be non-specific since HI activity was present for several group A and B antigens, and at the same time no neutralizing activity could be demonstrated for the same antigens. The table appearing in Infoexchange No. 11 also shows that those sera which were negative for one antigen were negative for most others.

The same hedgehog sera have since been retested for HI activity after acetone extraction (technique of Clarke and Casals). This treatment removed the HA inhibitors. Extraction with Freon was not satisfactory.

These observations make it clear that kaolin treatment of hedgehog sera is not sufficient for removal of non-specific inhibitors for arboviral HA. We do not know if the occurrence of these inhibitors is related with the physiologic status of hedgehogs at the time of bleeding.

The program on the possibility of installing latent infections with arboviruses was continued using the following combinations of (experimentally inoculated) animals and viruses: hedgehogs inoculated (S.C.) with RSSE virus and babychicks injected with Getah and Uruma viruses. Tissue cultures of the kidneys of these animals after the end of the viremic period were observed for periods up to 2 and 3 months but failed to reveal any reappearance of virus (by subinoculation into susceptible animals or tissue cultures.)

A study was made of the plaque-variants of Middleburg (MB) virus. From a strain of large plaque purified MB virus (MB1) obtained from Dr. Porterfield, a mutant producing small plaques (MBS) was isolated, and the 2 strains compared.

It was found that there were small but constant biological differences between the two strains. Both multiplied in embryonated eggs, MB1 giving slightly higher titers of infectious virus and interferon. Virus titer in chick embryo tissue culture (CETC) was also slightly higher for MB1, IF production was not different. Neither of the strains produced a clear CPE on HeLa and KB cells, plaques were not produced on these cells. Sensitivity for Semliki Forest interferon was the same.

Virusmultiplication in CETC of both was not influenced by IUDR, nor was that of IF.

Ten successive transfers in CETC in high titers in the presence or absence of 10% calfserum showed the strains to be very stable.

In some titrations of MB1 intermediate sized plaques were observed but these proved to be phenotypically induced by unknown environmental factors since subculture of such plaques gave rise to the original MB1.

Adsorption on CETC monolayers was more rapid for MB1 during the first half hour, although maximal for both variants after 40 minutes. Noble agar in petridishes (Porterfield's overlay) gave clearer and greater plaques for both MB1 and MBS as compared with ionagar. Petridishes gave better results than bottles (Melnick's techniques).

Addition of DEAE, protamine or $MgCl_2$ to the agar had no effect on the plaques of MB1. The addition of DEAE and protamine increased plaquesize of MBS to the same size of MB1. Agarose (Industrie Biologiqu francaise) however did not

influence plaquesize of MBS virus. Addition of DEAE to agarose increased somewhat the size of plaques of MBS but was rapidly toxic for the cells when the concentration was increased.

Titers in babymice (I.C. inoculation) were constantly 1 log higher for MB1 than for MBS.

REPORT FROM PROF. D. BLASKOVIC, M.D., DIRECTOR,
INSTITUTE OF VIROLOGY, CZECHOSLOVAK ACADEMY
OF SCIENCES, BRATISLAVA, CZECHOSLOVAKIA

Persistence of TBE virus in tick Ixodes ricinus and the transmission of this virus to the hedgehog (Erinaceus roumanicus).

The transmission of TBE virus by nymphs, females and males of Ixodes ricinus to the hedgehog and the interstadial virophory were confirmed.

Viraemia after tick-bite lasted from 2-8 days. The titer of virus in blood reached $10^{2,8}$ LD_{50} on the 7th day.

In the first experiment the premolting period from larva to nymph lasted 26 days, the prefeeding period 92 days, the virophoric period including infection period of larva on the white mouse lasted 121 days. The experiments with the transmission of TBE virus by infected nymphs to hedgehogs were successful.

In the second experiment the premolting period from larva to nymph lasted 26 days, the prefeeding period 212 days, virophoric period 241 days.

In the third experiment the premolting period lasted during the autumn and winter. (The males hatched on 3/14/1964 and the virus was transmitted, by males 6/26/1964.) The prefeeding period lasted in males 104 days, the virophoric period 285 days. In the next experiment the nymphs have been infected on the white mouse 7/4/1964, the females hatched in 33 days, the virophoric period lasted 149 days.

In the experiments of the transmission of TBE virus by a female of I. ricinus to the hedgehog the prefeeding period lasted 21 days only, the premolting period 178 days and the virophoric period 202 days.

The presistence of TBE virus in the nymphs of Ixodes ricinus reached 241 days, in the males to 285 days and in the females 202 days. The embryonal diapausa (premolting period) from larva to nymph lasted 26 days; in adults in autumn 178 days.

This investigation carried out in the recent years contributed a great deal to the knowledge of the circulation of the TBE virus under natural conditions of Central Europe.

Reference: Kozuch, O., Nosek, J., Lichard, M.: Uberleben des Zeckenencephalitis Virus in der Zecke Ixodes ricinus und die Ubertragung dieses Virus auf den Igel (Erinaceus roumanicus), Zentralblatt fur Bakteriologie, Parasitenkunde, Hygiene und Infektionskrankheiten (in press).

Study of Detection of TBE Virus in Blood of Wild Ducks

The long-term observation of TBE virus in blood of experimentally infected wild ducks was carried out.

After 9 days lasting viraemia the virus was recovered in blood in irregular intervals: in first duck on the 11th week, in the second duck on the 14th and 29th week after infection the virus was detected. The titre of this virus was $10^{1,2}$ to $10^{1.7}$ LD_{50} per 0.03 ml interperitoneal for suckling mice. In two other ducks no virus was recovered.

After reinfection of these ducks on the 62nd week a resorbtive viraemia was observed on the first day. Further, the virus was detected from the blood of one duck on the 7th day.

Virus-neutralizing antibodies varies and on the 52nd week after first infection their levels were 1:32 to 1:128. An increasing of antibodies level was observed on the 7th day after reinfection.

REPORT FROM DR. V. BARDOS, CHIEF, VIROLOGICAL DEPARTMENT,
RESEARCH INSTITUTE OF EPIDEMIOLOGY AND MICROBIOLOGY
BRATISLAVA, CZECHOSLOVAKIA

Tahyna virus.

An extraneural variant of Tahyna virus strain 236 showed a significantly lower cytopathic activity in primary chick embryo cell and hamster kidney cell cultures in comparison with the neuroadapted variant of the same virus strain (Dr. L. Sefcovicova).

After 2-3 successive passages of the extraneural variant in these cell cultures a cytopathic line of virus could be selected.

In the course of intracerebral passages of the extraneural variant in weanling mice marked cytopathic effect on hamster kidney and chick embryo cell cultures was noted from the 6th mouse passage.

The reproduction of the two strains of the Tahyna virus in chick embryo cell cultures by the direct fluorescent antibody technique and by morphological methods was studied (Dr. Z. Wallnerova, in collaboration with Dr. P. Albrecht+). The neuroadapted variants of the strain 92 and 236 were compared with the extraneural variant of the strain 236.

The neuroadapted variants of the Tahyna virus multiplied readily in chick embryo cell cultures with complete cytopathic effect and localization of the fluorescent viral antigen was typically cytoplasmatic. Infected cells showed also typical morphological changes - dispersed nucleoli.

The reproduction of extraneural variant of the strain 236 in chick embryo cell cultures was different: it multiplied without cytopathic effect and titres of the infectious virus were low. Infected cells did not show any morphological changes and no fluorescent antigen could be detected by the direct fluorescent antibody technique.

A study was undertaken to determine Tahyna virus concentration threshold producing viraemia in suckling pigs (Dr. V. Bardos and Dr. E. Cupkova, in collaboration with Dr. J. Jakubik from the Veterinary Research Institute).

+ From the Virological Institute of the Czechoslovak Academy of Sciences, Bratislava.

Ten suckling pigs of a weight 2.2 kg to 3.4 kg were infected with various doses of virus. Viraemia was daily measured by i.c. inoculation in weanling white mice. Tahyna virus concentration threshold producing viraemia from traces to 0.6 log. LD_{50} of 3 to 5 days duration was observed in sucklings infected with $10^{1.3}$ LD_{50} but not with $10^{0.3}$ LD_{50} of Tahyna virus.

The possible participation of the bat species Nyctalus noctula in the various phases of the year-round transmission cycles of Tahyna virus in nature was studied (Dr. A. Simkova). In habitats with the occurrence of Tahyna virus, N. noctula is frequently and regularly attacked by mosquitoes.

The extraneural variant of Tahyna virus strain 236 was used in the 7th passage. Fourteen bats were inoculated subcutaneously in summer months with doses varying from 32 to 320 i.c. mouse LD_{50} of virus. The animals were maintained at 27 + 2° C. Forty bats were inoculated in winter. Immediately after s.c. inoculation with 100 to 100,000 i.c. mouse LD_{50} of Tahyna virus, the bats were placed into an environment at 80% humidity and about 0° C, and groups of 10 or 20 bats were removed from the simulated hibernation 14, 21 and 46 days after inoculation.

Viraemia was assayed in each bat by i.c. inoculation of young mice daily for 14-21 days. The brains, lungs, brown fat and blood from 15 bats were assayed for virus immediately after awaking of the animals. Blood taken from the bats before and at intervals after inoculation was examined for neutralizing antibody in chick embryo cell cultures.

None of the bats showed signs of disease, no virus could be demonstrated in either blood or tissues from these animals at any time during the course of the experiments, and in no instance were virus neutralizing antibodies detected in any of the 54 experimentally inoculated bats.

Nor were Tahyna virus neutralizing antibodies found in the sera from 82 Nyctalus noctula collected in habitats with mass occurrence of mosquitoes.

These results suggest that the hibernating species N. noctula of the mammalian order Chiroptera represents a species resistant against the strain of Tahyna virus used in the present experiments.

The possible clinical manifestation of Tahyna virus infections were studied in a serological study on patients hospitalized in years 1960 to 1964 on the interne ward for adults of a hospital on the Czechoslovak-Austrian frontier (Dr. V. Bardos, Dr. E. Cupkova, Dr. L. Sefcovicova, in collaboration with Dr. F. Sluka, Hospital - Valtice). Paired sera from patients with febrile illnesses and without fever (as control) during the whole year (including also winter months) were tested. CNS infections were occasionally admitted to this ward. Tahyna, Calovo, Semliki forest and dengue 1 antigens were used in the hemagglutination-inhibition (HI) test. Tahyna positive sera were also tested in a virus neutralization test. The results are seen in the table 1. The results demonstrated that 23.4% of 141 exposed and susceptible febrile patients were showed a significant increase (conversion or fourfold increase) of HI antibodies against Tahyna virus.

HAI TESTS WITH VIRUS **ŤAHYŇA** ON PATIENTS (1960-1964) IN ONE HOSPITAL

RESULT OF HAI TEST		PATIENTS			TOTAL
		WITH FEVER		WITHOUT FEVER „CONTROL"	
		WITHOUT COMPLICATIONS	WITH COMPLICATIONS		
NEGATIVE [1]		54	83	114	251
POSITIVE	NO RISE	8	8	5	21
	IN-SIGNIFICANT RISE [2]	17	7	1	25
	SIGNIFICANT RISE [3]	20	13	1	34
TOTAL		99	111	121	331

1) = < 1:20
2) = TWOFOLD
3) = FOURFOLD OR > OR CONVERSION FROM NEGATIVE TO POSITIVE

From the 13 febrile cases with complications there were 7 cases of bronchopneumonia (radiographically confirmed). Acute infections with tularemia, Eaton agent, influenza, adenovirosis, Q fever, ornitosis, parainfluenza 1,2,3 and RS virus so far tested were excluded. In the rest of the patients pleuritis was observed three times, pseudorheumatic pains in the joints twice, and serious meningitis once.

Of 53 bronchopneumonia cases, 13.2% showed a significant increase of HI antibodies against Tahyna virus.

The first cases were diagnosed in May with a peak in July and with last cases in October. Out of 33 patients, only 6 were females.

The HI titer in the first week was equal to or less than 1:20. In the second week mostly 1:20. In the third to the sixth week from 1:20 to 1:80. Virus neutralizing antibodies appeared faster than HI antibodies and were already positive in the first week in the hospitalized patients.

The Calovo virus.

The pig kidney stable cell line (PK cells) turned out to be useful for experimental work with Calovo virus (Dr. L. Sefcovicova). Mouse brain propagated virus caused a complete cytopathic effect in 2-4 days in tube cultures. The titers of virus expressed in CPD_{50} correspond with those in i.c. inoculated mice (LD_{50}).

The formation of plaques under agar overlay in prescription bottles was achieved without difficulty. The clear-cut plaques 1.0-1.5 mm in diameter appear after 2-3 days of incubation.

The susceptibility of PK cells to Calovo virus infection contrasts with the unsuitability of these cells for the cultivation of Tahyna virus. Therefore, this observation could be useful as one of the criteria for differentiation of these viruses in areas of their common occurrence.

References: Wallnerova, Z., Albrecht, P.: Reproduction of the Tahyna virus in chick embryo cell cultures studied by the biological and morphological methods. (To be published.)

Bardos, V., Cupkova, E., Jakubik, J.: Determination of Tahyna virus concentration threshold producing viraemia in suckling pigs. Acta virol., 1965, in press.

Simkova, A.: Tahyna virus in bats. Acta virol., 9, 285, 1965.

Bardos, V., Cupkova, E., Sefcovicova, L., Sluka, F.: A serological study on the medical importance of the Czechoslovak mosquito-borne viruses Tahyna and Calovo. Presented at the XIII. National Congress of Microbiology in Parma-Salsomaggiore, Italy, 3.-6. May, 1965, in press.

Sefcovicova, L.: Use of pig kidney stable cells in experiments on Calovo virus an arbovirus of the Bunyamwera group. Acta virol., 1965, in press.

REPORT FROM HYGIENE-INSTITUT OF THE UNIVERSITY OF VIENNA, AUSTRIA

Mosquito-borne Viruses in Austria. (Horst Aspock)

Previous serological investigations (Ch.Kunz, Zbl.Bakt., I,Orig., 190, 174-182, 1963; Ch.Kunz, Zbl.Bakt., I,Orig. in press, 1965) have shown that the occurrence of Tahyna virus (and probably some further mosquito-borne viruses) in Austria is definitely to be reckoned with.

During the period from May 1 to October 31, 1964, studies on Culicidae were carried out in three localities in the east of Austria (Leithagebirge, about 5 km north of Eisenstadt; Klosterneuburg, about 20 km west of Vienna; Fischamend, about 20 km east of Vienna). Mass collections for virus isolation experiments were performed in these localities from May 15 to August 27, whereby altogether about 80,000 mosquitoes were collected. From these, 54,900 female specimens were pooled (each pool containing 100 individuals) and virologically examined in suckling mice by intracerebral inoculation.

It was neither possible to isolate a virus from 13,800 mosquitoes (mainly comprising Aedes cantans, Ae. annulipes, Ae. excrucians, Ae. communis, Ae. cataphylla) collected in the Leithagebirge in the period from May 17 to June 6, nor from 27,500 mosquitoes (mainly comprising Ae. vexans and Ae. sticticus), collected in Klosterneuburg in the period from June 15 to July 29.

However, from 14,800 mosquitoes (mainly Ae. vexans), collected in the meadows along the Danube near Fischamend, one virus strain could be isolated which proved to be identical with the prototype strain of Tahyna virus (Bardos 92) by the immunofluorescent method as well as in the CF. As regards the pathogenesis of this "Fischamend" strain in suckling mice, however, distinct differences from the prototype strain were found as the virus causes a severe hemorrhage in the mouse brain which leads to a marked purpura cerebri.

The results of these field investigations emphasize our previous assumption that the Tahyna virus does not occur in the meadows along the Danube west of Vienna every year while the areas east of Vienna seem to represent stable foci.

At the moment, extensive field studies on Culicidae and their role as vectors of arboviruses are being carried out in Fischamend. It is, particularly, intended to reveal the cycle of the Tahyna virus which definitely cannot be maintained by the verified vector Ae. vexans exclusively but involves probably further Aedes-species (Ae. cantans, Ae. excrucians, Ae. annulipes) during spring and fall (H. Aspock, Wien.Klin. Wschr., 77, 165-169, 1965).

Hemagglutinating Property of TBE Virus. (W. Frisch-Niggemeyer)

On the strain Vie 415 B of TBE virus, subtype CEE, some investigations were undertaken concerning the stability of the hemagglutinin under different conditions.

The virus was grown in brains of baby mice, purified by protamin-treatment (Clarke and Casals 1958) and was then inactivated by heat (Salminen 1962).

Centrifugation in a sucrose-gradient proved that the hemagglutinating property sedimented homogeneously to the same depth as did infective virus.

By adding different proportions of "borate-saline-albumin-buffer" (BSA) and "virus adjusting diluent" (VAD) (solvents according to Clarke and Casals 1958) it was possible to adjust the pH of the virus solution to a series of selected pH-values. The HA-test was then performed with goose erythrocytes suspended also in a mixture of BSA and VAD but in reversed proportions.

After incubation at 0° C at pH values 9.0, 8.5, 7.9 and 7.4, there was no decrease in the HA-titer; at pH values 6.4 and 6.0 the HA-titer fell to 1/2 to 1/4 in 1 hour, to 1/8 in 2 hours and to 1/16 of its original value in 4 hours. At pH 7 we got intermediate values. This result could be obtained only with protamin-treated preparations.

In a similar way it was possible to test the heat-sensitivity of the TBE virus hemagglutinin at a pH-value of 7.5. Up to 45° C there was no decrease of HA-titer. At 30 minutes at 50° C, the HA-titer was less than 3% of its original value. An exposure time of 15 minutes brought the HA-titer at 50° C to 6% and even at only 5 minutes at 50° C, the titer was depressed to 1/8 of its original value.

The stability of the hemagglutinin in weak alkaline solution made it possible to observe its behaviour under the influence of trypsin. We incubated our preparation with 0.01% trypsin, i.e., 20 units/ml at pH 7.9 and 40° C. The hemagglutinin was degraded by trypsin.

It appears therefore that the hemagglutinating property of TBE virus is dependent on an acid sensitive protein which is situated on the surface of the virus particle.

REPORT FROM DR. P. BRES, CHIEF LABORATORY AND DR. L. CHAMBON, DIRECTOR, THE PASTEUR INSTITUTE, DAKAR, REPUBLIC OF SENEGAL

Clinical Findings.

For two years, we have been trying to demonstrate arboviruses as a cause of human disease. Two methods were used: isolation of virus from blood or brain, and serological conversion with paired sera.

Up to now, 257 isolation attempts, the most part from children under five, have been unsuccessful, even though we had frequently made blind passages.

We had only one serological conversion in an adult European woman 23 years old living in Senegal for one month and vaccinated against yellow fever. After she had spent 15 days in bush villages working on a psychological survey, she felt suddenly ill and was admitted to the hospital in Dakar with symptoms of dengue. We obtained a pattern of "secondary infection" in group B, with antibodies for Ntaya, Wesselsbron, Usutu, Yellow Fever, Dakar Bat, Uganda-S, West-Nile and Zika by IH, CF and NT in 3rd, 17th, 25th, 88th, and 365th days

sera. For example, IH antibodies for NTA reacted on 17th day to 1:1.310.720. (Complete results and graphs will appear in "Bulletin de la Societe Medicale d'Afrique Noire de Langue Francaise, Dakar).

Serological examinations have always been negative in African children under 15 living in the capital of Dakar:

Single blood specimens: 199 negative/209 examined(*)

Paired blood specimens: 10 negative/ 10 examined

(*) 10 positive YF after vaccination.

They are in sharp contrast with results from the bush. During our last survey in January 1965 in Eastern Senegal we found:

Age	No. examined	Positive IH CHIK	ONN	YF	UGS	DAK	WN	ZIK	BUN
0-4	13	4	4	8	6	5	4	4	0
5-9	39	23	23	31	28	25	16	18	0
10-14	35	29	29	32	24	18	13	25	1

Most of group B positive sera had a "secondary infection" pattern.

Cells Cultures.

We have studied some properties of virus from large size plaques (L) and small size plaques (S) that we obtained from chicken embryo cells under agar, according to Porterfield's method, with a sub-strain of SINDBIS Ar 339 virus. We have obtained 5/100 plaques (L) and 95/100 plaques (S) with original material.

We have inoculated 3-day-old suckling mice by i.c. with cloned virus from plaques L and from plaques S and the brains were harvested when the animals paralyzed. In several experiments, brains with virus L produced 100/100 L plaques, and brains with virus S produced 100/100 S plaques. Suckling mice were not an efficient L - S dissociating factor.

Meanwhile, virus from plaques L is more virulent for suckling mice than virus from plaques S.

Type of virus inoculated. (a: in mice b: in plaques	a: DL_{50}/1ml x 10^2 i-c	b: PFU/1ml x 10^2	DL_{50}/PFU
L	194.0	14.0	13
	10.0	0.7	14
	114.0	4.5	25
	3.0	0.1	30
S	4.7	3.4	1.3
	12.5	3.9	3.2
	30.8	33.4	0.9
	0.6	0.5	1.2

In correlating these results, we noticed that the average survival time for suckling mice inoculated with $10^{-0.7}$ of virus L was 2.8 days, and 6.0 days for virus S. (Complete results will be published next time in "Annales de l'Institut Pasteur").

REPORT FROM DR. OTTIS R. CAUSEY, ARBOVIRUS RESEARCH PROGRAM, UNIVERSITY OF IBADAN, NIGERIA

The mouse colony virus IB 1141 apparently was effectively controlled by the immunization program reported in the last Infoexchange. The new immune stock was put into production in isolated quarters at the rate of 100 additional females per day. By the end of February when the last of the old colony was destroyed and the old premises cleaned up, there was available for virus studies a regular supply of immune litters from vaccinated mothers. A determined number was sexed daily and raised to furnish regular replacement of breeding stock. Virus IB 1141 has not been encountered in the new colony during these five months of its existence.

Surveillance for virus infection of vertebrate and arthropod potential hosts has resulted in the isolation of a substantial number of viral agents. Isolates identified as Chikungunya and Bwamba (or viruses closely related to them) have been

obtained from human sources. Chikungunya and a group B agent (probably West Nile) were isolated from two of 250 sentinel mouse groups exposed in a forest near Ibadan. Examination of specimens from sentinel monkeys, sentinel chickens, wild rodents, bats, mosquitoes, phlebotomus, fleas and mites has so far yielded no isolates.

On the other hand, more than 270 agents were obtained from about 2000 pools of ticks picked from cattle and other animals coming to the Ibadan markets. Another 54 agents were isolated from the blood of 539 market animals. The relatively small portion of these which has been studied serologically fall into three groups. The one most commonly encountered is represented by type IB AR 1792 and also includes the biologically distinct type IB AR 2484 for which an HA can be prepared at pH 6.6. To date, 53 strains from ticks of the species Amblyoma variegatum, Boophilus decoloratus and Hyaloma truncatum and from cattle blood have been classified with these by CF and HI testing.

Another group also includes two biological types represented by AR 2709 and AR 3108. An HA is readily prepared from these agents at pH 6.0 and seven other tick isolates have so far been identified with them. All, except one pool from Hyaloma truncatum, were from Boophilus decoloratus.

A third group is serologically related to, if not identical with, Thogoto of East Africa. The HA (pH 6.0) and CF antigen for these is present in liver and only rarely found in brain tissue. Failure to use liver in early tests greatly delayed recognition of the relationship to Thogoto virus. The isolates IB AR 2012 and IB AR 2671 belong to this group, which now includes 14 agents from Boophilus decoloratus and one from a pool of Amblyoma variegatum.

Virus IB AN 5077 was isolated on two occasions from the blood of cattle at the slaughterhouse. An HA can be demonstrated by sucrose-acetone extraction of infected mouse brain and treatment with protamine sulphate. Maximum titer is at pH 6.0. This agent is unrelated to other isolates in Ibadan and is not yet identified.

REPORT OF THE PASTEUR INSTITUTE OF BANGUI

I. Isolations of the Virus.

Since our last report to the Infoexchange, we have inoculated the salivary glands, brains, and in a certain number of cases the sera of 439 bats grouped in 24 pools.

These bats came from Bambari for the most part, and include mainly Tadarida (identification by F. Petter, subdirector at the Museum of Natural History at Paris-Laboratory of Mammalogy).

Three new strains of the salivary virus provisionally named Bangui M 23, M 20 and M 28, have been isolated. Two among them have given an hemagglutinating antigen reacting with an arbovirus Poly-B immuneserum of reference, furnished by the Pasteur Institute of Dakar.

Inhibition of hemagglutination and crossed seroneutralization are in course in order to compare these strains to that isolated previously in the forest zone (Bangui M 7).

They have been sent to the WHO Regional Reference Center for Arthropod-borne Viruses (Pasteur Institute of Dakar).

II. Immunological Study of Yellow Fever.

The first results, presented in the previous reports, have been completed during this year. These results are tabulated by age and by region. The vaccination campaigns were conducted regularly throughout the country until 1960. Only the pygmies show evidence of low incidence of vaccination.

The results of the HI (hemagglutination inhibition) test agree with the 96 seroneutralization (NT) reactions in mice. Only one serum negative to the HI test is positive to the NT.

In the whole Central African population, theincrease of the number of "protected" subjects is proportional to age. There is noted beginning at 10 years in all the zones (except among the pygmies), an abrupt increase of the number of carriers of yellow fever antibodies, which reflect effectiveness of the vaccination campaigns. Among the children from 1 to 9 years who present HI anti-yellow fever antibodies, some have not been vaccinated and do not present any other antibodies of Broup B, which suggests without being able to be affirmative,

the possible existence of virus in circulation (notably the zones of Yaloko, Obo and Lobaye). In the adult population the percentage of carriers of anti-yellow fever antibodies varies, according to zones, from 50% (Bambari) to 73% (Bangui) if the pygmies (15.3%) are not counted. Heterologous reactions with Zika West Nile and, more rarely Uganda S, antigens are frequent. The table attached summarizes these different elements and groups all of the results obtained since 1962.

An immunological and epidemiological inquiry has been made at the start of the year 1965 in common with researchers Pasteur Institute of Cameroons, and the Center of the O.R.S.T.O.M. of Yaounde in the border regions Cameroons-Central Africa (Garoula Boulai and Boular). The sera collected and the mosquitoes captured are handled in the two Institutes of Yaounde and Bangui.

(A. Chippaux and C. Chippaux-Hyppolite)

SERA POSITIVE IN HI WITH YELLOW FEVER ANTIGEN

Groupes d'âge	PYGMEE-PABINGA de LOBAYE (forêt équatoriale)		OUBELLA-M'POKO et LOBAYE (forêt et savane en bordure)		BANGUI et "BANLIEUE"		YALOKE (savane) à mi-chemin entre Bangui et Bouar		BOUAR (savane) à 400 km. au N.-O. de Bangui	
	Nombre de serums examinés	Serums positifs	Nombre de serums examinés	Serums positifs	Nombre de serums examinés	Serums positifs	Nombre de serums examinés	Serums positifs	Nombre de serums examinés	Serums positifs
1 à 4 ans	18	0 %	23	0%	23	0 %	11	9 %	0	-
5 à 9 ans	61	2 %	77	14 %	158	16 %	85	16 %	24	8 %
10 à 14 ans	54	0 %	94	48 %	134	43 %	66	45 %	38	42 %
15 à 19 ans	60	5 %	63	49 %	29	62 %	98	60 %	15	40 %
20 à 39 ans	277	15 %	109	63 %	127	76 %	57	65 %	19	63 %
40 ans et +	61	28 %	53	62 %	0	-	0	-	44	80 %
TOTAL	531	11,7 %	419	45 %	471	41,6 %	317	44,5 %	140	50,7 %

Groupes d'âge	BALBARI (savane) à 350 km. au N.-E. de Bangui		BANGASSOU, sur l'Oubangui, à 500 km. à l'Est de Bang.		OBO (savane) à 900 km. à l'Est de Bangui		TOTAL	
	Nombre de serums examinés	Serums positifs	Nombre de serums examinés	Serums positifs	Nombre de serums examinés	Serums positifs	Nombre de serums examinés	Serums positifs
1 à 4 ans	0	-	3	0 %	6	17 %	84	2,4 %
5 à 9 ans	1	0 %	0	-	17	18 %	423	13,2 %
10 à 14 ans	18	17 %	0	-	34	35 %	438	37,2 %
15 à 19 ans	67	46 %	0	-	48	38 %	380	43,7 %
20 à 39 ans	9	77 %	81	59 %	18	39 %	698	45,5 %
40 ans et +	0	-	0	-	0	-	158	53,8 %
TOTAL	95	43,1 %	84	57 %	123	33 %	2.180	36,2 %

N.B.: Positive sera are those which give an inhibition complete at 1:20, which represents the first dilution examined.

A quantitative dose has been made for all these sera.

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

Chikungunya.

As mentioned in a previous contribution (Infoexchange Number 10), a population of vervet monkeys in a riverine gallery forest in Northern Zululand is being sampled for the incidence of chik antibodies. Since December 1963, when a start was made trapping monkeys there, 48 blood samples have been obtained from 36 monkeys; there being 12 bloods from recaptured animals. Excluding the results on "repeat" bleedings from recaptured animals, among which there were no conversions, 20 monkeys were positive for chik HI antibody. The results of HI and NT tests on these 20 sera with the five group A viruses known or suspected to occur in the area are shown in the Table. From the results it is reasonably certain that the antibody is in fact the result of infection by chik virus. Six of the positive monkeys were estimated to be one to three years old. One was only nine months. This virus has therefore apparently been active in the locality very recently.

Observations are also being conducted on the species of mosquito feeding on monkeys in this locality in the forest canopy at night. The results showed that Culex univittatus, Mansonia africana, Aedes scataphagoides and Aedes furcifer/taylori, in descending order of prevalence, were the mosquitoes significantly involved. Recent work by us has shown that C. univittatus is unable to transmit chik virus. M. africana is only a moderately efficient vector. The two Aedes species mentioned are rather seasonal in prevalence. Therefore the primary vector involved in these primate infections is still far from clear.

However, as these vervet monkeys spend a great deal of time during the day on the groun, food-hunting, the possibility exists that they are becoming infected at this time. This would suggest Aedes circumluteolus, the common ground-haunting mosquito in the area, as a possible vector.

TABLE ANTIBODY RESULTS WITH SEVERAL GROUP A VIRUSES ON MONKEY SERA FROM NDUMU

Virus	HI test		N test	
	No. sera positive	Mean titre	No. sera positive	No. sera inconclusive
Chikungunya	20	306	15	5*
Semliki Forest	9	88	0	7
Sindbis	5	48	2	0
Ndumu	1	20	0	0
Middleburg	7	34	3	0

* These sera were diluted 1/5 which may explain the inconclusive result.

REPORT FROM DR. SRIDHAR UPADHYAYA, DIRECTOR, VIRUS DIAGNOSTIC LABORATORY, SHIMOGA, MYSORE, INDIA

The following is the information with regard to isolation of Kyasanur Forest Disease virus from human and dead monkeys for the year 1965:

Among 30 monkeys autopsied, 14 yielded Kyasanur Forest Disease virus.

From January 1965 up to end of September 1965, Kyasanur Forest Disease virus has been isolated from 14 human cases.

REPORT FROM THE VIRUS RESEARCH CENTRE, POONA, INDIA

Outbreak of a Dengue-like Illness in Nagpur City, India.

The Virus Research Centre (VRC) Poona, undertook investigations on an outbreak of a dengue-like illness in Nagpur city, India

(Pop. 690,302). The outbreak occurred mainly from April to June 1965 and the investigations were made in June and July.

The proportion of the population affected is not known but rough estimates placed the incidence between quarter to half of the population of certain sections of the city. The symptoms of the illness in a majority of the cases were those usually associated with chikungunya virus. Haemorrhagic manifestations were not common. In a few cases bleeding from the gums and epistaxis occurred. Very rarely cases of hematemesis, blood in the stools and vaginal bleeding were reported.

Sixty acute sera were inoculated into infant mice and 26 agents were isolated. Fifty-eight of these same sera were also inoculated in tissue culture (BS-C-1 cell line) and 24 agents isolated. Two of the mouse isolates were from sera not tested in tissue culture. Twenty of the isolates in mice have been identified as chikungunya virus by complement fixation tests. The tissue culture isolates thus far identified were seven chikungunya, one agent that behaves like dengue in tissue culture and two probably a new agent (VRC No.653514 and 653703). Thirty-two of 48 pairs of sera (acute and convalescent) showed conversion in CF antibodies to chikungunya virus. These included 20 pairs from whose acute sera chikungunya virus had been isolated. One pair showed clear conversion to a Group B arbovirus and another to the new agent (VRC No. 653514).

Seventy-eight single convalescent sera were tested in complement fixation using the antigens of JE, dengue 1, dengue 2, KFD, Sindbis, chikungunya and the new agent. Fifty-eight had significant CF antibody titres to one or the other Group B arboviruses and 69 to chikungunya virus. Ten of the sera had CF antibodies to the new agent.

Of the 32 sera from contacts tested, 12 had significant CF antibodies for chikungunya, 22 for a Group B arbovirus, and eight for the new agent.

From Aedes aegypti mosquitoes collected by the Maharashtra State Public Health Department (552 adults in 34 pools) 14 isolations have been made of which five have been identified as chikungunya and five as dengue 4 by CF tests; Seven out of nine non-chikungunya isolates behave like dengue viruses in tissue culture.

These investigations have shown that in Nagpur city there was an outbreak of febrile illness during which three viruses seem to have been active at the same time, chikungunya (accounting for most of the cases), dengue and a new agent still to be identified and if necessary named.

This outbreak is interesting coming as it does in the wake of an outbreak of chikungunya in Madras State in July to November 1964, of dengue in Visakhapatnam, Andhra Pradesh, in July to November 1964 and of dengue and chikungunya in Calcutta in July to November 1963.

The new agents (VRC No. 653514 and 653703) referred to above were isolated from the acute sera of two patients.

Neutralizing antibodies were found to have developed in their convalescent sera, though not CF antibodies. A third patient from whom virus was not isolated showed conversion in the convalescent serum. Sera of several persons in Nagpur city have shown antibodies to this virus. Preliminary studies on human sera from other parts of India in the collection of the VRC indicate that the occurrence of antibodies to this virus seems to be widespread.

In infant mice this agent produces sickness similar to that produced by chikungunya virus. In tissue culture (BS-C-1 cells) the CPE in the primary isolations occurred between 36-48 hours but in subsequent passages with more concentrated virus suspensions the CPE is produced within less than four hours as compared to other viruses handled at the VRC which take a much longer time (over 36 hours). The characteristics of the CPE are also different. Preliminary tests in tissue culture indicate that the virus is not neutralized by anti-sera of JE, WN, dengue 1, dengue 2, dengue 4, KFD, Ntaya, Sindbis, chikungunya, G 1424 (Umbre), G 3159, G 2464, G 9601 (Umbre), G 14132 (Kaisodi), G 20207 (Chittoor), 616313, 62220 (Rabies), P 20323 (Herpes simplex) and 633970. The new agent is being compared also with other previously isolated but unidentified viruses available at the VRC.

REPORT FROM DEPARTMENT OF BACTERIOLOGY, FACULTY OF MEDICINE, UNIVERSITY OF SINGAPORE

Isolation of Dengue Virus Type 3.

Twelve strains of dengue virus were isolated in 1963 from acute sera of patients who have been diagnosed clinically as having haemorrhagic fever. Two of the viruses have now been identified as strains of dengue virus type 3 by HI, CF and microprecipitin agar gel diffusion tests. High HI and CF antibody titres to dengue virus were demonstrated in the convalescent serum of one patient, and a significant antibody rise was found in the paired sera of the other patient.

With the isolation of dengue virus type 3 strains in 1963 all the four types of dengue virus have been shown to occur in Singapore. The number of identified dengue virus strains in the 5-year period since the first outbreak of haemorrhagic fever in 1960 is shown in the accompanying table.

DENGUE VIRUSES ISOLATED IN SINGAPORE, 1960–1964

Virus	Number of strains isolated in					Total
	1960	1961	1962	1963	1964	
Dengue type 1	1	0	2	2	12	17
Dengue type 2	1	2	9	5	4	21
Dengue type 3	0	0	0	2	0	2
Dengue type 4	0	3	0	0	0	3
Not yet typed	0	0	0	3	10	13
Total	2	5	11	12	26	56

REPORT FROM DR. DORA TAN, INSTITUTE FOR MEDICAL RESEARCH
KUALA LUMPUR, FEDERATION OF MALAYSIA

Haemorrhagic Fever in Malaya.

The investigation of haemorrhagic fever in Malaya was initiated by Dr. Albert Rudnick of the University of California, Hooper Foundation in 1962. It was continued after he left for the U. S. in June 1964.

From November 1962 to May 1965, a total of 80 cases were found positive. Of these 75 were from Georgetown, Penang, a northwestern island city in the Straits of Malacca, and 5 from Kuala Lumpur. Although the Chief Physicians in the main hospitals throughout Malaya were alerted, most of the specimens received were from Penang and Kuala Lumpur. A few blood samples were received from other towns but all were found negative.

Analysis of the 75 cases in Penang showed that a vast majority of them were Chinese, the incidence rate per 1,000 population being 0.156 compared with 0.030 to 0.086 in the other racial groups (table 1). In Kuala Lumpur the 5 cases were comprised of 3 Chinese, 1 Malay and 1 Indian. No cases were observed among foreign visitors.

TABLE 1

RACIAL DISTRIBUTION OF 75 LABORATORY CONFIRMED CASES

OF HAEMORRHAGIC FEVER IN PENANG FROM NOVEMBER 1962 to MAY 1965.

Racial Group	Total No. of Cases	Estimated population for 1963	Incidence Rate per 1,000 population
Chinese	61	389,929	0.156
Indian	7	81,074	0.086
Malay	6	197,214	0.030
Others	1	12,648	0.079

A study of the age and sex distribution revealed that the highest percentages of total positive were seen in the age group between 6 and 8 years (27.5%) and in females (60.0%). It is believed that the preponderance of females over males may be because young girls tend to spend more time than boys during the day in or about the home, where exposure to the diurnal-biting vector, Aedes aegypti is greatest. The fatality rate was 6.1% (5/80), all the deaths occurring prior to 1964 among children age 6 to 8 years in Penang.

TABLE 2

AGE AND SEX DISTRIBUTION OF 80 LABORATORY CONFIRMED CASES OF HAEMORRHAGIC FEVER FROM NOVEMBER 1962 TO MAY 1965.

Groups	Total no. Cases	% of total positive
- 2 years	0	0
- 4 "	8	10.0
- 6 "	19	23.8
- 8 "	22	27.5
-10 "	17	21.2
-40 "	14	17.5
Totals	80	100.0
Male	32	40.0
Female	48	60.0
Totals	80	100.0

Table 3 presents the clinical features in descending order of frequency. Among the prominent features observed were fever, vomiting, nausea, haemorrhagic signs, lethargy and abdominal pain. In severe cases there might be circulatory collapse and shock which usually preceded death. Enlargement of the liver, spleen and lymph nodes might be present. Haemorrhagic signs (table 4), almost always accompanied by a positive tourniquet (Hess's) test, usually consisted of petechiae, melaena and ecchymoses. Less often haematemesis, bleeding gums, epistaxis and purpura might occur. A marked thrombocytopaenia and appearance of Turk cells were typical clinical laboratory findings.

TABLE 3

CLINICAL FEATURES OF 80 LABORATORY CONFIRMED CASES OF HAEMORRHAGIC FEVER IN MALAYA NOVEMBER 1962 TO MAY 1965.

(IN DESCENDING ORDER OF FREQUENCY).

Feature	No. positive	% of total positive
Fever	80	100·0
Vomiting & Nausea	54	67.5
Haemorrhagic signs	54	67.5
Lethargy	38	47.5
Abdominal pain	32	40.0
Shock-like state	27	33.7
Hepatomegaly	27	33.7
Lymphadenopathy	21	26.2
Headache	21	26.2
Restlessness	14	17.5
Diarrhoea	9	11.2
Cough	6	7.5
Muscle pain	5	6.2
Stiff neck	5	6.2
Conjunctival injection	5	6.2

TABLE 4

HAEMORRHAGIC SIGNS IN 80 CASES OF HAEMORRHAGIC FEVER IN MALAYA, NOVEMBER 1962 - MAY 1965.

Haemorrhagic Sings	No. positive	% of total positive
Petechiae	42	52.5
Melaena	20	25.0
Ecchymoses	18	22.5
Haematemesis	6	7.5
Bleeding gums	6	7.5
Epistaxis	4	5.0
Purpura	2	2.5

REPORT FROM DEPARTMENT OF MICROBIOLOGY,
JOHN CURTIN SCHOOL OF MEDICAL RESEARCH,
AUSTRALIAN NATIONAL UNIVERSITY, CANBERRA, AUSTRALIA

Dual Infection of Aedes Aegypti with Arboviruses

West Nile, Semliki Forest, and Sindbis viruses have been used in various combinations to study the outcome of dual infections in the mosquito, Aedes aegypti. The growth cycles of both the infecting and the challenge viruses appear to proceed normally or with only slight modification in the presence of the other, and this relationship is not influenced by varying the dose, the routes of infection, and the time intervals between infection and challenge.

When dually infected mosquitoes are individually fed on suckling mice, it is found that when transmission occurs both viruses are usually transmitted concurrently. The only exception to this is in mosquitoes with salivary glands severely damaged by Semliki Forest virus, in which case neither virus is transmitted.

A summary of the results obtained with 2 group A arboviruses is presented in Table I, the infecting Sindbis virus followed by Semliki Forest. Table II shows the same two viruses in the reciprocal combination. Growth and transmission occur concurrently in dually infected mosquitoes except in the case where SFV is the infecting virus, in which case transmission is not achieved 25 and 32 days after infection in both singly and dually infected mosquitoes.

Table III and IV show the results obtained with a group A (SFV) and a group B (WNV) virus. The findings are essentially the same as that obtained with the two group A viruses.

Except for the aberrant behaviour of SFV in Aedes aegypti, the results are in accordance with those of Chamberlain and Sudia (J. Infect. Dis. 101, 233 (1957)), who found no apparent interference to the multiplication and transmission of WEE and EEE in dually infected C. tarsalis, but are at variance both with the report by Altman (Amer. J. Trop. Med. Hyg. 12, 425 (1963)) that MVE and JE interfere with each other in C. tritaeniorhynchus, and with Sabin's observations on Y.F. and dengue viruses in Ae. aegypti (Amer. J. Trop. Med. Hyg. 1, 30 (1952)).

If we can generalize from these results, it seems likely that at times of intense arbovirus activity, such as the current Asian epidemics of Chikungunya and dengue, individual mosquitoes will sometimes become infected with two viruses during their life span, and will subsequently transmit these two viruses simultaneously. The finding also seems to refute the idea that an arbovirus can be excluded from an endemic area through viral interference in mosquitoes. The ability of arboviruses to grow in close association with one another in their vector might lead to possible genetic exchange, resulting in recombinants invested with new properties.

The finding of interference in the transmission but not the growth of the challenge virus following prolonged incubation of SFV in Ae. aegypti supports the observation by Mims and Day (Infoexchange No. 8, 122 (1963)) that extensive histological changes are evident in the salivary glands of Ae. aegypti 21 days or more after an infective feed of SFV.

(K. Lam)

TABLE I

Aedes aegypti are infected by feeding on Sindbis viremic mice circulating 9 log PFU/ml of blood. At suitable intervals the mosquitoes are dually infected by feeding on SF virus infected mice with a viremic titre of approximately 10 log PFU/ml. 6 days after dual infection, singly and dually infected mosquitoes are tested for virus multiplication and transmission.

Sindbis infected only		Dually infected*		SF infected only	
		Sindbis	SF		
Day 12	5.09ø	4.58	4.20	Day 6	4.84
Transmission	+	+	+		+
Day 15	5.30	3.89	4.33	Day 6	5.75
Transmission	+	+	+		+
Day 28	3.45	3.92	5.50	Day 6	6.32
Transmission	+	+	+		+

* Incubation times of each virus in dually infected mosquitoes correspond to times given for appropriate singly infected controls.

ø Median log P.F.U. per mosquito, assayed on chick embryo fibroblast monolayers.

TABLE II

Aedes aegypti are infected by feeding on SF viremic mice and at suitable intervals dually infected by feeding on Sindbis viremic mice. After an extrinsic incubation of the challenge virus, viral multiplication and transmission are determined in singly and dually infected mosquitoes.

SF infected only		Dually infected*		Sindbis infected only	
		SF	Sindbis		
Day 13	4.81ø	4.87	5.40	Day 9	5.65
transmission	+	+	+		+
Day 17	4.96	4.78	5.72	Day 10	5.78
transmission	+	+	+		+
Day 25	4.33	3.71	4.92	Day 11	5.39
transmission	-	-	-		+
Day 32	4.88	4.10	5.25	Day 11	5.75
transmission	-	-	-		+

* Incubation times for each virus in dually infected mosquitoes correspond to times given for appropriate singly infected controls.

ø Median log P.F.U. per mosquito, assayed on chick embryo fibroblast monolayers.

TABLE III

Aedes aegypti infected by inoculating each mosquito with approximately 150 PFU WN virus. At various time intervals the mosquitoes are dually infected by inoculating each with 100 PFU of SFV. The mosquitoes are tested for virus multiplication and transmission.

WN infected only		Dually infected* WN	Dually infected* SF	SF infected only	
Day 7	4.69 ø	4.50	5.45	Day 4	5.77
transmission	+	+	+		+
Day 10	4.65	3.33	3.76	Day 7	4.55
transmission	+	+	+		+
Day 23	4.21	3.31	4.00 (feeding)	Day 9	5.08 (feeding)
transmission	+	+	+		+

* Incubation times of each virus in dually infected mosquitoes correspond to times given for appropriate singly infected controls.

ø Median log P.F.U. per mosquito, assayed on chick embryo fibroblast monolayers.

TABLE IV

Aedes aegypti are infected by feeding on SF viremic mice and subsequently challenged with a low dose of WNV by injection (approximately 150 PFU/mosquito). 6 days after challenge, dually and singly infected mosquitoes are tested for multiplication and transmission.

SF infected only		Dually infected* SF	Dually infected* WN	WN infected only	
Day 11	4.58ø	4.69	4.77	Day 6	4.70
transmission	+	+	+		+
Day 20	4.06	4.48	4.22	Day 6	5.35
transmission	-	-	-		+
Day 27	3.04	3.61	4.20	Day 6	4.42
transmission	-	-	-		+

* Incubation times of each virus in dually infected mosquitoes correspond to times given for appropriate singly infected controls.

ø Median log P.F.U. per mosquito, assayed on chick embryo fibroblast monolayers.

REPORT FROM QUEENSLAND INSTITUTE OF MEDICAL RESEARCH, BRISBANE, AUSTRALIA

Studies of Isolated Viruses.

The MRM 1178 strain, previously considered to be distinct from arboviruses previously isolated in Australia (Infoexchange No. 10) was adapted by serial passage to kill suckling mice inoculated intraperitoneally. Neutralization tests to arbovirus antiserum were repeated by IP inoculation but no relationships demonstrated.

The In 1074 strain, isolated from a mixed species pool of mosquitoes collected in October 1963 near Innisfail, North Queensland, was studied. No haemagglutinin could be obtained but neutralization and CF tests suggest that it is also distinct from other viruses found in Australia.

Studies are in progress to determine whether MRM 1178 and In 1074 will multiply in inoculated C. fatigans. Both strains have been sent to the regional and world reference centres for further study.

Surveys of Arbovirus Antibody.

Tests of sera from man and other animals for group A antibody (Infoexchange No. 10) have continued, with further demonstration that antibody in birds reacts to Sindbis virus and in mammals to Ross River virus, but antibody apparently to Sindbis has also been found in sera from man and dogs. The M 78 strain, isolated by Professor J.A.R. Miles in New Zealand, was obtained; HI, CF and neutralization tests confirmed his finding that it is related to but distinct from the Australian MRM 39 strain of Sindbis. A group of field sera of known reactivity were tested to M 78 by neutralization test; more reacted to MRM 39 than to M 78.

Review of 1954-1955 Dengue Epidemic.

Previous studies suggested that the most recent epidemic of dengue in Queensland was due to dengue type 1, but types 3 and 4 were not then available. Paired sera from that epidemic have now been retested to dengue types 1-4 by HI and plaque inhibition tests on PS cells. The results indicate that the epidemic was due to type 3, or to a virus closer to it than to types 1, 2 and 4.

Field Programmes.

In two expeditions in October-November 1964 and in March-May 1965, 34,251 mosquitoes and blood and organs from 160 wild birds were collected at Mitchell River Mission in North Queensland. No virus was isolated from the 1964 material; the 1965 collection is still being studied.

Sera were collected in May, 1964 from domestic fowls in south-west Queensland; 114 of 438 had HI antibody to MVE; neutralization tests suggest that most if not all antibody was due to infection with Kunjin; 162 of 436 had HI antibody to Sindbis. Sentinel fowls were stationed at 12 sites in that area in October 1964, and bled in May 1965, but sera from them have not yet been tested. This evidence of arbovirus activity in an area of low rainfall is of interest and further studies are planned.

Experimental Mosquito Inoculation.

Corriparta virus, previously considered of interest because of its relative lack of sensitivity to SDC and ether, was maintained through five passages in mosquitoes inoculated with infected mosquito salivary gland. This is taken to confirm that it is indeed an arbovirus.

Clinical Virology.

Rising titres of antibody to Ross River virus were found in six patients with clinical diagnoses of epidemic polyarthritis - one from Cairns (ill in January 1964), one from Charleville (May 1964) and four from the Murray Valley of Victoria or New South Wales (early 1965).

Other Studies.

During the year other investigations were concerned with tissue culture techniques for group B arboviruses, with laboratory studies of mosquito behaviour, with biochemical properties of several ungrouped viruses, and with chemical studies of haemagglutination. Details of this work may be found in the Annual Report of the Institute.

(R. L. Doherty, J. G. Carley, H. A. Standfast, E. G. Westaway, B. M. Gorman, R. H. Whitehead)

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

Field studies have continued in South Westland in the South Island of New Zealand. Further isolations of the Sindbis-like agent, M 78 have not been made since 1963, although similar numbers of mosquitoes have been processed in each year. The serology of wild birds trapped bled and released has shown that virus has been present in each year, but the proportion of seropositive birds has been substantially lower in 1965 than in the earlier years (Table 1). Further in 1963, 73% of bird sera gave a titre of over 1:200 in a plaque reduction neutralization test, while in 1964 and 1965 the percentages giving such high titres were 26 and 37%.

In each year direct evidence of infection was obtained either from rising titres in sera of birds caught on more than one occasion, or from a sentinel fowl or from both. It appears that virus activity has been present each summer but that, at least in 1964 and 1965, the level was lower than 1963 and probably lower than 1962.

The largest proportion of positive sera has come from cleared or partly cleared country in pastoral use, while the proportion of positives in native bush has been very low. The bush catch contains a high proportion of strictly territorial birds which are not widely ranging at any time of year. In 1963 the few shore and water birds bled showed a very high positive rate, but this was not confirmed in later series. The probable reason for the difference is that the 1963 birds were shot whereas the later specimens, except for some of the duck bloods, came from birds trapped or captured when unable to fly either before being fully fledged or when they were in moult.

Investigations on the introduced Australian brush-tailed possum (Trichosurus vulpecula) which is the most numerous mammal in the area, have shown that specimens often have antibodies to M 78, but laboratory investigations have failed to demonstrate viraemia at any stage in the infection, and it seems unlikely that this species is ecologically important to the virus. Rattus rattus is sometimes locally abundant, but the relatively rare occasions on which this mammal is abundant and the low susceptibility found in rats to laboratory infections, does not suggest that they are ecologically significant.

Virus isolations have been made in 1964 from birds and mosquitoes and in 1965, in studies not yet complete, from birds only. Ten isolations were obtained from mosquitoes at four different sites in January and February, 1964. The two main species in the area, Culiseta tonnoiri and Culex pervigilans, both carried virus. All strains were re-isolated from the original material. Eight virus isolations have been made from bird bloods during 1964 and 1965 as shown in table 2. Reisolation of some of the 1965 strains has not yet been attempted.

All eighteen strains behave similarly in mice and are probably the same. Only a few have been studied in detail so far. They are ether and DCA resistant. By filtration they are 80-100 mu in diameter. The one mosquito strain studied under the electron microscope has spherical or polyhedral particles 70-75 mu in diameter. Very large cytoplasmic inclusions are found in nerve cells in mouse brain, and similar neutrophilic cytoplasmic inclusions containing large amounts of virus, sometimes in crystalline array, are found in culture of BHK21 and P.S. cells. CPE is produced in primary monkey kidney and human amnion cells. Neither CPE nor inclusions have been found in duck embryo cell cultures. The three 1964 and two 1965 strains so far studied are reovirus type 3.

We are not aware of any previous reports of isolations of reovirus from birds or from blood and consider the large number of isolations from mosquitoes of considerable interest.

Our laboratory in Suva, Fiji is now in operation, but has not yet any results to report.

TABLE 1. NEUTRALISING ANTIBODIES AGAINST M78 VIRUS IN BIRD BLOODS.

Year	Habitat Type	- Species -																	
		Black bird (Turdus merula)	Thrush (Turdus ericetorum)	Chaffinch (Fringilla coelebs)	Red Poll (Carduelis flammea)	House Sparrow (Passer domesticus)	Hedge sparrow (Prunella modularis)	Starling (Sturnus vulgaris)	White eye (Zosterops lateralis)	Tui (Prosthemadera novaezeelandiae)	Bell bird (Anthornis melanura)	Pukeko (Porphyrio porphyrio)	Ducks Anas spp.	Black swan (Cygnus atratus)	White fronted tern (Sterna striata)	Black billed gull (Larus bulleri)	Red billed gull (Larus novaehollandiae)	Godwit (Limosa lapponica)	Domestic Fowl (Gallus gallus)	Other species
1963	Open Country	35/148 24%	21/43 49%	2/9 22%	2/5	10/51 21%	0/3	0/1	8/74 11%	1/3	2/10 20%	-	-	-	-	-	-	-	1/46 2%	0/16
	Lagoon	-	-	-	-	-	-	-	-	-	-	1/1	1/1	1/2	-	-	-	2/2	-	-
1964	Open Country	10/33 30%	9/33 27%	3/16 19%	-	-	-	0/2	1/15 7%	1/8	4/14 29%	-	-	-	-	-	-	-	6/18	0/26
	Bush	4/26 15%	0/1	0/6	-	-	0/3	1/1	0/135	1/5	0/20	-	-	-	-	-	-	-	-	0/67
	Lagoon	-	-	-	-	-	-	-	-	-	-	0/4	0/33	2/92 2%	-	-	-	-	-	-
1965	Open Country	10/72 14%	4/26 15%	1/39 2.5%	0/17	9/100 9%	1/20 5%	0/1	0/97	0/2	0/7	-	-	-	-	-	-	-	1/20 5%	0/40
	Bush	0/1	-	0/7	-	-	-	-	0/72	-	0/1	-	-	-	-	-	-	-	-	0/13
	Lagoon	-	-	-	-	-	-	-	-	-	-	-	-	0/5	0/37	3/6	0/19	-	--	-

REPORT FROM DR. MARSHALL Y. KREMERS
CLARK AIR FORCE BASE, PHILIPPINES

Serologic Survey of Clark - Preliminary Results.

A rough census was made of both American and Filipino populations within the survey area. The total population was broken down by categories as follows:

Americans on base	11,349		
Americans off base	11,668		
Total Americans		23,017	
Filipinos on base - Negritos		800	
Domestics		3,306	
Filipinos off base within survey area		26,224	
Total population sampled			53,347

A completely random sample was taken of the entire population. A sample size of 100 was chosen arbitrarily as being a large sample, since with no previous experience from which to anticipate how many would be positive, it was not possible to calculate the sample size mathematically. The assistance of the provincial health department was enlisted and obtained for collecting the Filipino samples.

Portions of each sample were tested by hemagglutination-inhibition by Dr. Howard Jenkin at NAMRU-2, Taipei, Taiwan; by Dr. Scott Halstead at the SEATO Medical Research Laboratory, Bangkok, Thailand; and in our laboratory. These samples were thus also used for standardization of our own serology section. The results which were in very close agreement between the three laboratories are as follows:

Americans: All positive samples were caucasoids.

Number	Age	Sex	Time at Clark	Group B Titer*
8	28	M	41 mo.	40
10	36	M	12 mo.	20
22	34	M	15 mo.	20-40
25	35	M	16 mo.	20
32	28	F	9 mo.	320-640

Filipino:

Number	Age	Sex	Time at Clark	Group B Titer*
16	21	F	3 yr.	80
17	30	F	1 yr.	80-160
18	24	F	4 yr.	40
19	24	F	2 yr.	80
23	28	F	7 yr.	80
24	24	F	2 yr.	320
26	34	M	4 yr.	80
44	43	M	19 yr.	80
45	44	M	19 yr.	40
47	42	F	18 yr.	40
48	33	F	3 yr.	40
49	23	F	4 yr.	40-80
51	37	M	37 yr.	20
53	14	F	14 yr.	0-40
55	20	M	18 yr.	40
56	52	M	4 yr.	80
57	36	M	2 yr.	40
59	59	M	17 yr.	0-80
60	38	F	23 yr.	80-160
61	50	F	18 yr.	20
62	32	F	21 yr.	40-160
63	10	M	10 yr.	160
64	42	F	17 yr.	80-320
66	40	M	26 yr.	80
67	16	M	16 yr.	20
68	34	F	10 yr.	80
69	52	M	21 yr.	20-40
70	23	F	23 yr.	80
71	36	F	8 yr.	40
72	30	F	18 yr.	40-160
73	26	F	19 yr.	160
74	40	F	15 yr.	320
75	15	F	15 yr.	320
76	61	M	35 yr.	40
77	34	F	19 yr.	80-320
78	34	M	19 yr.	80
79	42	M	40 yr.	40
80	48	F	20 yr.	40
81	34	F	20 yr.	40-160
82	20	F	3 mo.	0-160
83	47	M	47 yr.	80
84	21	F	21 yr.	80
85	16	M	16 yr.	320

Filipino: (Continued)

Number	Age	Sex	Time at Clark	Group B Titer*
86	35	F	2 yr.	20
87	14	F	14 yr.	640-1280
88	78	M	78 yr.	0-40
89	18	F	18 yr.	160
90	32	M	14 yr.	40
91	28	F	28 yr.	160-640
92	54	F	14 yr.	80
93	75	M	15 yr.	80-320
94	36	F	15 yr.	20
95	21	F	9 mo.	160
96	24	F	8 mo.	40
97	38	F	18 yr.	40
98	34	M	4 yr.	320
100	44	M	44 yr.	0-40

All titers are expressed as the reciprocal of the highest dilution of serum to completely inhibit hemagglutination in the test system. Where two numbers are listed, differences are between laboratories and/or differences using different antigens. Correlation of results between different laboratories is well within normal limits, and reproducibility in our laboratory has been excellent. This test is a non-specific screening test and can be expected to show antigenic experience with a variety of group B arbovirus infections. In addition, eight Filipino sera, including all three Negrito sera tested, showed antibody to group A arboviruses. These were tested by Halstead using Chikungunya as an antigen. Complement fixation tests were done by Halstead using a battery of dengue and Japanese encephalitis viruses, but the results were not much more specific, indicating infection by viruses of this group. The above data can be broken down as follows:

		No. Positive			
	No. Tested	Male	Female	Total	% Positive
American	36	4	1	5	14 (4.67-29.50)
Filipino	64	22	35	57	89 (78.75-95.49)
TOTAL	100	26	36	62	62 (51.75-71.52)

The percentage of infection is higher in the Filipinos. The percentages in parentheses represent the 95% confidence limits for binomial distributions. Since there is no overlap between these percentages, the differences between Americans and Filipinos are significant. It would not be possible to show

differences between males and females, due to inadequate numbers of replicates, or inadequate sample size. The same thing holds true for other factors such as length of time at Clark, living on or off base, etc.

These data were used to compute a variance from which the actual sample size necessary to show differences at the 95% level of confidence could be calculated. The calculated sample size for a population with this variance was found to be 62. Thus, for this problem of binomial distributions, our sample size of 100 was more than adequate.

It should be possible to tell by surveys presently planned and underway, whether differences in infection rates are due to differences between Americans and Filipinos, or reflect differences in living conditions or length of time in the Philippines. An important role that a preliminary survey of this nature can play is in guiding the direction and scope of future work.

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

Immune Adherence Hemagglutination With Japanese B Encephalitis Virus.

Immune adherence hemagglutination (IAHA) was applied to JBE virus. JBE virus was harvested in mouse brain homogenate and was partially purified by protamine sulfate and ethanol treatment. Immune serum was obtained from Japanese monkeys (Macaca fuscata) receiving a subcutaneous injection of live mouse passaged JBE virus. Prior to use, the serum was absorbed three times with packed human 0 type red blood cells (RBC) at 0° C for 10 minutes each, then was inactivated by heating at 56° C for 30 minutes. Complement (C') was from donors from whom RBC were obtained. Its concentrations were adjusted to contain 8,000 x 50% units ($C'IAHA_{50}$/ml.). Freshly prepared Veronal buffer (pH 7.5) containing 0.1% gelatin, 4% sucrose, 0.00015M Ca^{++}, 0.0005 M Mg^{++}, AND).)(M NaCl was used as a diluent.

Two-tenth ml. of antigen was mixed with 0.2 ml. of serially diluted antiserum. Two-tenth ml. of C', usually diluted 400-fold, were added. After being placed in a water bath at

37° C for 30 minutes, the mixture was added with 0.5 ml. of RBC suspension (4 x 10^7 cells/ml.). The tubes were shaken in the water bath for 10 minutes and incubated for further 60 minutes. Patterns of settled RBC were recorded.

The following controls, which must be negative, were included in every test: antigen, antiserum, and C' each alone with RBC, and RBC alone in diluent. Controls were also placed to assure the absence of IA due to soluble antigens in the sera used. It was also necessary to exclude natural antibodies in the C' serum.

JBE virus, in the presence of anti JBE monkey serum and human C', agglutinates human RBC by IA. Both JBE virus antigen and antiserum can thus be titrated. Typical examples of the results obtained as well as comparative examinations with C' fixation, hemagglutination and neutralization tests are shown in tables 1 and 2.

Table 1. Chessboard Titration of JBE Antigen and Antiserum by I A H A Patterns.

Antigen dilution	Antiserum dilution								
	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	None
1/2	4*	4	4	2	1	0	0	0	0
1/4	4	4	4	4	4	2	1	0	0
1/8	4	4	4	4	4	1	0	0	0
1/16	4	4	4	4	2	1	0	0	0
1/32	4	4	4	4	2	1	0	0	0
1/64	3	3	3	3	1	1	0	0	0
1/128	0	2	3	2	1	0	0	0	0
None	0	0	0	0	0	0	0	0	0

* Numerals 4-1 indicate grades of agglutination.
0 means negative.

Table 2. I A H A titers as compared with titers obtained by C' fixation, hemagglutination, hemagglutination inhibition, neutralization tests and mouse-infectivity.

	IAHA	C'F#	HA##	HI##	NIx	LD_{50}xx
Antigen titer	128	30	4,096			$10^{7.0}$
Antibody titer	160	80		1,280	3.0	

The method of Kabat and Mayer, using 5 $C'H_{50}$ of C'.

Clarke-Casals' method.

x Neutralization index obtained in diduted virus-constant serum system.

xx Mouse LD_{50}/0.02ml.

Mosquito Tissue Culture

Attempts have been continued to search for an improved medium for Culex molestus mosquito ovarian tissue culture. A medium of the following composition has been found to be satisfactory for not only longer survival but also marked outgrowth of ovarian tissues as well as for proliferation of several cell types in vitro.

Basal Solution		Final Culture Medium	
NaCl	0.65 g	Basal solution	80 parts
KCl	0.05 "		
$CaCl_2$	0.01 "	Synthetic mixture	
KH_2PO_4	0.01 "	No. 199	20 parts
$NaHCO_3$	0.01 "		
Sucrose	1.00 "	Calf serum	10 parts
Lactalbumine hydrolysate	2.00 "		
Aq. dest.	100.00 ml	pH 7.0 - 7.2	

Calf serum showed accelerating effect(s) for cellular proliferation, compared with ovine, bovine serum or chicken plasma. The ovarian tissues could survive for more than one month and cellular proliferation was observed over 3 months, when the medium was replaced every 6 days. Besides the primary cultures, sub-cultures were also possible, and up to the present time eight serial sub-cultures have been attained during 2 months.

(S. Hotta, H. Aoki, and T. Yasui)

Reference: S. Kitamura: The *in vitro* cultivation of tissues from the mosquito, *Culex pipiens* var. *molestus*, II. An improved culture medium useful for ovarian tissue culture. Kobe J. Med. Sci. Vol. 11, 23-30, 1965.

SPECIAL NOTICES

The IX International Congress of Microbiology will be held in Moscow 24-30 July 1966. Requests for registration forms and tentative program should be addressed to

Professor V.M. Zhdanov
Secretary General
USSR National Organizing Committee
IX International Congress for Microbiology
Institute of Virology
Moscow D-98, USSR

Professor M.P. Chumakov, Director, Institute of Poliomyelitis and Viral Encephalitis, Academy of Medical Sciences, Moscow B-27, USSR, is in charge of program items dealing with arboviruses.

Of special interest will be meetings and a special arbovirus committee report on an evolving official international classification and nomenclature of viruses. A conference for WHO Regional Reference Laboratory Directors is under consideration, prior to the Congress.

A number of matters of concern to arbovirologists may also come up for consideration, if special arbovirus meetings similar to those held in 1958 at the International Congresses of Tropical Medicine and Malaria in Lisbon and in 1963 in Rio de Janeiro are considered feasible and can be organized. The feasibility of such meetings is largely dependent upon presence of enough arbovirologists to make them worthwhile. Therefore, information regarding the intent of participants and associated workers should be conveyed to the Subcommittee on Information Exchange. If enough persons will be present, efforts will be made to schedule several special sessions during the Congress in Moscow, or immediately following, ie 31 July through 3 August.

An International Congress on Tropical Biology is being planned for the period 6 through 11 June 1966 to be convened at Belem, Para, Brazil. A two-day section for Parasitology, Bacteriology, and Virology is being planned

by Dr. Manuel Bruno-Lobo. Any arbovirus investigator who is interested in submitting a paper on a pertinent subject should contact:

Dr. Manuel Bruno-Lobo
Simposio Sobre A Biota Amazonica
Belem, Para, Brazil

The next Pacific Science Congress is scheduled for Tokyo, 22 August through 10 September. The Section on Arthropod-borne Viruses is being headed by Dr. Masami Kitaoka, National Institute of Health, 284 Kamiosaki-chojamaru, Shinagawa-ku, Tokyo, Japan. Three special programs on arboviruses are already in the final stages or organization and a tentative program will be distributed in a subsequent Information Exchange.

Any readers who are interested in attending or participating in the Congress should communicate with Dr. Kitaoka.