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

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

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

"Effective July 1, 1968, Dr. R. M. Taylor who has directed the Catalogue of Arthropod-borne Viruses of the World since the inception of the Catalogue in 1960 will relinquish the day-to-day operation of it. Dr. Taylor requested that he be relieved of this duty for health reasons, and his resignation was accepted with considerable regret by the A.C.A.V. Executive. Happily, Dr. Taylor has kindly agreed to remain a member of the A.C.A.V. Subcommittee on Information Exchange, and he will serve as Consultant to the Catalogue."

Dr. Trygve O. Berge has been appointed to membership of the Subcommittee on Information Exchange and will assume direction of the Catalogue. The Catalogue operation will be transferred from Berkeley, California to Dr. Berge's office in Rockville, Maryland. Hence forth all correspondence concerning the Catalogue should be directed to him at the following address: Dr. Trygve O. Berge, Curator, Collection of Animal Viruses and Rickettsiae, American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A.

The A.C.A.V. Executive expresses its gratitude to Dr. Berge for his ready and cheerful acceptance of direction of the Catalogue in succession to Dr. Taylor whose services have been appreciated most gratefully during the past eight years.

REPORT OF THE CHAIRMAN OF THE SUBCOMMITTEE ON ARBOVIRUS INFORMATION EXCHANGE

The Arthropod-borne Virus Catalogue

Since the report on the Catalogue in the last issue (#16) of the Information Exchange, 15 "new" viruses have been registered, making a total of 222 registered in the Catalogue at the close of 1967.

Catalogues Distributed

One hundred thirty-seven catalogues have now been issued, 56 sent to addresses in continental U.S.A. and 81 to foreign addresses involving 46 countries. The distribution by continent is as follows: Africa 11; Asia 18; Australia 7; Europe 23; North America 62; South America 16.

Abstracts and Current Information

A total of 4, 456 abstract and current information slips have been issued

as of January of this year, consisting of 2, 504 from Biological Abstracts; 1, 707 from Bulletin of Hygiene and Tropical Diseases Bulletin; and 245 personal and other communications.

Publication of Catalogue

Notifcation of the intended publication of the Catalogue was given in the last (#16) Information Exchange. It has now been published and may be obtained from the Government Printing Office under the designation:

> Taylor, R. M. (comp.) Catalogue of Arthropod-borne Viruses of the World. PHS Publication no. 1760. First ed., 1967. U.S. Government Printing Office, Washington, D.C. 908 pages. Available from Superintendent of Documents, $5.25.

REPORT OF SIRACA ON THE REGISTRATIONS OF THE GROUP C VIRUSES NOW IN THE CATALOGUE

After consideration of the serological evidence submitted with the registration cards, as well as other available relevant information, the following conclusions were reached and recommendations made.

1. Patois and Zegla viruses should not be included in Group C; they were therefore not considered further.
2. The Group C viruses now registered are: Apeu, Caraparu, Gumbo Limbo, Itaqui, Madrid, Marituba, Murutucu, Nepuyo, Oriboca, Ossa, and Restan.
3. For the purpose of differentiating these viruses only the results of hemagglutination-inhibition and neutralization test were considered, not those of complement-fixation and cross-challenge tests, for these reasons:
    a) The HI and N tests were generally concordant and, furthermore, afforded a clearer, sharper separation of the viruses.
    b) The CF tests results were, on the whole, less discriminatory than those of the HI and N tests; in addition, when the CF discriminated between viruses it did so in a manner that conflicted, in part, with the HI and N tests' results.
    c) Cross-challenge tests based either on survival of challenge animals or on viremia titrations are too incomplete to use as a general basis for comparison; furthermore, as far as

they go they appear to give an even wider overlap than the CF test, hence even less usable for separation of viruses.

4. There are no duplications among the examined cards, the 11 registered viruses being distinguishable from each other.

5. After a thorough examination of the serological evidence, the Subcommittee suggests the following scheme of classification of Group C viruses, with a view to stress areas of similarity and separation in the group.

Complex	Virus (or type)	Subtype
Caraparu	Caraparu	1 (Belem)
		2 (Trinidad)
		3 (Ossa)
	Apeu	
	Madrid	
Marituba	Marituba	1 (Marituba)
		2 (Murutucu)
		3 (Restan)
	Nepuyo	1 (Nepuyo)
		? 2 (Gumbo Limbo)
Oriboca	Oriboca	
	Itaqui	

The scheme attempts to recognize the fact that there are gradations in the reported distinctness between agents. The Subcommittee recommends that, for the time being, subtypes be designated both by number and, in parentheses, by the name given, with the expectancy that future usage will settle for either one or the other. As examples, Ossa would be called "Caraparu virus, subtype 3 (Ossa)" and Murutucu as "Marituba virus, subtype 2 (Murutucu)."

Gumbo Limbo is entered with a question mark, not in order to question its distinctness but rather to indicate that its position in the scheme is unclear to the Subcommittee; evidently, additional work is required to settle this matter.

6. The scheme is an attempt to express the various magnitudes of the quantitative differences in the results of tests between viruses. For reasons already expressed by the Subcommittee in its previous report, June 14th, 1967, on Group B viruses, it is not possible to establish a set of definite rules.

Evidently, the differences in the N and HI values between members of a complex and those of another are greater than those among the members of a complex; and in a given complex, the serological differences between viruses or types are greater than those between subtypes. The precise numerical value of the reciprocal differences, in terms of log N indices in the neutralization test or serum titers in the HI test, in the comparison of two strains, that are required at each level of the scheme, cannot be stated. In general, differences in log NI between types were 2 or higher, reciprocally; and 4-fold at least, usually greater, in the HI titers, also reciprocally. The differences were smaller between subtypes.

7. The Subcommittee urges ACAV to bring the scheme to the attention of interested and knowledgeable investigators, in order to generate and promote feed-back; the tenor of the latter will undoubtedly influence future recommendations by SIRACA.

8. The above, or a similar scheme, if found satisfactory, might be applied to other arboviruses' groups. As an analogy only and with no other implications, for the present, it can be seen that the scheme follows a gradation in descending order as might exist at the corresponding level of the Linnean classification. As an example, one can place a strain, Murutucu Be An 974, in the scheme as follows.

Agent to be classified: Murutucu (Be An 974)	Steps in the classification of arboviruses	Linnean taxa
Bunyamwera	Antigenic supergroup	Supergenus
↓	↓	↓
C	Antigenic group	Genus
↓	↓	↓
Marituba	Complex	Subgenus
↓	↓	↓
Marituba	Virus (or type)	Species
↓	↓	↓
2 (Murutucu)	Subtype	Subspecies
↓	↓	↓
Be An 974	Strain	Individual

At the step between subtype and strain may be placed "variants"; the Subcommittee felt that this term might best be applied to characteristics other than serological.

(E. L. Buescher, J. Casals (Chairman), G. E. Sather, W. Scherer, R. E. Shope (ad hoc Consultant) and C. L. Wisseman, Jr.)

REPORT OF THE SUBCOMMITTEE ON BIRDS AND OTHER VERTEBRATES

Training Program Held 18-19 March, Laurel, Maryland

On 18-19 March 1968, the Subcommittee on Birds and Other Vertebrates of the ACAV, jointly with the Migratory Bird Population Station and the Patuxent Wildlife Center of the Fish and Wildlife Service, held at Laurel, Maryland, a two-day training program on the methodology and philosophy of studying vertebrates in public health research on arboviruses. Announcement of the meeting was sent in December to about 90 institutions and public health laboratories. Responses were encouraging and included suggestions as to aspects of field techniques they hoped to have covered by the program. The 32 individuals who attended the meeting were sponsored by their own institutions; no ACAV funds were spent on the program.

Dr. William Reeves presented the introduction to the meeting, discussing the need for the study of wild vertebrates in public health research, the fundamental questions asked, and the general methodology for obtaining the answers. Mr. Allan T. Studholm of the United States Fish and Wildlife Service, discussed Federal and State permits needed by field workers handling migratory birds. Mr. Earl Baysinger, Chief, Bird Banding Laboratory, Migratory Bird Populations Station, Patuxent, discussed the working philosophy, organization and problems of the Banding Laboratory - especially those caused by people in arbovirus research whose records are not well kept. He later led the group through the banding laboratory (see accompanying report). Vertebrate zoologists, specialists in their fields, discussed collecting methods and field experiences: birds, Mr. Chandler S. Robbins, Migratory Bird Populations Station, Patuxent; small mammals, Dr. Kyle Barbehann (U.S. National Museum); bats, Mr. Merlin Tuttle (Univ. Kansas Museum of Natural History) and cold-blooded vertebrates; Mr. Charles Meyers (Univ. of Kansas Museum of Natural History). Drs. Rex Lord and Althea Burton respectively, discussed the current status of knowledge of bats and cold-blooded vertebrates in relation to arboviruses. Dr. Robert Dickerman concluded the program by discussing the use of hamsters as sentinels for isolation of arboviruses, and by summarizing tissue collection techniques, including those for both blood and organs.

(R. Dickerman)

Arbovirus Research and the Bird Banding Laboratory of the United States Fish and Wildlife Service Migratory Bird Population Station

The Bird Banding Laboratory is presently processing about a million and a half new bandings each year and almost 100,000 recoveries from these bandings. Along with this work, more and more research is going into the aging and sexing of the species being banded. The number of people requesting data from the Banding Laboratory for associated research purposes continues to increase each year. For this reason, the data submitted to the Banding Laboratory must be as biologically correct as the bander can make it.

The submission of records at the appropriate time in a form that can readily be handled by the Automatic Data Processing Section is also of paramount importance. This is almost as important as being sure the data is biologically correct. It should be remembered that these are scientific documents and if they are made out in such a manner that they cannot be used readily, the data on them is of little value.

At the present time, there is some discussion as to the value of banding birds that have been bled. The thought here is that a bird that has had a blood sample taken is less likely to survive, perhaps, than a normal bird. This is particularly true in cases where the person doing the bleeding is not highly skilled or the bird is stressed due to being handled for a long period of time or in conditions that are detrimental to the bird's survival, such as heat, cold, etc.

Birds that have been bled or handled in an experimental manner must be coded in such a manner that this will be apparent to later researchers. Many of these later researchers will be using banding data for life history studies, movements, mortality, etc., and they should be aware that birds handled in other than normal manners are marked so they can be excluded or included as the investigator may wish.

The "additional information" codes that we are presently using on our banding schedules are very much outdated, but until we can update and modify them, they are all we have to use. At the present time, we ask anyone who is taking blood samples to code such birds 6-18 so they will be recognized and can be handled in the computer. Along with this coding in the "status" column, it is very helpful if it is noted in the "remarks" section on the back of each banding schedule that the birds were bled or handled in an experimental manner.

Those people that are bleeding large numbers of birds should remember

that at the present time the Banding Laboratory has requested that once a bird has been banded, released into the wild and a record for that banding submitted to the Banding Laboratory, the bird must be considered untouchable by persons subsequently recapturing it. If a banded bird is taken in an arbovirus program, this bird should not be bled. The additional manipulations or treatments of previously banded birds may seriously jeopardize the project of the person who originally banded the bird or greatly change the data pertaining to these birds that have already been sent into the Banding Laboratory and later passed on to other investigators. Anyone who would like further information about the problem of handling previously banded birds should write to the Bird Banding Laboratory and request a copy of "Bird Banding Notes", vol. 6, #1, March 1966, which outlines the present banding policy on such birds.

In the future, with our automatic data processing system and computers, we will be able to process birds that have had status changes made on them. This would mean that a bird banded and released as "normal" and later bled could be processed in our system and the code change shown on the report for this bird. However, at the present time, our system is not well enough developed to allow us to handle such changes. For this reason we cannot stress too strongly the very serious problem caused by persons who handle previously banded birds in such a manner as to change their present status or condition.

Another problem that has developed in the past is with arbovirus research teams apparently not having a qualified orinthologist in the group. In cases where a reseacher is contemplating marking and bleeding large numbers of birds and no one is qualified to identify all species, it would be wise (and the Banding Laboratory cannot stress too strongly the advisability) to contact someone in the area who is either a bird bander or a qualified authority on bird identification to help with the program. This is particularly true at the present time with the stress being put on proper identification of different ages and sexes of the birds being handled. Most people working in the banding program would be only too happy to assist in an arbovirus study as a part-time consultant and help with the identification, aging, sexing, etc.

(Earl Baysinger)

THE RICHARD MORELAND TAYLOR AWARD FOR ACHIEVEMENT IN ARBOVIROLOGY

The Richard Moreland Taylor Award was established by the American Committee on Arthropod-borne Viruses in 1966 with a generous donation of $1, 500 given by Abbott Laboratories of Chicago, Illinois. Recognition by Abbott Laboratories of the significance of achievement in arbovirology puts in new perspective in medical science and tropical medicine recognition of those who have devoted attention to this complex area.

The initial Award was conferred upon Dr. Richard M. Taylor, after whom the Award is named, at the 1966 Puerto Rico meeting of the American Society of Tropical Medicine and Hygiene. It is intended that additional awards of the medal and certificate will be made from time to time. The next Award will be presented at the open meeting of the American Committee on Arthropod-borne Viruses at the Biltmore Hotel in Atlanta, Georgia on 30 October 1968.

The substantial material recognition by Abbott Laboratories, a pharmaceutical establishment known well and long for its work in parasitology and tropical medicine, is especially appreciated by those engaged in the laboratory and field investigation of arboviruses and the diseases they cause. The Abbott donation largely paid for design and preparation of the original dies from which each awardee's medal will be struck. For continuation of the Award program, ongoing resources will be required and it is considered that those active in this scientific field would like to personally participate in this new recognition in tropical medicine.

Individual contributors should mail their cheques to the A.C.A.V. Treasurer, Dr. William C. Reeves, School of Public Health, University of California, Berkeley, California 94720. Contributors should:

1. Make cheques payable to The Regents, University of California.

2. Attach a note containing the donor's name and address, also indicating that the funds are for deposit in the Richard Moreland Taylor Award Account. A receipt should be requested.

All contributions are tax deductable.

REPORT FROM THE DEPARTMENT OF ENTOMOLOGY, LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE, LONDON, UNITED KINGDOM

I. Cell culture: arthropods and lower vertebrates

Fibroblasts from metamorphosing tadpoles of the common frog, Rana temporaria, were grown at 28° C in a medium containing NCTC . 109 medium and foetal calf serum. Louping ill virus and Langat virus increased by 4-5 logs. There was no cytopathic effect.

In a few preliminary experiments, liver cells from the wall lizard, Lacerta muralis, supported growth of Quaranfil virus, without cytopathic effects. The medium used was Hank's BSS with lactalbumin hydrolysate and foetal calf serum.

Numerous primary cell cultures of the tick Hyalomma dromedarii have been set up for infection with arboviruses. Besides Rehacek's original medium, several other media are being tested. Some of these media keep the cells dividing for extended periods - numerous mitoses can be seen as late as 30 days after seeding.

Embryonic cells of the blood sucking bug, Triatoma maculata, are also being grown as primary cultures for virus infection experiments.

Cell line from deermouse kidneys

A cell line from suckling deermouse (Peromyscus maniculatus bairdii) was established and has been stored in liquid nitrogen. The cells supported the growth of Langat virus and Semliki Forest virus, the latter killing the cells in 2-3 days with a TCD_{50} of 10^6. The results will be published.

(M. G. R. Varma)

II. Field work in British Honduras, 1967

From January to September, 1967, collections were made of mosquitoes, ticks, human and animal sera in British Honduras over a sector from Belize City on the coast westwards 70 miles to the vicinity of Cayo, and from there southwards into Mountain Pine Ridge (3,000 ft.) as far as Augustine. Laboratory facilities, including a Revco at -70° C, were at Central Farm, near Cayo, the base of the Dermal Leishmaniasis Research

Unit. Sera on ice in thermoses, and sera and arthropods on liquid nitrogen, were sent by air, via Miami, for analysis by the Trinidad Regional Virus Laboratory, using Union Carbide LR-10A-6 refrigerators. These were not reliable for this arduous journey although, in fact, most of the material was, fortunately, transported successfully to Trinidad.

Preliminary analysis of 277 human sera, broadly representative of the sector from coast to mountain, gave 27.9% positive reactions, by HI test, mainly for Group B antibodies with a few for Mucambo VEE. Other groups were rarely represented. Reactions were absent below 10-15 years of age but incidence rose in higher age groups to about 75-90%; this applied to both sexes. Neutralization tests are in progress.

Horses, mules, cattle and pig reacted mainly to VEE antigen in HI tests, and equines to Cache Valley.

Tests for tick-borne-type virus antibodies in human sera are being arranged for at Yale Arbovirus Research Unit.

No virus isolations were obtained from about 10,000 mosquitoes or 7,000 ticks (about 60% of them larvae).

The indications are that active arbovirus transmissions to man are negligible at the present time.

About 70 species of mosquito were taken, of which eight were anophelines, and twelve species of ixodid tick. Ground-breeding Aedes serratus, Ae. scapularis and, at the coast, Aedes taeniorhynchus, were common, this abundant mangrove-species, however, being taken also in small numbers as far as 70 miles inland. Widely prevalent in appropriate environments were Ps. ferox and Ps. cingulata, Mansonia spp., Deinocerites (in coastal mangrove) and Culex fatigans (domestic), C. coronator and C. taeniopus/opisthopus. Few species of Culex (Melanoconion) were taken, and only small numbers usually. Sabethines include Limatus spp., Sabethes chloropoterus, Trichoprosopon magnum, and Wyeomyia spp. Haemogogus include H. andinus. Material is still being studied, and collections sent for opinion, and the records, of the project for Mosquitoes of Central America.

Human bait collection was particularly successful, and CDC miniature light traps productive for C. taeniopus/opisthopus, other Culex, and Mansonia spp. particularly. The collections, when worked out, should provide useful information for a species list, for species distribution in the territory, and from sequential hourly or half-hourly catches in bush, and on platforms of a tower in a forest, data on their biting cycles.

Ticks were collected by dragging, and from domestic animals and wild life. The most widely prevalent species were Amblyomma cajennense and Amb. oblongaguttatum. The majority of ticks collected belonged to the genus Amblyomma. The ticks most commonly found on equines were Anocentor nitens and Amb. cajennense, and on cattle Amb. cajennense. Attachment of larval Amblyomma and nymphs of Amb. cajennense to men moving in bush and rough pasture were frequent.

(D. S. Bertram and M. G. R. Varma)

REPORT FROM THE MICROBIOLOGICAL RESEARCH ESTABLISHMENT, PORTON, ENGLAND

Assay of virus infectivity by formation of negative plaques

The procedure for the assay of the infectivity of group B, tick-borne viruses by the enumeration of plaques formed in agar suspensions of chick embryo cells (Information Exchange #14) may be usefully extended to viruses or virus strains showing little or no cytopathogenic effect (CPE) by using a suspension of a second virus (Semliki Forest virus or Vesicular Stomatitis) as a lytic stain. Thus if dilutions of a virus (Langat, louping-ill) normally showing poor CPE in the CEC agar suspension system are set up in the normal manner, but without actinomycin-D, few or faint plaques may be seen when plates are stained by neutral red at five days. If however, 1,000 infective units/plate of the stain virus are applied 12-24 hours before the normal staining period, and the plates further incubated to the normal time, then a totally lysed background will be observed except where the cell suspension has been protected by the diffusion zone of interferon which marks every site at which the initial virus has multiplied. The large negative plaques of up to 10 mm diameter of interferon protected intact cells are as clear as the normal plaques of the stain virus and their enumeration gives a valid plaque count for the inital virus dilutions.

Although it is an interference method the counting of negative plaques is fully quantitative and is thus distinct from quantal procedures for interference of growth or CPE in roller tubes. The value of this negative plaque procedure will be tested with some other viruses (yellow fever, chikungunya) which are associated with interferon production but do not easily form plaques by normal methods.

Virus production in rolling multi-layers of chick embryo cells

With the finding that at suitably high cell densities it is possible to form adherent multi-layers of primary chick embryo cells on the interior walls of slowly rotating (1 rev/min.) cylindrical bottles (2 in. - 6 in. diameter) it became feasible to test such multi-layers for the production of a virus. Virus production in rolling multi-layers of cells offers a simple culture and containment system together with the promise of high infectivity and low initial protein content. The medium (Parker's medium 199, pH 7.4 with or without 5% calf serum) cells and assay procedures were as described previously (Information Exchange #14).

In a typical roller culture in 18 inch long x 6 inch diameter bottles an input of $10^{8.4}$ cells and $10^{7.5}$ infective units of Semliki Forest virus in 15 ml of medium forms a cylindrical multi-layer which yields 3,000 infective units/cell or 15 ml of $10^{10.7}$ infective units/ml after 18 hours at 34° C.

For the group B tick-borne viruses (Langat and louping ill) the optimum yield is currently only about 200 infective units/cell. This requires the inclusion of 5% calf serum in the growth medium which cannot be replaced by bovine serum albumin. A typical roller culture of the size defined here yields at 48-72 hours a volume of 15 ml of about $10^{9.5}$ infective units/ml or $10^{10.7}$ units/bottle. The inclusion of Actinomycin-D at a concentration of 0.025 μg/ml enhances significantly the sensitivity of the plaque assay of the tick-borne viruses but is without influence on the production of virus in roller cultures. The poor yield of infective units/cell for the group B, tick-borne viruses (up to 200) as compared with that for the group A virus (1,000-3,000) may be due to the relatively inefficient early infection of the cells by the former.

When Semliki Forest virus is produced in chick embryo cell cultures at 34-37° C in the absence of calf serum, or with calf serum replaced by bovine serum albumin, the supernatant fluid at the optimum time contains over 10^{10} infective units/ml but no detectable inhibitors of haemagglutination. Despite this high initial infectivity such fresh samples show no, or very low, haemagglutinating activity unless extracted by tween-80 and ether. These and other data indicate that haemagglutination may not be an intrinsic property of the initial, undegraded infective particle but may be uncovered by treatment. Alternatively, the infective particle may have intrinsic haemagglutinating activity but this may be suppressed by an adsorbed inhibitor which does not interfere with infectivity and is lost on extraction or degradation.

Quaranfil Virus in Mice, Hamsters and Guinea Pigs

The virus used was the 27th mouse passage of the EGAR 1095 strain.

Mice: Intraperitoneal (IP) inoculation of mice with 10^{-1} suspensions of infected brain produced no signs of disease or inflammatory changes in the brain. Intracerebral (IC) inoculations of mice, on the other hand, produced symptoms and mortality within five days. The appearance of symptoms was usually scattered over 14 days (from five to 19 days following inoculation). As a whole the first to go down were mice inoculated with 10^{-2} and 10^{-3} dilutions of infected brain, then followed 10^{-1} and 10^{-4}. The LD_{50} varied from $10^{-6.1}$ to $10^{-7.5}$. The first sign of the disease was hyperexcitability, the animals trembling, running and jumping at the slightest provocation. When left alone they remained curled up with the hind legs tucked under. Sooner or later they developed spasms of violent trembling, and at various times, especially when excited by a strange sound or noise suddenly became stiff and stretched out; next the head would start twisting sideways, followed by rolling and twisting of the whole body around the long axis. Occasionally animals in the course of a jump were seen to start rolling in mid-air. Some animals die during these spasms, other survive several spasms and finally became comatose and die. A small proportion of mice became moribund without rolling symptoms. Histological examination of brains of affected mice revealed severe inflammatory lesions in all subcortical centers and there was regular involvement of the cochlear nuclei of the pons and medulla.

Hamsters: inoculated IC became comatose on the sixth day without showing the symptoms observed in mice. In hamsters symptoms developed more or less simultaneously and CNS lesions were usually very extensive involving all parts of the brain.

Guinea-pigs: inoculated with 10^{-1} dilutions of mouse brain IC or IP as a rule showed no disease or mortality up to six weeks. However, when guinea pigs were destroyed seven days after IP or IC inoculation, virus was recovered from the brain and early histological lesions were observed in it. On the other hand when guinea-pigs were destroyed after two weeks, virus could be recovered from only half of the IC animals, and from none of the IP animals, but inflammatory brain lesions were found in all of both groups. Three weeks after inoculation, no virus could be recovered from the brains.

Arbovirus investigations in Sarawak

A total of 57 virus isolations have been made from 879 pools of mosquitoes collected in Sarawak during the period September - early December 1966. Thirty-four isolations were made in suckling mice and forty-one

isolations were made in tube cultures of chick embryo fibroblasts. Twenty-three of the viruses isolated in tissue culture were not isolated in parallel attempts in mice. Sixteen viruses were isolated from 274 pools of Culex tritaeniorhynchus (an isolation rate of 1:1700), 11 from 229 pools of C. gelidus (1:2100) and 17 from 75 pools of C. pseudovishnui (1:440). Identification of the virus isolates has been performed using multiple antigen-antiserum HI tests, cross challenge tests in mice and by plaque-inhibition tests in chick embryo fibroblasts. Seven strains of Sindbis, 7 strains of Japanese encephalitis and 10 strains of Tembusu have so far been identified, all in Culex species. Double infection with Sindbis and a Group B virus was found in two mosquito pools.

A previous hypothesis that flooding of ricefields following the monsoons in late October led to a rise of C. tritaeniorhynchus populations was disproved in that C. tritaeniorhynchus numbers increased rapidly some weeks before the ricefields were flooded. The increase may be associated more with the intensity of the rainfall rather than the overall amount, as very heavy rain washes out breeding places while the same amount of rain falling over a longer period does not.

REPORT FROM ARBOVIRUS UNIT, DEPARTMENT OF VIROLOGY, STATE INSTITUTE OF HYGIENE, WARSAW, POLAND

I. Serological survey of healthy population

The studies were made in 1965 through 1967 to obtain data on the occurrence and dissemination of encephalitic arboviruses. The work was conducted in cooperation with the Provincial Sanitary-Epidemiological Stations/PSES/. It was supported by a Grant No. CDC-VR-P1 from USA, PHS, CDC, Atlanta, Georgia.

Sera negative in the Wasserman test taken at random / about 50 sera from each district, e.g., 35 from adults and 15 from children and adolescents to 18 years of age / served as material for studies. Great care was taken to obtain an even distribution of samples over district territory. HI tests according to Clarke and Casals were used in the studies. In 1966 and 1967 the micromethod of Takatsy in adaptation of CDC Arbovirus Unit, Atlanta, was applied for tests performed in our laboratory. The positive results were confirmed by neutralization test in mice or tissue culture.

A total of 17.215 sera was tested. The highest percentage of arbo A antibodies/antigens used: WEE, EEE / was found in the provinces of Zielona

Góra and Gdańsk, of arbo B antibodies/mainly to TBE virus which is known from the neutralization and HI tests/in the provinces of Lublin and Szczecin.

The detailed analysis of areas with the highest percentage of antibodies to TBE virus shows that following regions are highly suspected of the presence of endemic foci: /1/ eastern and central districts of the province of Lublin connected with southern districts of the province of Białystok and a central band of districts in the province of Warszawa/on the average 6.5% of positive results/ ; /2/ two regions in the province of Szczecin: north-eastern region connected with north-western districts of the province of Koszalin/on the average 6% of pos. res. /and south-eastern one connected with northern districts of the province of Zielona Góra/on the average 6.4% of pos. res./; /3/ northern part of the province of Olsztyn connected with northern districts of the province of Białystok/on average 4.8% of pos. res./.

In the rest of the country territory the percentage of antibodies to TBE virus was from 0.5% to 2.1%.

The percentage of persons with arbo B antibodies is increasing with age. A statistically significant difference was found in the percentage of persons with antibodies between urban and rural population, the percentage being higher in the latter.

The results concerning arbo A antibodies demonstrated that two regions in this country may be suspected of the presence of arbo A endemic foci: /1/ the region comprising southern districts of the province of Szczecin and northern and central districts of the province of Zielona Góra/about 7.2% of pos. res./; /2/ the region of southern and central districts of the province of Gdańsk and Braniewo district of the province of Olsztyn/on the average 5.5% of pos. res./. In addition in three districts of the province of Warszawa 5.6%, 6.0% and 8.6% of the sera tested were positive. No statistically significant differences were found in the percentage of antibodies to group A between different age groups or between rural and urban population. The statistically significant difference was found between male and females, the percentage being greater in males.

The results concerning group B antibodies were confirmed by epidemiological data. As has been known, in the district of Hajnówka in the province of Białystok a focus of TBE has been detected/Białowieża National Park/. However, its size and connection with the territory of the province of Lublin was unknown, although the epidemiological data and the results of previous serological investigations of the forest workers in the province of Lublin suggested the presence of the focus. In the province of Szczecin epidemiological data seem to confirm the presence of the focus of TBE.

In the province of Olsztyn and the northern part of the province of Białystok cases of TBE were noted and strains of the virus isolated from ticks and patients. The periodical check of vectors and reservoir animals will be performed in the next years.

There is a lack of epidemiological data on the occurrence of human or animal infections etiologically connected with group A arboviruses in Poland. In areas with a high percentage of arbo A antibodies virological check of suspected cases will be conducted.

(Z. Wróblewska-Mularczykowa, L. Dobrzyński, D. Olkowska, and W. Magdzik)

II. Preparation of non-infectious HI antigens

On the basis of data published by M. Mussgay, R. Rott/Virology, 1964, 23, 573/we prepared several lots of HI antigens for TBE, WEE, EEE viruses. Tissue culture fluids were concentrated 30 x by ultricentrifugation in Spinco L 50 model, then treated with Tween 80 and ether/15 minutes each treatment/. After centrifugation the water phase at the bottom of the tube was collected as HI antigen.

The specificity of antigens was checked using reference sera from CDC, Atlanta. No infectivity of the antigens was found when tested by intracerebral inoculation of mice.

HI titers were table during at least six months of storage in 4° C. Tween antigens used in serological tests in 1966 and 1967 proved to be a valuable diagnostic preparation.

(Z. Wróblewska-Mularczykowa, and L. Dobrzyński)

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

Hemagglutination-inhibition antibodies to arboviruses in human population in Thailand

One hundred sera samples from Thailand were tested for the presence of antibodies to arboviruses. The sera were obtained from WHO Serum Bank

through the Institute of Epidemiology and Microbiology, Prague, thanks to kindness of Dr. K. Žáček. The sera were examined in hemagglutination-inhibition tests using the following virus antigens: Western equine encephalomyelitis /WEE/, Eastern equine encephalomyelitis /EEE/, Sindbis and Chikungunya - group A arboviruses; dengue type 1, 2, 3 and 4, Japanese B encephalitis, West Nile, Yellow fever and tick-borne encephalitis - group B arboviruses.

Antigens were prepared from the brains of suckling mice in 8.5% sucrose solution by aceton extraction after Clarke and Casals. The sera were extracted by kaolin and adsorbed with goose erythrocytes. Four-eight hemagglutination units were used in the hemagglutination-inhibition test.

The results obtained revealed that children up to two years of age do not possess antibodies to group A arbovirus antigens. Forty-four percent of sera reacted with B group antigens and reached the highest titre with Japanese B encephalitis virus.

Twenty percent of sera from the studied group of three year old children reacted with EEE antigen at low titre - 1:20 and 40% with group B antigens at highest titre with type 1 dengue virus.

In the group of four and five year old children, 30% of sera reacted with group A arbovirus antigens at maximum titre with EEE virus - 1:160 - 1:320. Sixty percent of sera reacted with group B antigens at highest titres with type 1 dengue virus.

In the group of six-ten year old children, 58% of positively reacting sera with group A antigens at maximum titre with EEE and Chikungunya virus were found. Seventy-three percent of sera reacted with group B antigens at maximum titres with type 1 dengue and type 3 Japanese B encephalitis, West Nile, Yellow fever, and tick-borne encephalitis. It may be assumed that the individuals included might have mixed infections with dengue and other group B arboviruses.

From the serological survey of Thailand human population could be concluded that EEE and Chikungunya viruses are of epidemioloigcal importance among group A arboviruses for this region. From the group B arboviruses, namely dengue and Japanese B encephalitis viruses probably play an important role in the epidemiology of this country. Considering the fact that sera exhibited cross reaction with all antigens of group B arboviruses, it seems likely that the adults were exposed to mixed or double infections with arboviruses. According to the antibody level it may be supposed that

mixed infection is due to dengue and Japanese B encephalitis viruses.

(M. Sekeyová and M. Grešíková)

Transmission of tick-borne encephalitis virus by nymphs of Ixodes ricinus and Haemaphysalis inermis to the common shrew, Sorex araneus

Tick-borne encephalitis /TE/ virus was transmitted to the shrew/Sorex araneus/ by nymphs of the ticks Ixodes ricinus and Haemaphysalis inermis. The virus was found in the shrews blood three-five days after the bite of I. ricinus nymphs, reaching the maximal titre of 10^1 intracerebral LD_{50} per 0.03 ml on the third day after the bite. Neutralizing antibodies were found on the 14th day in a titre of 1:16. The transmission of TE virus after the bite of infected H. inermis nymphs was demonstrated on the third and fourth day, the maximal titre being $10^{3.5}$ mouse intracerebral LD_{50} per 0.03 ml on the third day after the bite.

Based on the laboratory results and ecological observations the shrew may be considered the main reservoir of TE virus in natural foci.

(O. Kožuch, J. Nosek, M. Lichard, J. Chmela, and E. Ernek)

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

It was shown by previous investigations that in Hungary neutralizing antibodies are present against the TBE virus /KEm_1 strain, isolated in 1952/. In the years of 1966 and 1967 field trials were carried out in two different parts of the country.

In 1966 experts of the WHO Regional Reference Laboratory for Arboviruses, Bratislava, assisted in examining material collected from the north-eastern part of Hungary for virus isolation and serological investigation. Blood samples from 126 humans, 125 cows, 21 goats and 50 sheep were examined for the presence of HI antibodies against certain arboviruses /Table 1/. Isolation of virus was attempted from 1,500 unfed and 600 engorged Ixodes ricinus ticks, 160 small rodents and 54 moles. From groups of unfed ticks two strains of TBE virus were isolated in suckling mice.

In 1967 in the south-western part of Hungary blood samples of 147 humans, 40 cows and 34 small mammals were examined for HI antibodies to TBE

and West Nile /WN/ viruses /Table 2/. - In the same area 3,000 Ixodes ricinus ticks and 3,800 mosquitoes were collected. Virus isolation was attempted from 79 tick and 31 mosquito groups in suckling mice. From a group consisting of 100 nymphs a virus strain was isolated, which seemed to be identical with our TBE virus strain in neutralization test carried out in mice. From a group consisting of seven mosquitoes supposedly belonging to species Theobaldia annulata, another strain was isolated, which seemed to be in a close relationship with the WN virus. The identification of a third virus strain isolated from a mosquito group consisting of 150 Aedes vexans is still in progress. The first two strains mentioned are sensitive to sodium deoxycholate and both are pathogenic for suckling as well as for adult mice. The third strain is pathogenic for suckling mice but not for adult ones.

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

Table 1

Prevalence of antibodies to certain arboviruses in blood samples collected in Hajdu-Bihar County, Hungary 1966

Source	# of Blood Samples	Percentage incidence of HI antibodies to:					VN antibodies to:
		WEE	WN	TAH	Calovo	TBE	TBE
Human	126	3.1	10.3	4.7	0	4.7	4.0
Cow	125	0.8	8.0	5.6	47.6	38.8	23.0
Goat	21	0.0	9.5	14.0	9.5	19.0	15.0
Sheep	50	0.0	0.0	0.0	0.0	0.0	0.0

Table 2

Prevalence of HI antibodies to certain arboviruses in blood samples collected in Zala County, Hungary, 1967

Source	# of Blood Samples	Percentage incidence of HI antibodies to:	
		TBE	WN
Human	147	20	2
Cow	40	10	5
Mammal	34	6	32

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

Isolation of Tahyna virus in Italy

As referred in the sixteenth issue of the Arthropod-borne Virus Information Exchange, two strains of viruses were isolated from pools of mosquitoes (Aedes caspius 90% and Aedes vexans 10%), caught on September, 1967. Mosquitoes were collected at two places, a farmhouse at "Cona Spigolo" and along the river Isonzo, both at about 30 km. south of the city of Gorizia, north-eastern Italy. Both strains were reisolated from the original suspension and identified as Tahyna virus by neutralization test. This is the first isolation of Tahyna virus in Italy.

Preliminary serologic survey of human sera showed that Tahyna virus is active in the area.

REPORT FROM THE LABORATOIRE DES ARBOVIRUS, INSTITUT PASTEUR, PARIS, FRANCE

Argas borne viruses

Identification of several strains isolated from pools of Argas reflexus reflexus collected in pigeon houses in Camargue during the year 1966 was performed. Two different viruses were found: seven strains of GRAND ARBAUD and one strain of PONTEVES. These two viruses show a one-way serological relationship by CF tests. Both are related to a virus isolated in Finland: UUKUNIEMI (Table 1). The first virus was obtained from adult females and from different stages of nymphs, the second from unfed larvae hatched in the lab from eggs laid by collected engorged females.

Another field trip was made in January 1968 for new collections in the same pigeon houses. Inoculation of the first pools resulted in isolations of two strains of GRAND ARBAUD virus from nymphs collected at Grand Arbaud and isolation of one strain of PONTEVES virus from nymphs collected at Ponteves.

Serological examination of human and animal population of the same area are undertaken.

Serological surveys

Systematic serological surveys in different areas in France resulted in the finding of a focus in Alsace, near Colmar, where Tahyna antibodies are prevalent. It is a small size focus: in three villages the percentage of HAI positive tests in adults is above 50% although in the neighbouring region it is only 10% or less. This study is continued and a field program is prepared for next summer.

So far, the known distribution of the Tahyna infected regions was limited to the coast of the Mediterranean between Marseille and the Spanish border. Some areas show a broad distribution of virus activity (Languedoc), some others show small foci (near the Spanish border).

Tick transmission studies

An artificial feeding technique was designed for Argas reflexus in order to maintain a colony and to perform infection of the ticks, using capillary tubes containing normal or infected bird blood. This technique was used to infect ticks with West Nile, Tahyna and Grand Arbaud viruses. So far, multiplication of virus in the ticks and transmission of the virus to chicks by natural feeding was demonstrated for West Nile and Tahyna two months after the infecting meal. Transmission was demonstrated by isolation of virus from the chick or by antibody production. Long term experiments are in progress.

(Claude Hannoun)

Table 1

FC Cross reactions between Argas viruses and Uukuniemi

Antigen/Serum	Grand Arbaud 2	Ponteves L6	Uukuniemi A 21
Grand Arbaud 2	64/32*	0/0	64/8
Ponteves L6	16/32	128/32	4/8
Uukuniemi A 21	2/8	0/0	32/64

* reciprocal of titer of serum/reciprocal of titer of antigen

REPORT FROM THE VIROLOGY DEPARTMENT, PRINS LEOPOLD INSTITUUT VOOR TROPISCHE GENEESKUNDE, ANTWERPEN, BELGIUM

Observations on behaviour of small and large plaque variants of group A arboviruses in mosquitoes were continued. During our observations related in the foregoing issue of arbo exchange, the average meal of A. aegypti had been found to be 10^{-4} - 2.10^{-4} / mosquito. However, for practical reasons harvests of the mosquitoes occurred only 1 - 1.30 hr. after feeding. New observations in which mosquitoes were harvested immediately after feeding gave results of 8.10^{-4} - $1.6\ 10^{3}$ / mosquito. Observations were made on the behaviour of small and large plaque variants of Sindbis and WEE viruses in A. aegypti. In each instance the large plaque variants had a lower infection threshold than the corresponding small plaque variant. This phenomenon has now been confirmed for three arboviruses: WEE, Sindbis and Middelburg.

Some of the small plaque type virus (designated as Q9 in the former issue) isolated from mosquitoes originally infected with the large type Middelburg virus, has been studied more fully and the conclusion is now that this virus is probably in fact of an intermediate plaque size type but with all other in vitro markers of the large type virus. Studies are now in progress on the possibility of such intermediate plaque size virus occurring in mosquitoes infected with WEE-1 virus.

REPORT FROM THE VIRUS RESEARCH LABORATORY, UNIVERSITY OF IBADAN, NIGERIA

Light-trapped and aspirated collections of haematophagous arthropods were made during November and December 1967 at the University of Ibadan Dairy Unit. The light-trap (Toshiba Circline, modified by substitution of black light with standard white light) was operated for 12-hour periods from 1900 to 0700 hours. Attracted insects are blown into the collecting bag by a motor-driven fan. The collections included large numbers of Culicoides spp. but few mosquitoes. Mosquitoes were collected by aspiration of resting specimens from the cattle and from supporting framework of their open sheds during the first hours after sunset.

Each light trap collection was liberated into an 18" cubic gauze cage with glass top, and cloth sleeve on one side. The uninjured specimens would fly to the side facing a window and could then be sucked into a tube connected to a vacuum pump. The damaged and dead specimens falling to the bottom of the cage were preserved in alcohol for eventual identification. These constituted perhaps one half of each catch. The living specimens were knocked down by tobacco smoke or by exposure to freezing temperature, sorted into pools containing approximately 250 individuals each and held at minus 70° C until inoculated into baby mice. A portion of some catches was stored unpooled at minus 70° C for future study. Numbers of flies were estimated by volume rather than by actual counts. It was determined that approximately 2,000 Culicoides occupied one cubic centimeter. Small vials were calibrated to contain approximately 250 and 500 specimens and calculations of numbers were then made by multiple volumes of these lots. The largest 12-hour collection consisted of probably around 100,000 specimens. The number collected in each of the twenty-two 12-hr. collections varied widely and no connection was evident between either meteorological conditions or methods of manipulation. Approximately 118,000 specimens in 449 pools were inoculated for virus isolation attempts. Of these pools, 377 were from collections made between November 2 and 28. Forty-three viruses were isolated from the November collections. One viral agent was obtained from the 72 December samples, but has not yet been studied.

Three isolates were identified by CF testing as apparently identical with agents known to be active in the Ibadan area. One of these is a Simbu group virus and two are agents previously associated with ticks and viremia in market cattle. Thirty-seven of the agents (prototype AR22388) are related to, or identical with, each other insofar as tested to date. By complement fixation they are related to Bluetongue virus in preliminary screening test by

EAVRI. Four isolates remain untyped.

Also during November, one virus strain was isolated from a pool of twelve blood-engorged Aedes (Aedimorphus) fowleri. This isolate is tentatively placed, by CF testing, with the isolates from Culicoides related to Bluetongue. No isolates were obtained from the other 556 mosquitoes representing 14 species and seven genera, or from Phlebotomus spp. collected in November, nor from the 443 mosquitoes in December collections.

The presence of virus in Culicoides was discovered at a time of transition from the wet to dry seasons, the dryest months being December, January and February. It is significant to find that the Culicoides were such good indicators of virus activity, regardless of whether or not they actually serve as vectors. The flies were processed without regard to state of feeding so that blood engorged individuals were included with non-engorged flies.

Several trials were made during December to hold groups of Culicoides for three days prior to processing to allow time for fresh blood meals to be digested. Although 2,500 individuals survived and were processed in 25 pools, no virus was isolated. Unfortunately, these trials were made at a time when it appears that virus activity in the flies had diminished.

It is planned to return to the unpooled material from November collections held at minus 70° C storage to select engorged and non-engorged flies for processing separately. In this way it is hoped to see whether the viruses are present only in the fresh blood meals or in the flies showing no evidence of recent blood feeding, or in both groups.

Surveillance for virus activity in the Culicoides at the Dairy Unit is continuing. Parallel studies are planned on various vertebrate hosts in the area to determine, if possible, the arthropod (Culicoides) - vertebrate cycle by virus isolation attempts and antibody studies in frequent samples over a period of several seasons. The University cattle have protective antibodies for AR22388, conversions have been demonstrated in paired samples from young heifers. All sheep and lambs tested to date show CF antibodies for AR22388 at titers greater than 1:16. No isolations of this virus have been made from cattle or sheep.

REPORT FROM THE ARBOVIRUS LABORATORY, PASTEUR INSTITUTE OF BANGUI, REPUBLIC OF CENTRAL AFRICA

I. Exanthemic Fevers of Central Africa

Exanthemic fevers are frequently encountered and present an etiologic problem. Attempts at virus isolation and etiologic studies have yielded three different viruses, but others certainly exist because these three viruses used as antigens do not establish a diagnosis of all the "red fevers".

Clinical signs do not permit the separation of the different etiologies. Illness begins with a fever, arthralgia and backpain associated with a maculopapular eruption. They are of small size, a little raised and pale red in color, in clusters with intervals of healthy skin.

The virus isolations carried out from sera taken very early from febrile patients have permitted isolation of: - Bwamba virus, - Tataguine virus.

Serologic studies on the paired early and late sera employing H.I., C.F., and neutralization reactions permit etiological agents to be identified : by the H.I. reaction coupled with a quantitative sero-neutralization, the virus O'nyong 'nyong; the C.F. reaction confirmed by sero-neutralization, Bwamba virus; the neutralization reaction, Tataguine virus.

II. Virus Isolations

Diverse strains have been isolated from previously sampled humans or pooled mosquitoes.

a) Strains already catalogued:

from human beings: one strain of Bwamba, two strains of Tataguine

from mosquitoes: three strains of Sindbis, five strains of Mossuril, two strains of West Nile and one strain of Pongola

b) Strains provisionally new

from mosquitoes: one strain of BA . 209 from group B, and one strain of BA . 365 ungrouped

III. Serologic Examinations

The serologic investigation begun in 1966 was continued in 1967. Five

thousand, seven hundred eleven (5, 711) sera have been collected at 52 different points distributed over the territories of the Central African Republic and Tchad. Four large zones are represented.

1) The Forest zone

Situated in a hot, humid climate of the equatorial type, this zone is characterized by the scarcity and sudden late appearance of antibodies from group A and an almost complete absence of group B antibodies in children less than ten years old. In older persons the antibodies correspond to old yellow fever vaccinations. One observes, however, some very, very rare Zika antibody positives.

2) The zone of Prairie - Savannah

Chikungunya and O'nyong 'nyong antibodies appeared as elevated titers in a significant proportion of the population with hyperendemic ecologic foci. The viruses are still actually in circulation because a certain number of serologic conversions have been observed. Evidence of group B virus activity is rare. One can, however, observe Zika antibody positives. In 1966 yellow fever antibody has been evident in non-vaccinated children in the region of Berberati and in four villages to the north of Bangui. The children living north of Bangui again this year have rise in titer of neutralizing antibodies. These children have never been vaccinated and confirm therefore the presence of sylvan yellow fever virus.

3) The region on the shores of the River Oubangui

Chikungunya and O'nyong 'nyong antibodies reflect a transitional nature of this zone. Low incidence in the forest zone, but much higher incidence in the swamp.

While group B positives are rare, West Nile and Zika antibody has been detected. The presence of West Nile virus has in addition been confirmed by the isolation of a strain at the confluence of the M'Poko and Oubangui Rivers. Yellow fever antibodies appeared in children under ten and correspond to the vaccinations.

4) The region sahelo-soudanese, situated southeast of Tchad between Biobe and Am Timam

This region is characterized by a relatively low incidence of chikungunya and O'nyong 'nyong viruses. By contrast, group B antibodies appear very early and in a very significant proportion of the population. Eighty percent

of children between 10 and 15 years of age and nearly 100% of children below 15 and adults possess antibodies for group B viruses, with heterologous reactions for yellow fever virus. It or the viruses in question can only be determined through isolation from the sera of sick or of febrile children, or from arthropod vectors.

REPORT FROM THE MEDICAL ZOOLOGY DEPARTMENT, UNITED STATES MEDICAL RESEARCH UNIT NUMBER THREE (NAMRU-3), CAIRO, EGYPT, UNITED ARAB REPUBLIC

New Tick Virus Vector Laboratory

The new Tick Virus-vector Laboratory of the NAMRU-3 Medical Zoology Department is now being activated. It is headed by Dr. Robert E. Williams. Research will be directed to: (1) Investigate biological inter-relationships between tickborne viruses and their hosts in Egypt; including transmission studies in experimental animals. (2) To isolate previously unknown viruses from Egyptian ticks and conduct epidemiological studies of each virus. (3) To examine sera from persons and animals exposed to infection due to proximity to infected ticks. (4) To obtain serological and virological evidence implying previous exposure of migrant birds to virus infections, and the ability of these birds to sustain infections. (5) To make preliminary isolations of viruses from exotic tick species and furnish specialists in viral characterization and identification with comparative research material in this field.

Virus isolations from Argas ticks (Thailand and Nepal)

Since the last reporting period a virus has been isolated from Argas (Persicargas) robertsi Hoogstraal, Kaiser and Kohls (1968, Ann. Ent. Soc. America, 61 (2): 535-539). The ticks were collected by one of us (MNK) from a rookery of an open-bill Stork, Anastomus oscitans, at Wat Phai Lom temple, Pathumthani, 60 km north of Bangkok, Thailand. Suckling mice inoculated intracerebrally (ic) with tick suspensions showed signs of virus infection on day eight till day 16 after inoculation. This period shortened to days eight or nine in the third passage. The virus isolation was designated Virus Prototype Tick 39. Other isolations were made from Argas (Argas) reflexus subsp. collected by one of us (HH), R.M. Mitchell, and D. Kent, from pigeon lofts in Patan and Katmandu, Nepal. Two pools of ♂♂ were positive, apparently infected by two viruses as evidenced by the wide difference in incubation period. The first virus, which kills mice on day four after intracerebral inoculation in the second passage, is designated Prototype Tick 89. The second virus, which kills mice from day 10 to day 14 on the second passage, is designated Prototype Tick 94. Virus prototype isolations are being forwarded to Dr. Casals at Y.A.R.U. for identification.

(H. Hoogstraal and M. N. Kaiser)

REPORT FROM THE EAST AFRICAN VIRUS RESEARCH INSTITUTE, ENTEBBE, UGANDA

Isolation studies with mosquitoes collected at Zika forest and the West Nile district, Uganda have continued. Studies were started this year at Kamese Forest, Mawokota county some 15 miles west of the Institute, near the homestead of a jaundiced patient who had a rising titre of group B antibodies unlike that associated with yellow fever. Viruses identified are:

Code	1967	Mosquito	Place	Virus
MP 6088	Jan.	Culex annulioris	Kamese F.	Ntaya
MP 6138	Jan.	Aedes circumluteolus	Kamese F.	Pongola
MP 6186	Feb.	Culex annulioris	Kamese F.	Kamese
MP 6397	June	Mansonia uniformis	Kamese F.	Sindbis
MP 6440	June	Mixed mosquito spp.	Kika F.	Sindbis
MP 6830	Oct.	Aedes abnormalis/tarsalis gp.	Kamese F.	BUN gp.

Kamese virus which is related to Mossuril virus appears to be hitherto undescribed. In addition six strains from mosquitoes are still being identified.

Patients with the following arbovirus infections have been seen during 1967 at the Institute clinic: Congo virus (x2), Rift Valley fever, (x2), and chikungunya.

Ticks continue to prove a fruitful source of viruses. Thogoto virus has been isolated from Amblyomma variegatum collected in West Nile. Two strains of Group B virus hitherto undescribed as occuring in Africa have come from Rhipicephalus pravus collected in Karamoja. This virus (AMP 6640) is provisionally named Kadam virus after a mountain near the site of collection. During the last three years 10 closely related strains have been isolated from Amblyomma variegatum or A. lepidum collected in Karamoja and Entebbe. These agents appear to be unrelated to any described African arbovirus; AMP 5435 has been selected as the prototype of these agents and is provisionally named Karamoja tick virus.

Another antigenic variant of the group B viruses from the salivary glands of bats has been isolated from an insectivorous bat not previously tested,

Otomops martiensseni, collected on Mt. Suswa, Kenya. This strain (BP1912) should perhaps be named Mt. Suswa bat virus. It is, however, doubtful if the proliferation of exotic names for these agents is of value.

Human serology survey in the Northern Frontier district, Kenya, has shown that yellow fever has recently been active near Lake Rudolph and as far south and east as Marsabit. There is, however, no serologic evidence of recent yellow fever activity in Karamoja in the north-east of Uganda. Whether the activity in Northern Kenya is a direct extension of the recent yellow fever epidemic in Ethiopia or represents separate foci of yellow fever has not been determined. Sera are now being collected at the Kenya coast and at Garissa lying between the coast and Marsabit.

The yellow fever vector A. simpsoni has been collected biting man during the day in a Colocasia plantation near Kampala where the mosquito population is considered to be essentially non-anthropophilic. Precipitin studies on blood meals from wild fed A. simpsoni collected in the same area, show that the mosquito frequently feeds on rodents.

(M. C. Williams, G. W. Kafuko and B. E. Henderson)

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

Mosquito transmission experiments

The vectorial capacity of Culex univittatus with Sindbis and West Nile viruses has been determined in quantitative experiments. Mosquitoes were infected with varying ten-fold doses of virus by feeding them on a virus-blood mixture through freshly prepared chicken skin. After 18-21 days transmission to chickens by individual mosquitoes was attempted usually only with the group of mosquitoes infected with the strongest virus concentration, which was in the region of 5.0 logs. After the transmission attempt mosquitoes from all groups were tested individually for virus to determine an infectivity rate for each dose of virus. From these rates an infectivity threshold (dose infecting 10% of mosquitoes) was calculated. The table shows the results of one West Nile experiment and three with Sindbis. It can been seen that with both viruses the infectivity thresholds are low, but probably slightly lower with West Nile in which an endpoint was not obtained. However, on the basis of transmission rates vectorial capacity was superior for West Nile virus. These results seem to agree

with field observations in which the antibody conversion rate among sentinel fowls has nearly always been higher for West Nile despite an average higher frequency of isolation of Sindbis virus from Culex univittatus in the same study areas. The higher prevalence of Sindbis virus in the mosquito could perhaps be accounted for by a higher average level of viraemia by Sindbis virus in the birds of the area. An unresolved question in these experiments is whether a mosquito which failed to transmit to a chicken would succeed in infecting a more susceptible avian species.

Summary of quantitative infectivity/transmission experiments with West Nile and Sindbis viruses and Culex univittatus

Virus	Infecting Dose. Logs	Transm^{n}. Rate x	Infectivity Rate ‡	Infectivity Threshold (10%). Logs *
West Nile	4.7	4/4 (100%)	14/15	
	3.7	8/9 (88%)	15/16	< 1.7
	2.7	-	20/25	
	1.7	-	6/15	
Sindbis	5.6	2/3 (66%)	10/12	
	4.6	-	3/6	< 3.6
	3.6	-	3/5	
	2.6	-	None fed	
"	3.9	0/2	7/12	
	2.9	-	2/3	2.0
	1.9	-	1/16	
	0.9	-	1/19	
"	5.1	12/22 (55%)	23/32	-

x numerator = number of infected mosquitoes transmitting virus to chicks; denominator = number of infected mosquitoes feeding.

‡ numerator = number of mosquitoes infected; denominator = number of mosquitoes tested.

* calculations based on Reed and Muench method.

REPORT FROM THE SCHOOL OF TROPICAL MEDICINE, CALCUTTA, INDIA

Susceptibility of some laboratory animals to chikungunya virus

Animals employed in this study were mouse, rat, fowl, rabbit and guinea pig. Both newborn and adult animals were tested. Only subcutaneous inoculations were made and the dosage used was arbitrary, although bigger animals received higher doses and newborn animals received proportionately lower doses of the virus. Dose of inoculum was calculated in terms of LD50/0.02 ml inoculated I.C. in infant mice. Samples of blood were collected at intervals to detect viraemia, HI and neutralizing antibodies. In case of death, autopsy was performed for isolating the virus from blood and internal organs.

Results, in summary, have been shown in the Table:

		Dose in LD50/0.02 ml in mouse	Sickness & death	Viraemia	Antibody response	Virus in internal organs.
Rabbits	Adult	1000,000	Nil	+(upto 48 hours)	+	NT
	2-day old	250,000	+	+	NT	+
Guinea-pig	Adult	500,000	Nil	+(upto 48 hours)	+	NT
	2-day old	200,000	+	+	NT	+
Rat	Adult	100,000	Nil	+(upto 48 hours)	+	NT
	2-day old	1000	+	+	NT	+
Mouse	Adult	10,000	Nil	+(upto 48 hours)	+	NT
	2-day old	100	+	+	NT	+
Fowl	Adult	500,000	Nil	Nil	Nil	NT
	2-day old	200,000	Nil	+(upto 72 hours)	NT	+

NT = not tested

Isolation of dengue virus from an outbreak of fever in a small town in West Bengal

There was an outbreak of dengue-like fever in Asansol, a small town at a distance of about 100 miles from Calcutta. Seventy-five samples of blood from acute and subacute cases were brought in wet ice by train. By inoculating the sera in 2-day old mice, 10 strains of virus, apparently dengue, have been isolated. The strains are being typed.

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

As usual the surveillance for detection and follow up of Kyasanur Forest Disease (KFD) was continued during 1967-68.

The human incidence of KFD during 1967 was restricted to the period from February to May. Blood specimens from 69 human cases and autopsy materials of nine dead monkeys yielded KFD virus during the year 1967.

The incidence of KFD during 1968 has been promptly evidenced by human cases and monkey deaths starting from January 1968. KFD virus has been isolated among 60 human cases and 20 dead and dying monkeys upto middle of March 1968.

KFD involvement during 1968 has been extensive though essentially in the old known area. Apparently certain localities which did not report in the previous years are involved during this period. It is likely, at this rate, 1968 may record the highest incidence so far.

During 1968 insecticidal spraying of forest at the sites of dead monkeys has been taken up as an experiment to study its effect on control of ticks.

(D. P. Narasimha Murthy)

REPORT FROM THE VIRUS RESEARCH CENTRE, POONA, INDIA

1. Susceptibility of Cell Lines from Aedes albopictus and Aedes aegypti Larvae to Infection with various Viruses

Cell lines of Aedes albopictus and Aedes aegypti established in this laboratory (Reference - Number 16, Arbovirus Information Exchange), were tested for their capacity to support the multiplication of various viruses. None of the non-arthropod-borne viruses (Polio I, Coxsackie B.3, Encephalomyocarditis (EMC) and hepatoencephalomyelitis (HEV)) showed any multiplication in either of the cell lines. Of the arboviruses tested, chikungunya and Sindbis viruses of group A, Japanese encephalitis, West Nile, dengue 1, dengue 2, dengue 3 and dengue 4 viruses of group B, Chittoor (Bunyamwera group), Sathuperi (Simbu group), Chandipura (Piry group) and Ganjam (ungrouped) multiplied in Aedes albopictus cell line. Aedes aegypti cell line supported the multiplication of chikungunya, West Nile, and Chandipura viruses only. The known tick-borne viruses like Kyasanur Forest disease, Kaisodi and Dhori failed to multiply in either of the cell lines.

The above observations suggest that Aedes albopictus cell line is more suitable for the multiplication of arboviruses. Also these cell lines appear to be selectively susceptible to infection with mosquito-borne arboviruses.

Cytopathic effect was observed with Japanese encephalitis, West Nile, dengue 1, dengue 2 and dengue 4 viruses in Aedes albopictus cultures only. The details of CPE are being studied and the main features appear to be: (1) formation of large syncytial cell masses; (2) development of multinucleate giant cells, and (3) cytolysis of phagocytosis of small cells. If it is confirmed after studying a larger number of arboviruses, that the CPE is limited to members of mosquito-borne group B arboviruses, this finding may be of help in distinguishing this group of arboviruses from others.

Incidentally, the cell lines were in the following passages:

	Line No.	Passage level
Aedes albopictus	ATC-15	45
	ATC-16	38
	ATC-18	35
Aedes aegypti	ATC-10	45
	ATC-23	22

Establishment of Carrier Cultures of Aedes albopictus Cell Lines Infected with Arboviruses

Aedes albopictus cell cultures were infected with $10^{3.0}$ - $10^{3.5}$ mouse LD_{50} of Japanese encephalitis (P20778 strain), West Nile (G2266 strain) and chikungunya (634029 strain). In the cases of cultures infected with Japanese encephalitis and West Nile viruses cytopathic effect was observed four to five days after the inoculation of the virus and within ten days most of the cells came off the glass surface. When the cells were further incubated the remaining cells multiplied and formed a monolayer. Serial subcultures were made from this and virus was demonstrable in the tissue culture fluid in the subsequent passages. In the first 3-4 passages a diminishing number of cells showing cytopathic effect was observed with the cultures but in the subsequent passages the cells seemed normal in appearance.

With chikungunya virus no cytopathic effect was observed either in the primary infected culture or in the subsequent passages.

List of publications on work done with mosquito cell cultures

1. SINGH, K.R.P. (1967)

   Cell cultures derived from larvae of Aedes albopictus (Skuse) and Aedes aegypti (L). Curr. Sci., 36: 506-508.

2. SINGH, K.R.P. and PAUL, S.D. (1968)

   Multiplication of arboviruses in cell lines from Aedes albopictus and Aedes aegypti. Curr. Sci., 37: 65-67.

3. SINGH, K.R.P. and PAUL, S.D. (1968)

   Susceptibility of Aedes albopictus and Aedes aegypti cell lines to infection with arbo and other viruses (Manuscript ready for publication).

4. PAUL, S.D., SINGH, K.R.P., and BHAT, U.K.M. (1968)

   A study on the cytopathic effect of arboviruses on cultures from Aedes albopictus cell line (Manuscript ready for publication).

5. BHAT, U.K.M. and SINGH, K.R.P. (1968)

   Structure and development of vesicles in larval tissue culture of Aedes aegypti (Manuscript ready for publication).

6. BANERJEE, K. and SINGH, K.R.P.

Establishment of carrier cultures of Aedes albopictus cell line infected with arboviruses (Accepted for publication with Indian J. Med. Res. - June, 1968).

II. Isolation of KFD virus from Rattus blanfordi

The involvement of rodents, mice and shrews in the non-primate cycles of KFD in the natural environment had been suspected in the past because isolations of KFD virus had been made from the spleens of three Rattus rattus wroughtoni, four Rattus blanfordi and three Suncus murinus. Further, sera of some species of rats, mice and shrews collected in areas with evidence of active transmission in man and monkeys and also from some areas without such primate involvement had neutralizing antibodies to KFD virus.

During January-February 1967 many human cases had occurred at Kodakani and a number of monkey deaths had also occurred in the neighboring forest. Many pools of ticks had been found virus positive in the area. KFD virus was isolated from the spleens of four out of 12 Rattus blanfordi trapped there during that period and the ticks collected off these rats were also found to be virus positive. Since this was the first time when multiple isolations from the same species of rat had been made from a small area it was considered necessary to study the matter further. For this purpose 29 sera from Rattus blanfordi were collected during March 1967 from the same area and 17 were found to neutralize KFD virus (13 completely and 4 partially).

During January-February 1968 when KFD virus was being isolated from ticks, monkeys and humans in Halagalale and Uppahalli forests, rodents were trapped and their sera and spleens tested for virus. KFD virus was isolated from sera of two Rattus blanfordi from Halagalale (February 1, 1968; February 3, 1968) and another isolation made from the spleen of another Rattus blanfordi at Uppahalli (January 30, 1968). Therefore, up to date KFD virus has been isolated eleven times from Rattus blanfordi collected in nature. These findings throw further light on the probable role of small mammals in the maintenance of KFD in nature.

III. West Nile Virus in Sagar-Sorab Area of Mysore State

Among the brains of sick mice received on August 25, 1967 from the Assistant Director of Public Health, Virus Diagnostic Laboratory Shimoga, for routine identification of agents isolated from human sera of the KFD area, one was found to contain an agent behaving differently from the KFD

virus. Both by complement fixation and neutralization tests it was identified as West Nile virus. The serum had been collected by a Medical Officer of the Mysore State Public Health Department from a febrile patient at Kaisodi village, Sorah Taluk in Mysore State on April 16, 1967. The original serum which had been stored at the Virus Diagnostic Laboratory, Shimoga, was also received on request and the same strain of the virus was reisolated. As this was the first instance in India of isolation of West Nile virus from a naturally infected human patient, convalescent serum sample was also requested. The convalescent sample collected on November 7, 1967 neutralized the homologous virus.

Blood samples were collected from a representative group of persons in the locality in collaboration with the staff of the Mysore State Public Health Department. Sixteen of the 117 such survey sera collected in December 1967 neutralized West Nile virus (Strain No. G22886 first isolated from mosquitoes in Vellore). West Nile virus had been previously isolated from mosquitoes both at Vellore, South India and Manjri, near Poona. Serological evidence for the prevalence of West Nile had also been detected in many parts of India including the KFD area in Mysore State but no isolation of West Nile from naturally acquired infection had been recorded. However, one West Nile virus isolation had been made from a case of presumed laboratory infection in a staff member of the VRC. Further, a recent case of encephalitis in a child (in Vellore) in which serological evidence of West Nile infection was inferred was reported in the Information Exchange Circular #16. Further, more detailed serological studies in the KFD area are in progress.

REPORT FROM THE C.S.I.R.O. DIVISION OF ANIMAL HEALTH, ANIMAL HEALTH RESEARCH LABORATORY, PARKVILLE, VICTORIA, AUSTRALIA

Preliminary report on outbreak of Ephemeral Fever

An outbreak of Ephemeral Fever commenced in Northern Australia in November 1967 and, since then, has spread over most of Queensland, New South Wales and Northern Victoria. The virus from early cases in the Northern Territory was transmitted to cattle in our laboratory and these cattle subsequently resisted challenge with the 1956 strain of Ephemeral Fever virus.

Attempts to isolate the Ephemeral Fever virus in tissue culture and to confirm the observations of van der Westhuizen (Ond. J. Vet. Res., 34 (1):

1967, 29) are under way in several laboratories in Australia. Some entomological observations are being made on the present outbreak. It is likely that a report will be available for the #18 issue of the "Information Exchange."

(E. L. French and W. A. Snowdon)

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

Ephemeral fever of cattle

A large epizootic has spread through northern and eastern Australia in the summer of 1967-68, and has been studied by several veterinary laboratories in collaboration with this Institute. Studies here have led to the isolation of several virus strains in infant mice. The agents are not yet fully characterized but one strain has been shown to pass APD 450 mμ Millipore filters, is sensitive to deoxycholate and ether, is neutralized by serum from convalescent cattle but not by serum from unaffected animals or by antisera to arboviruses previously isolated in Australia. It is hoped to compare the strains with those isolated from cattle with ephemeral fever in South Africa by van der Westhuizen (Onderstepoort J. vet. Res., 1967, 34: 29).

(R. L. Doherty and H. A. Standfast)

The ease of preparation of 19S (IgM) antibody in rabbits, either by collecting immune serum at about the fifth day or by separation from 7S (IgG) antibody in later bleeds by ultracentrifugation or gel filtration, facilitates the application of this rapid diagnostic HI test.

(E. G. Westaway)

A rapid diagnostic HI test for closely-related group B arboviruses:

Although the HI test is generally regarded as the least specific of the serologic tests available for group B arboviruses, recent results with separated immunoglobulins from rabbit antisera to Kunjin virus (Westaway, E.G., J. Immunol.). In the press (February, 1968) indicated that 19S (IgM) antibody may be a useful diagnostic reagent. This finding has now been considerably extended, proving particularly useful for the "MVE subgroup",

which includes Murray Valley encephalitis (MVE), Japanese B encephalitis, (JBE), St. Louis encephalitis (SLE), West Nile and Kunjin viruses (Westaway, E.G., Amer. J. Epidemiol. 84: 439 (1966)), plus a new Queensland member, Alfuy virus.

Rabbits were bled seven days after intravenous immunization with approximately 10^9 mouse ID_{50} of infected mouse brain pools. The 19S and 7S globulins were separated by ultracentrifugation in sucrose density gradients; non-specific inhibitors remained at the top of the gradient. All rabbits produced 19S and 7S antibodies in seven days. Whereas whole antisera and the 7S fractions were extremely cross reactive in HI tests, all the MVE subgroup viruses were clearly differentiated by the 19S antibodies (Table 1). The observed difference in specificity was not confined to the MVE subgroup (see results with Kokobera virus antibodies). Serial bleeds from a rabbit immunized with MVE virus contained relatively specific 19S antibody from day three to at least day 28, whereas 7S antibody which first appeared on day six was of poor specificity. Similar results were obtained using guinea pig antisera to Kunjin virus.

TABLE 1

CROSS REACTIVITY IN HI TESTS OF IgM AND IgG ANTIBODIES IN RABBIT SERA AT 7 DAYS AFTER IMMUNIZATION WITH GROUP B ARBOVIRUSES

	19S (IgM) antibodies						
Antigen	JBE	MVE	Kunjin	WN	Alfuy	SLE	Kokobera
JBE	160*	80	40	< 20	< 10	40	<10
MVE	20	640	160	20	10	80	10
Kunjin	< 20	160	640	20	10	160	<10
West Nile	<20	160	80	160	<10	40	<10
Alfuy	40	320	80	40	80	160	10
SLE	<20	80	40	< 20	< 10	640	< 10
Kokobera	<20	80	20	< 20	< 10	20	80

	7S (IgG) antibodies						
Antigen	JBE	MVE	Kunjin	WN	Alfuy	SLE	Kokobera
JBE	80	160	40	20	20	80	< 20
MVE	160	5120	1280	160	320	640	80
Kunjin	80	1280	640	160	160	640	80
West Nile	40	1280	320	160	80	640	20
Alfuy	160	10240	640	160	320	1280	80
SLE	40	1280	160	80	80	640	20
Kokobera	20	640	80	80	40	160	20

* HI antibody titre versus 4 units of HA in Microtiter plates, incubated for 1 hour at room temperature.

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

Arbovirus Infections in Chickens

Non-specific inhibitors to group A and group B arbovirus were present in all of 50 chicken sera tested. Inhibitors to group B viruses appeared to increase with age. The titre of non-specific inhibitors was increased by heating at 60° C, 80° C or 100° C. After heating, removal of inhibitors by acetone or kaolin treatment was less successful than for unheated serum. Non-specific inhibitors in serum were separated from antibody by ultracentrifugation, and they appeared in the lipoprotein fraction.

Normal agglutinins to goose erythrocytes were present in all 70 chickens studied. They were unaffected by kaolin treatment, but reduced in titre or removed by acetone treatment. The agglutinins were stable at 60°C for 30 minutes, and entirely destroyed by 10 minutes exposure to 70° C. On ultracentrifugation most of the agglutinins occurred in the 19S fraction, but a small peak was also present in the 7S fraction.

A total of 967 chicken sera collected in Queensland were tested for haemagglutination-inhibition (HI) antibodies. Seventeen were HI positive for group A viruses (Sindbis, Ross River or Getah) and 148 were positive for group B viruses (Stratford, Edge Hill, Kunjin, Kokobera and Murray Valley encephalitis). Neutralization tests have not been completed.

(Y. Chung and P. Spradbrow)

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

New Zealand Arbovirus Survey

Mosquito Catching: Trapping was continued in the Whataroa area during January, February, March and December, 1967. Fowl baited stable traps were used and nine sites were studied. The January-March collections were from a variety of sites, but the December collections came from the Mt. Hercules circuit area, where the largest catches of the year were made, and from three other sites where much smaller catches were made.

As in last season's catches, *Culiseta tonnoiri* was the predominant species

captured, but this year the ratio of C. tonnoiri to Culex pervigilans was even higher (4:1). This may be a reflection of the introduction of the Mt. Hercules catching site, since very few C. pervigilans were captured there.

The total catch for the year was 17,036 mosquitoes, this being made up of 13,045 C. tonnoiri and 3,991 C. pervigilans. The mosquitoes were divided into 251 pools, processed as in previous years, then inoculated i.c. into suckling mice onto primary duck-embryo monolayers for virus isolation. No virus has been isolated so far, but some of the December material has yet to be processed through the duck embryo system.

Serological Testing:

Plaque-reduction neutralization tests were carried out on all plasmas from birds bled in Westland during 1967. All plasmas were tested for antibody against Bebaru and Semliki Forest viruses (see below), some 1966 plasmas being included in this study.

Antibody against Whataroa virus was detected in 67 of the 1,201 plasmas collected in 1967. Although the figures are barely significant, there does seem to be a consistently higher antibody incidence in the summer months (see Table 1).

Table 1

Monthly Incidence of Whataroa Virus Antibody in 1967 Bird Plasmas

Month:	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
Tested:	190	186	-	143	73	103	99	113	66	78	18	132	1201
Positive	19	10	-	3	2	3	5	6	2	4	0	13	67
%	10	5.4	-	2.1	2.7	2.9	5	5.3	3	5.1	-	10	5.6

The average incidence rate of 5.6% for the whole year is lower than that found in previous years. Since the bird netting circuit giving the lowest proportion of positive sera in previous years has not been used, but has been replaced by another circuit near an area where virus isolations have been obtained, it is probable that the low proportion of positives does signify a low level of virus activity in 1967.

Studies on Specificity of Group A Antibody in Bird Plasmas

In order to ascertain the degree of specificity of the Whataroa virus-inhibiting antibody in the bird plasmas collected in Westland, neutralization tests were carried out simultaneously using Whataroa and Bebaru virus with 82 routine bird plasmas collected in 1966, and in a second series using Whataroa and Semliki Forest viruses on 141 routine bird plasmas collected in 1967. There is no other evidence to suggest that either Bebaru or Semliki Forest viruses occur in Westland. The results of these tests are shown in Table 2.

Table 2

Neutralising Substances found against Whataroa, Bebaru and Semliki Viruses in Westland Bird Plasmas

(a) 1966 Plasmas:

Plasmas Tested against Bebaru and Whataroa	Bird species represented	Positive Whataroa only.	Positive Bebaru only.	Positive to both.
82	12	2	35	7

Percentage of all plasmas tested against both viruses which were positive for Bebaru antibody = 35 + 7 = 42/82
= 51%

Percentage of all plasmas tested against both viruses which were positive for Whataroa antibody = 2 + 7 = 9/82
= 11%

(b) 1967 Plasmas:

Plasmas Tested against Semliki and Whataroa	Bird species represented	Positive Semliki only.	Positive Whataroa only.	Positive to both.
141	12	9	18	9

Percentage of all plasmas tested against both viruses which were positive for Semliki antibody = 9 + 9/141
= 12.8%

Percentage of all plasmas tested against both viruses which were positive for Whataroa antibody = 18 + 9/141
= 19.1%

The results shown in the above Table suggest that some of the "Whataroa antibody" recorded in routine screenings of Westland bird plasmas, may be non-specific Group A neutralizing substance. The high incidence of Bebaru "antibody" cannot be explained by suggesting that it is cross-reacting Whataroa antibody since many of the Bebaru "antibody" levels were very high in plasmas which contained no detectable Whataroa antibody. Conversely, not all the Whataroa "antibody" could be non-specific neutralizing substance, since many plasmas contained very high levels of Whataroa antibody in the absence of either Bebaru or Semliki antibody. These studied are continuing.

(J. A. R. Miles, T. Maguire and R. W. Ross)

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

EEE Surveillance in Jamaica

Bird netting, banding, bleeding and releasing continued in the parishes of St. Elizabeth, St. Thomas where the 1962 EEE outbreak occurred, and in Portland. The sera of a migrant bird, *Vireo altiloquus*, captured in St. Elizabeth, demonstrated partial protection against 400 LD 50 of EEE virus in neutralization tests in baby mice. This is the first evidence of serological activity in an animal outside St. Thomas. A total of 319 birds were captured and 72 were recaptured during this period.

A total of 10,083 mosquitoes, with *Aedes taeniorhynchus* (6,161) being the dominant species, were caught in St. Thomas from donkey traps. Processing of 7,833 mosquitoes revealed no virus.

Sentinels

Sentinel mice and horses are being exposed in St. Thomas and Portland, while sentinel hamsters are used in St. Thomas and St. Elizabeth. No virus isolation or serological conversions have yet been achieved.

St. Louis Encephalitis Surveillance in Jamaica

Mosquito collections in the parishes of Clarendon and St. Catherine, SLE endemic areas, amounting to 6,408 with *Deinocerites cancer* and *Culex nigripalpus* the dominant species captured respectively in Clarendon and

St. Catherine, revealed no virus.

Clinical Specimens

Sixty-one human sera were screened by micro-HAI tests during this period. Antigens routinely used were St. Louis, Eastern equine and Venezuelan equine encephalitis, and dengue. Results are given in Table 1.

Table 1

Antigens	# Positives	# Conversions
Dengue	15	2*
SLE	11	1
EEE	0	0
VEE	0	0

*One specimen, CV 681/67, from a clinically diagnosed case of dengue, showed conversion to dengue and SLE.

Serological survey - School children

Sera were collected from children between the ages of three and 14 years in the Clarendon area. Results are given in Table 2.

Table 2

Number of specimens tested	238
Number of specimens positive to SLE only	53
Number of specimens positive to dengue only	13
Number of specimens positive to SLE and dengue	11

A total of 183 of these sera were tested against VEE and EEE, and all were negative.

Ticks - Survey

Fifty-four pools of ticks, predominantly *Boophilus microphilus* and *Amblyoma cajanense*, collected in Clarendon and St. Catherine and inoculated in-

tercranially into suckling mice produce no virus.

Foot Note

Several single specimens collected in Kingston from patients with dengue-like symptoms during November, 1967 showed high HAI titres (320 - 2,560) to SLE and dengue. HAI titres of 1,280 or greater are highly suggestive of recent infections. This was our experience in the 1963 dengue outbreak in Jamaica.

REPORT FROM THE P.A.H.O. INSECTICIDE TESTING UNIT, KINGSTON, JAMAICA

Among the obstacles that had delayed the *Aedes aegypti* Eradication Program in the Caribbean and in the extreme north of South American one of the most important was the resistance of this vector to chlorinated insecticides.

In order to study the resistance problem, P.A.H.O. established in Kingston, in 1962, in collaboration with the Government of Jamaica and the University of the West Indies, an Insecticide Testing Unit to investigate the susceptibility of strains of *A. aegypti* from that area to various insecticides and to evaluate new products that might be substituted for chlorinated insecticides in the eradication of the mosquito.

The Unit tested the susceptibility to chlorinated insecticides of *A. aegypti* from 66 localities in 18 countries and territories of the above mentioned areas, the results of these tests showing that the strains of the mosquito in those areas are resistant to DDT or dieldrin or both.

At the same time, the Unit investigated the susceptibility of several of these strains to certain insecticides that might possibly replace the chlorinated ones and assessed the residual action of these products in various types of receptables in which the *A. aegypti* breeding places in the Caribbean area and in South America are usually found.

In July 1966, a program was begun of field testing candidate insecticides and methods in villages in Jamaica. The candidate insecticides were chosen on the basis of results from previous laboratory work of the Unit. By the end of 1967, 11 tests had been run using abate, bromophos and fenthion with perifocal and intradomiciliary techniques. In addition, abate as 1% sand granules was used in drinking water in a number of tests. In the

small villages where the tests were carried out the intradomiciliary technique proved superior and abate and fenthion used in this manner proved the most effective.

A cholinosterase testing program is being carried out as part of the insecticide testing program to check the safety of the candidate insecticides. The spraymen are tested periodically and a sample of the villages in the test area is examined before the application of the insecticide. Toxicology problems were encountered with fenthion.

A program has been initiated for the monitoring of the insecticide susceptibility levels of Aedes aegypti in the Caribbean Area. The purpose of this program is to locate developing resistance at an early stage. The program consists of determining the baseline values for the species in the area, and then comparing with current values, periodically. Most of the baseline data has been obtained.

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

Bush Bush

Despite an apparent recovery of the rodent population and an increase in the number of Culex portesi caught and tested, no viruses were recovered from this area during 1967.

Turure Forest

Numerous isolates were made from sentinel mice and mosquitoes from this area. VEE, Caraparu, Restan, Oriboca, Guama, Bimiti and Catu were encountered frequently during the rainy season. It appeared that the field laboratory which was built on Turure Road was located in that area of the Forest in which virus activity was most pronounced. Studies on the ecology of Culex portesi were pursued actively during the year. The colonization of this species under totally artificial conditions has now been achieved and transmission studies are in progress.

Cumaca Studies

The Group B agent mentioned in our last report as having been isolated from a Marmosa mitis appeared on further study to be a Dengue type 1 strain and is now regarded as the result of a laboratory contamination.

Cedros

During the second half of 1967 a Guama strain was isolated from Culex portesi caught in the area and evidence of Group C virus activity was discovered in sentinel rodents.

In January operations in this area were discontinued. Next rainy season we hope to investigate the south-east corner of Trinidad.

Dr. Spence left the directorship of TRVL in January in order to take up the post of professor of Virology at McGill University, Montreal.

(L. Spence, A. H. Jonkers, E. S. Tikasingh, J. B. Davies and D. Bassett)

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

The Arbo-virus investigation program in 1967 was devoted to the isolation of viruses from mosquitoes. Nearly all the mosquitoes were caught by hand suction on human bait. Some catches with sentinel animals have been included but these are only a small portion in comparison with the catches on human bait.

The captured mosquitoes represent 49 species derived from 12 different places. The bulk of the mosquito catches comes from Leiding 16a, a place selected to investigate ecological circumstances.

Transport difficulties limited the investigation of reservoir animals but this program in still in progress.

From a batch of 22 Psorophora ferox caught on human bait at Leiding 16a one virus was isolated. This virus proved to be ether sensitive and according to the C. F. reaction it belongs to the Arbo group A viruses. It has been sent to the Trinidad Regional Virus Laboratory for further determination.

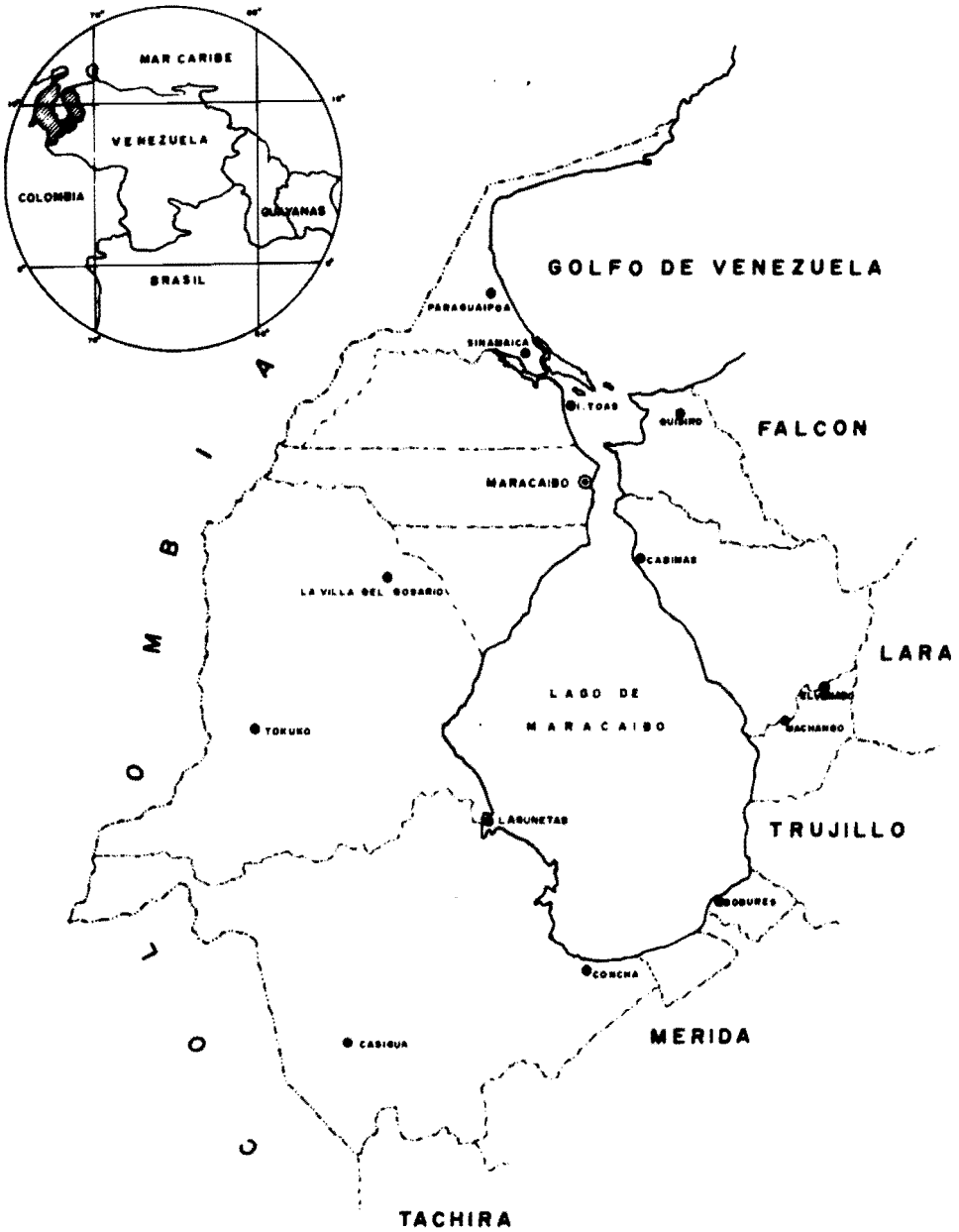
MOSQUITO CATCHES ON HUMAN BAIT	TOTAL NUMBER COLLECTED	NUMBER OF POOLS
Aedes species	31	3
serratus	4550	97
taenorrhynchus	249	10
scapularis	972	25
fulvus	694	13
angustivitatus	54	4
terrens	-	-
aegypti	90	2
Aedomyia squamipenni	1	1
Anopheles species	3	2
aquasalis	743	20
apicimacula	1253	10
Culex species	38	7
I	335	10
II	146	4
III	18	6
IV	156	11
V	50	6
VI	1	1
VII	14	1
VIII	627	10
IX	-	-
X	53	3
XI	-	-
XII	7	1
fatigans	798	15
portesi	882	16
spissipes	9	3
microculex	10	1
Haemagogus species	9	1
capricorni	64	5
Limattus species	16	3
flavisetosus	6	2
durhami	5	2
Mansonia titillans	5768	141
Venezuelensis	371	29
pseudo titillans	5	1
Psorophora species	20	3
ferox	2257	63
medio albipes	1985	55
Sabethes chloropterus	4	1
Trichoprosopon species	6	2
longipes	20	2
leucopus	4	1
Weyomyia species	831	32
medio albipes	510	13
TOTAL	23685	638

MOSQUITO CATCHES WITH SENTINELS

	Sentinel rats		sentinel monkeys		sentinel mice	
	Number collected	Number of pools	Number collected	Number of pools	Number collec-ted	Number of pools
Aedomyia squamipenni	15	2	1	1	27	2
Aedes species	36	2			11	1
serratus	101	6	14	1	206	7
taenorrhynchus	42	2			22	2
scapularis	19	3	1	1	32	2
ful fulvus	26	3			27	2
Anopheles Aquasalis	43	2	1	1	33	2
apicimacula	27	2	4	1	10	1
Culex species	16	2			4	1
I	86	3			86	5
II	283	6			229	9
III	103	3			63	4
IV	147	4			224	8
V	12	4			6	2
VI	16	4			6	1
VII	18	3			1	1
VIII	200	6			94	3
IX	7	1			-	-
X	4	2			2	1
XI	10	2			1	1
XII	18	2			11	1
XIII	3	1			-	-
XIV	8	1			-	-
fatigans	836	15			1463	29
portesi	32	3			6	1
spissipes	5	2			8	1
microculex	36	4			28	3
(culex) species	-	-			6	1
Limatus species	1				-	-
durhami	-	-	2	1	5	1
flavisetosus	1	1			1	1
Mansonia titillans	374	12	106	4	532	17
pseudo titillans	6	2	1	1	-	-
venezuelensis	-	-	-	-	4	1
Orthopodomyia species	-	-	-	-	2	1
Psorophora species	-	-			1	1
ferox	94	3	2	1	8	2
medio albipes	82	2	44	2	52	4
Sabethes chloropterus	1	1			2	1
cyaneus	-	-			2	1
Trichoprosopon longipes	1	1			1	1
Weyomyia species	12	3			22	5
medio albipes	1	1			3	1
	2722	117	176	14	3241	128

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

Serological survey of the Estado Zulia has been conducted during 1967. Seven hundred ninety-four human sera from 14 towns were collected. Specimens were tested for VEE HI antibodies.



Paraguaipoa, Sinamaica and Isla de Toas were affected by a severe epidemic of VEE in 1962. Five years later we found a 33, 44 and 20% of immunes respectively. Since the first town has a migratory population, percentage seems to be greater which is in accordance with the epidemiological data of the zone. No positive cases were encountered under five years of age.

No VEE virus activity has been reported before from Concha. In the present study we found 29% of immunes, the youngest being a 10-year-old girl. Lagunetas, Machango, El Venado and Bobures seem to have had VEE virus activity in the past since the positive cases are over 20 years of age. Besides El Venado has two positives ages 10 and 13, and Bobures had four positives under 5 years, which may represent recent activity. A 50 year old male was the only positive in Maracaibo. La Villa del Rosairo and Cabimas have 17 and 11% of immunes, all ages affected. (Table 1)

From 129 positives, 57 had titers of 1:40 or less, and those might be due to a drop in the titers (noted in the 1962 epidemic) or to the presence of a virus closely related to VEE. No differences in sex distribution were noted.

Neutralization tests are in progress.

AGES	Para-guaipoa *	Para-guaipoa +	Sina-maica		Isla de Toas		Mara-caibo		La Vi-lla del Rosario		Tokuko		Lagunetas		Casigua		Concha		Bobures		Machango		El Venado		Cabimas		Quisiro	
0 - 5	0/16	0	0/17	0	0/10	0	0/11	0	0/3	0	0/12	0	0/13	0	0/9	0	0/6	0	4/12	33	0/11	0	0/13	0	1/10	10	0/5	0
6 - 15	2/10	20	4/21	19	2/20	10	0/20	0	2/18	11	0/20	0	0/16	0	2/20	10	4/24	17	0/18	0	0/20	0	2/17	12	1/19	5	0/12	0
16 +	16/28	57	25/38	66	10/30	33	1/36	3	6/27	22	1/23	4	5/26	19	4/30	13	10/19	53	7/30	23	9/29	31	6/30	20	4/28	14	1/27	4
TOTALE	18/54	33	29/66	44	12/60	20	1/67	1	8/48	17	1/55	2	5/55	9	6/59	10	14/49	29	11/60	18	9/60	15	8/60	13	6/57	11	1/44	2

* POSITIVES/TOTAL.

\+ PERCENT OF POSITIVES.

REPORT FROM THE GORGAS MEMORIAL LABORATORY, PANAMA

Arbovirus Surveillance Activity, 1967

During the year 1967 our arbovirus work was conducted in several study areas under various research projects. Field collections were concentrated at both ends of the Republic (Almirante in the Northwest and Darien in the Southeast). Additional work in other areas was performed unsystemattically, as time permitted.Therefore, negative findings in these latter areas could not be interpreted as there being no virus transmission involved. Results of virus isolation attempts and the detection of virus transmission by sentinel animals performed during the year for the various localities are summarized as follows:

1. Almirante: In cooperation with P. Galindo, as part of the rodent-insect arbovirus cycles study in its third year program, 75 virus isolates were obtained from all sources, i.e. 1 isolate from a human fever case [VEE (1)]; 37 of 172 families of sentinel mice exposed [Ossa (2), Madrid (1), Guama (2), unidentified (32)]; 12 of the 39 sentinel hamster tissues processed [VEE (2), Ossa (2), Madrid, (2), unidentified (6)]; 22 of 532 mosquito pools inoculated [Ossa (2), Nepuyo (1), Guama (11), unidentified (8)]; and 3 of 9 tissue suspensions of hamsters bitten by wild-caught C. vomerifer mosquitoes brought into the insectary [Ossa (2), unidentified (1)]. In addition, two virus infections [Ossa (1), Guama (1)] were detected in 14 surviving sentinel hamsters, and two others [Patois (1), Ilheus (1)] from 40 surviving hamsters which were bitten by wild-caught mosquitoes.

2. Bejuco: A preliminary experiment using two sentinel hamsters and eight sentinel chickens in the Bejuco area, 50 miles west of Panama city, during the month of September showed negative results.

3. Albrook, Canal Zone: Eighteen families of sentinal mice and 17 sentinel hamsters were exposed at the Tropic Test Center's tower near Albrook air force base in the Canal Zone. No virus transmissions were detected.

4. Juan Mina: In an area on the Chagres river, 20 miles north of Panama city, a field project was initiated during the last half of the year. Two human infections with VEE virus were detected from six seronegative cases which had been bled a year earlier. No virus isolations were obtained from three families of sentinel mice, 16 sentinel hamsters, 15 sentinel chickens and 30 wild animals.

5. Province of Colon: One VEE virus was isolated from a fever case reported to the laboratory in September, 1967.

6. Sambu: Sentinel hamsters were placed by Dr. Ralph Comer in the Sambu area, where malaria drug therapy was being investigated. This region is a few miles south of the new proposed interoceanic sealevel canal route 17, 120 miles southeast of Panama city. Two Guama infections were demonstrated from 30 hamsters exposed during the year.

7. Darien: Extensive research projects of overall disease surveillance and medico-ecological studies, under the supervision of Drs. Martin D. Young and Graham B. Fairchild were established along the new proposed interoceanic canal route 17 in Darien and San Blas provinces, approximately 120 miles southeast of the present Panama canal. U.S. Army personnel from office of the Atlantic-Pacific Interoceanic Canal Studies were responsible for field collections and sentinel animals. During the first half of the year, field activity was concentrated along the Pacific of the proposed new canal route. Only the results of arbovirus work are reported here. In that period virus transmission was almost undetectable. However, numerous isolates were obtained during the second half of the year from areas near the Caribbean coast. A total of 16 virus strains were isolated on this route. One isolate of Ossa virus was obtained from a human fever case and another from 77 families of sentinel mice. Eleven isolates [VEE (4), Ossa (2), Guama (1), unidentified (4)] resulted from 29 sentinel hamsters tissues processed, and three virus strains [VEE (1), Nepuyo (1), unidentified (1)] were obtained from wild animals. Another unidentified isolate was obtained from 210 pools of mosquitoes processed by Dr. Grayson. In addition, virus transmissions were detected from sentinels as follows: three significant rises in HI titers for group B virus from 323 human serial bleeding samples tested; 1 Guama virus from 31 survived hamsters exposed; and seven infections in sentinel monkeys [VSV-Ind. (4), Mayaro (2), Changuinola (1)].

8. Upper Colombia: Parallel to the study in Darien, surveys on proposed sealevel canal route 25 in Upper Colombia were also conducted. Two human infections with VEE virus were diagnosed. Eight isolates [VEE (3), Madrid (1), unidentified (4)] were obtained from 23 sentinel hamster tissue suspensions, and three other unidentified strains were isolated from wild animals.

Results shown above indicated that arboviruses were highly endemic in both border provinces of Panama and Upper Colombia in 1967.

(Sunthorn Srihongse)

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

Mosquito collections were made in New Caledonia during January and February, 1968 as part of an attempt to isolate an arbovirus whose ecology could be studied in the simplified environment of a Pacific island. In all, more than 19,000 mosquitoes were obtained and preserved in liquid nitrogen. The distribution of species was as follows:

Culex annulirostris	105
Culex sitiens	6218
C. sitiens or *C. annulirostris*	420
Culex fatigans	3112
Aedes vigilax	6671
Aedes notoscriptus	450
Coquillettidia xanthogaster	2409
Others	34

It will be noted that *Culex annulirostris*, which is of special interest because of its role as a vector of arboviruses in neighboring Australia, was relatively rare but that the related *C. sitiens* was abundant. Mosquitoes were collected with human and animal bait, in animal shelters, and with chicken - and CO_2 - baited traps.

Sera from domestic animals and birds were also collected to supplement the extensive collection of human sera made previously. It is planned to test these sera (and others from Pacific Islands) for antibodies against arboviruses previously isolated in Australia, New Zealand, and southeast Asia.

Mosquito material will be tested in both suckling mice and cell culture systems.

Further work is also planned on the material from the 1964-65 Dengue outbreak in Tahiti - with emphasis on the use of the plaque system for virus isolation.

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

The following is a brief summary of the Hooper Foundation Arbovirus Research Unit Annual Report for 1967. Copies of the report are available to those interested.

Dengue in Malaysia

The last recorded dengue outbreak in Malaysia was the hemorrhagic fever epidemic in Penang in 1962, caused by dengue 2 virus. Previous to that there was a small, localized outbreak in a Kuala Lumpur girl's school in 1954. This epidemic was caused by dengue 1 and was a mild febrile illness with no deaths.

The most recent dengue epidemic in Malaya began during the latter part of 1966 and reached its peak in August and September 1967. The majority of laboratory confirmed cases came from the main urban centers of Kuala Lumpur and Penang, but a few came from other parts of the peninsula and one from Sarawak in East Malaysia. Between October 1966 and December 1967, 252 cases were confirmed either serologically or by virus isolation. Almost all the specimens received for laboratory diagnosis came from hospitalized patients. However, conversations with private physicians indicated that many cases diagnosed clinically as dengue were seen as out patients. The few blood samples submitted by private physicians were all serologically dengue positive.

Comparison of the recent dengue epidemic with the dengue hemorrhagic fever epidemic of 1962 in Penang reveals some striking differences. Clinically the 1967 epidemic was mild; very few cases showed any hemorrhagic signs and there were no deaths. In 1962 a high percentage of the cases showed severe hemorrhagic signs and the death rate of hospitalized cases was 8.2%.

The 1967 epidemic primarily involved young adults compared to 1962 when young children made up the bulk of the hospitalized cases. The sex ratio of cases was almost exactly the reverse in 1967, compared to 1962 - 63% males and 37% females compared to 38% males and 62% females.

In 1967 there were fewer cases among Chinese (53%) and more among Indians (23%) and Malays (22%) compared to the general census. In 1962 there were more cases among Chinese (80%) and fewer among Indians (10%) and Malays (8%) compared to the general census.

In 1967 the majority of the cases were in the outer urban and suburban areas of the cities. In 1962 the majority of cases were clustered in the crowded poorer central districts of the city where the Chinese are concentrated.

The serology of the 1967 cases did not differ significantly from that of the 1962 cases. The majority of cases in both epidemics responded with a broad, secondary type antibody response against group B arbovirus antigens, indicating previous experience with related group B viruses. Specific, primary antibody responses were seen mostly in children and in foreigners. This is the expected picture because of the wide prevalence of a number of group B arboviruses in Malaysia.

Over 50 strains of dengue virus have been isolated from the acute phase sera of patients. The only strain identified thus far is one (P6-1359) isolated from an acute phase serum sample collected in November 1966. It is a dengue type 1.

Mosquito collections made in Penang during the 1962 hemorrhagic fever epidemic showed that there were high populations of Aedes aegypti in the congested areas of the city from which most of the cases came. In contrast, Ae. albopictus occurred in considerably lower numbers.

During the 1967 epidemic of mild dengue, a larval survey was conducted in both urban and suburban sections of Kuala Lumpur, Petaling Jaya, Penang (Georgetown) and Malacca. Suburban collections were made in areas from which dengue cases occurred. In all instances the Ae. albopictus larval index was higher than that of Ae. aegypti in the suburban areas. The reverse was generally the case in the town centers.

Japanese Encephalitis in Malaysia

Over a 15 month period (April 1966 - June 1967) an extensive survey was made of evening biting mosquitoes coming to domestic animal shelters in rural Malaya. Collections utilizing Malaise traps and various battery operated suction devices were made from 27 areas scattered throughout peninsular Malaya. Although collections were not made in every month, the data indicate that large populations of Culex gelidus and mosquitoes of the C. vishnui group occur throughout the year. The most numerous species collected were Culex gelidus (43, 785), C. vishnui group (28, 047) and C. fuscocephalus (4, 150). Six other Culex species were represented by relatively few individuals.

Inoculation of these mosquitoes into suckling mice in pools of up to 400 per pool (but generally 300 or less) has resulted in the isolation of 33 virus

strains. Among them are 15 strains of JEV and two strains of Tembusu. The remainder are still in the process of identification, but most appear to be JE virus. Virus isolations were made from every state except Trengganu and Penang. At least one isolate from each of seven of the 11 states has been identified as JEV, demonstrating its peninsula-wide distribution.

Evidence for the prevalence of human JE infection comes from routine diagnostic tests on cases of encephalitis. Thirty-nine cases from seven of the 11 peninsular states and Sarawak in East Malaysia were serologically confirmed in 1967. At least one case occurred in each month of the year except January and February, but there were several cases in January and February 1966. These figures represent the minimum number of cases since only those referred to our laboratory are included. The age range of cases in indigenous people is from less than one year to 20 years of age. Over half the cases were in children 6 years of age or younger; 84% were in the 12 years or younger age group.

Philippine Hemorrhagic Fever Epidemic of 1966

This study by Dr. Venzon in our San Francisco laboratory was described in our 1966 annual report in which the results of HAI tests on serum samples from 399 cases were reported.

Serum samples from 243 patients collected within four or five days of onset of illness have been inoculated into one and 2-day old suckling mice for attempted virus isolation. Using the dengue-challenge technique and serial blind passage, 15 strains of dengue virus have been isolated and adapted to suckling mice. An additional 47 presumptive isolates (on the basis of dengue challenge resistance of surviving mice) are currently being processed. A tissue culture laboratory is now being established in our San Francisco laboratory (also one in the Malayan lab) and most of these presumptive isolates will be studied further in cell cultures.

In the meantime six of the isolates have been presumptively identified by cross neutralization tests in mice; five are similar or identical to dengue 4 and one appears to be similar but not identical to dengue 2.

Sentinel Animal Program

Sentinel animals (monkeys and chickens) were maintained for several months at a series of stations from 230 M to 1200 M elevation in undisturbed primary lowland and hill dipterocarp forests on Gunong Benom in central Malaya. Due to the isolation of this area and its distance from the laboratory at Kuala Lumpur, this study was conducted as a series of three

expeditions of a month or more each in February - March, May and July, 1967. Sentinels were stationed at various levels to 30 M above ground (into the mid-canopy). Each animal was bled three times weekly for virus isolation and at monthly intervals for serology. Sentinels were exposed for approximately 1,000 sentinel animal days.

None of the sentinels converted serologically. The large number of blood samples collected are still being processed for virus isolation, but it does not appear likely that any of them contracted a group B arbovirus infection. However, a number of wild leaf monkeys (Presbytis obscurus) collected from this area all contained high titer dengue and/or Zika antibody indicating that both these viruses have been active in the area. A virus (as yet unidentified) was isolated from Aedes (Finlaya) formosensis, the main primate-biting Aedes species at ground level in the area.

Tropical Canine Pancytopenia

In 1967 we were requested to assist in the study of an outbreak of a previously undescribed, fatal illness among Alsatian and other pure breed dogs in Singapore and Malaysia. This disease has been designated as Tropical Canine Pancytopenia from its most prominent manifestation. It appears to be caused by a virus and has been experimentally transmitted to beagles and ferrets at the Animal Health Trust, Small Animal Center, Newmarket, England. The dog population affected is continously exposed to heavy attack by local dog ticks, Rhipicephalus sanquineus, and we were asked to determine if they might be transmitting the infectious agent. Attempts to isolate viruses from these ticks have been unsuccessful so far. Serum samples from a number of normal and infected Alsatian dogs also were tested for complement fixing and neutralizing antibody against Seletar virus. This virus was previously isolated in our laboratory from cattle ticks, Boophilus microplus, collected in Singapore and Kuala Lumpur. It is very closely related to Wad Medani virus which was originally isolated from Rhipicephalus sanquineus ticks in Egypt. No CF or neutralizing antibody to this virus was detected in any of the dog sera. An attempt to produce infection with Seletar virus in mongrel dogs was not successful. One attempt to produce experimental infection in Malaysian cattle gave equivocal results.

Mosquito Bionomics

Comparative studies of the use of small bait traps with monkeys, chickens and other animal baits with and without dry ice were completed. One of the purposes of this study was to test ways and means of increasing the exposure of sentinel animals to mosquito attack, thus increasing their sen-

sitivity for the detection of mosquito-borne viruses.

Two species of monkeys, Macaca nemestrina and M. irus, and domestic chickens are used as the basic sentinel animals; the monkeys because of their apparent involvement in natural dengue infection and the domestic chicken as a general indicator of other arbovirus activity. The effectiveness of these or any other sentinel animals had not been tested previously in the Malaysian environment and it seemed desirable to know the extent to which sentinels were attractive to and being fed on by mosquitoes in various habitats.

In a series of carefully controlled experiments using small magoon-type bait traps, the effectiveness of CO_2 in attracting mosquitoes into the traps and increasing engorgement rate was studied in each of three areas -- freshwater swamp forest, mangrove swamp forest and lowland dipterocarp forest. Four bait traps of identical construction were used in each area; one was baited with a monkey or a chicken, one with CO_2 only (dispensed into the trap from an insulated box containing dry ice), one with monkey (or chicken) and CO_2, and one unbaited with either animal or CO_2. The traps were placed some distance apart, so they would not exert any influence on each other. Each trap was operated for a period of 22 hours each day and they were rotated so that each spent equal time at each location. The bait was also rotated so that every trap contained each type of bait (and no bait) in each location for an equal period of time. Several repetitions of this experiment in each area were made over a period of months. The mosquitoes were collected daily, identified to species, counted and their state of engorgement determined.

CO_2 alone was effective in attracting some mosquitoes into the trap, particularly in areas with dense populations. When used in combination with animal bait, CO_2 markedly increased the number of mosquitoes attracted to monkey-baited traps and produced a synergistic effect when used in chicken-baited traps.

Chickens consistently allowed a higher engorgement rate than did monkeys for the majority of mosquito species entering the traps either with or without CO_2 . The engorgement rate of mosquitoes entering the traps baited with monkeys alone or with monkeys and CO_2 varied considerably depending upon the particular individual used as bait. Certain individuals allowed mosquitoes to feed readily, while others actively prevented their engorgement. Further study of this phenomenon showed that it was not related to sex or species of monkey used. Observations indicated that some monkeys actively prevented mosquitoes from feeding by continously shifting the body and extremities, brushing, slapping or catching the mosquitoes with their

forefeet. They detect the mosquitoes visually and possibly by sound, but principally by the annoyance created when the mosquito alights and starts to probe with its mouthparts. This was demonstrated conclusively by the following series of experiments:

Monkeys treated with a corticosteroid to reduce inflammatory reactions due to repeated mosquito bites was not effective in increasing the engorgement rate on monkeys. However, when monkeys were sedated with a central nervous system depressant (Sernylan), the engorgement rate increased to a high level.

As a practical solution to the sentinel monkey problem, however, complete sedation leaves a great deal to be desired. The effect of blocking the sensory nerves to an extremity was then tested. Nerve blockage of a monkey's tail with xylocaine was quite effective in increasing the engorgement rate of mosquitoes coming into the bait trap even in those baited with monkeys which normally would not allow mosquitoes to feed on them. Unfortunately, xylocaine is effective for only a few hours at most and chemical blockage of the sensory nerves to the tail is not a practical procedure for sentinel monkeys. Sectioning all nervous pathways to the tail might solve the problem and is now being tested in the field.

Serology

HAI tests have been done on sera from a number of bats, mud skippers and monitor lizards not previously reported. About one third of the monitor lizards tested had low-titered dengue 3 antibody in the complete absence of antibody to dengue 1, 2, 4, Zika, Tembusu, JEV, Langat, Bunyamwera, Sindbis, Getah and Chikungunya. These have not yet been tested for presence of neutralizing antibody.

Over half the mud skippers (*Periophthalmus* spp.) had HAI antibody to group B arboviruses. It was generally low-titered and reacted only with dengue 2, dengue 3 and Langat antigens. The serum of one mud skipper had an HAI titer of 1/320 to dengue 2 and it neutralized greater than 2.3 logs of dengue 2 virus. Neutralization tests on 11 other HAI positive sera were negative.

Broad group B arbovirus HAI antibody was found in a variety of bats including the giant flying fox, *Pteropus vampyrus*. Neutralization tests have not been completed yet, but dengue 2, JEV and Tembusu neutralizing antibodies have been detected in some of the sera. Neutralizing antibody to Tembusu virus has also been demonstrated in six species of wild rodents, most of which were collected in rural areas in close proximity to

man and domestic animals.

Langat virus neutralizing antibodies were detected in the sera of Callosciurus notatus and Tupai glis.

Experimental

Experiments on rapid identification of dengue virus types are in a very preliminary stage but some promising results have been obtained. Dengue virus P6-1359, identified by standard cross-neutralization tests of mouse-adapted virus (ninth passage level) as type 1, was used in this preliminary experiment. At the third passage level, infective mouse brain material produced illness and a few deaths in 1 and two day old suckling mice and nearly complete resistance of survivors to dengue 2 challenge. This third passage material was diluted to 10^{-2} and 0.3 ml dispensed into each of four tubes, labeled A, B, C, and D. To tube A was added 0.3 ml of a dengue 1 hyperimmune mouse serum, to tube B was added dengue 2 serum, to tube C was added dengue 3 serum and to tube D was added dengue 4 serum. The hyper-immune sera were used undiluted and were heat inactivated at 56° for 30 minutes. Two litters of 2-day old suckling mice were inoculated (IC & IP) immediately with each mixture.

Over the 21-day observation period, none of the group A mice became ill and all subsequently died after challenge with 100 LD_{50} of dengue 2 virus. The other three groups reacted as if no hyper-immune serum had been added. Most became sick and over half of each group died. Almost all of the survivors resisted dengue 2 challenge.

On the basis of this preliminary experiment, P6-1359 is dengue type 1. This experiment is currently being repeated and amplified with original and first passage mouse brain material of recent dengue 4 and dengue 2 isolates from the acute phase serum of Philippine hemorrhagic fever patients.

Viruses Isolated in Malaysia

Recent virus isolations made from material collected in Malaysia are shown below. All but one of these isolations were made in our Kuala Lumpur laboratory; the other isolation (from Scotophilus temmenckii) was made in San Francisco from material sent from Malaysia.

Mosquitoes	
Aedes aegypti	Zika virus (1); unidentified (3)
Ae. mediolineatus	JEV (1)
Ae. pseudoalbopictus	unidentified (1)
Ae. (Finlaya) formosensis	unidentified (1)
Anopheles spp.	JEV (1)
Culex annulus	unidentified (1)
C. fuscocephalus	JEV (2)
C. gelidus	JEV (5); Tembusu (1); unidentified (14)
C. vishnui group	JEV (3); Tembusu (1); unidentified (4)
Mansonia uniformis	unidentified (2)
Ticks	
Dermacentor auratus	unidentified (1)
Haemaphysalis nadchatrami	Lanjan virus (1)
Haem. semermis	Lanjan virus (2)
Ixodes granulatus	unidentified (1)
Argas puisilus	unidentified (2)
Wild Vertebrates	
Scotophilus temmenckii (bat)	unidentified (1)
Periophthalmus sp. (mud skipper fish)	unidentified (1)
Pig	Pseudorabies virus (1)
Sentinel Monkeys	unidentified (7)
Humans	dengue 1 (3); JEV (1); unidentified (53)

The strain of Zika virus isolated from Aedes aegypti collected in a small town in central Malaya represents the first isolation of this virus outside of Africa and the first from any mosquito except Aedes africanus.

The pseudorabies virus was isolated by Mr. Cheong Sue Kheng, Director of the Veterinary Research Institute at Ipoh and referred to us for identification. We in turn referred it to Dr. J. Howarth, University of California, Davis, who made the specific identification.

The Scotophilus temmenckii isolate is the first reported virus isolation from a bat in Malaysia. It appears to be identical to the two virus strains isolated from Argas puisilus ticks, which parasitize this species of bat.

Some of the isolates from sentinel monkeys may not be arboviruses, but none have been identified yet.

Most of the 53 unidentified human virus strains were isolated from patients during the dengue epidemic and are probably dengue virus.

(Nyven J. Marchette, Elene Dukellis, and Eulalia Venzon* (San Francisco)

(Albert Rudnick, Richard Garcia and Duncan MacVean (Kuala Lumpur, Malaysia)

*N.I.H. Postdoctoral Research Fellow from Manila

REPORT FROM THE CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH, AND THE ROCKEFELLER FOUNDATION ARBOVIRUS STUDY UNIT, BERKELEY, CALIFORNIA

There were four confirmed and two presumptive cases of Western encephalitis in man in California during 1967. These cases occurred during July, August and September. There were 148 cases of encephalitis reported in horses in 1967 of which 31 were confirmed as Western encephalitis by serological studies and one by isolation of Western encephalitis virus from the brain. There was one case in June, 9 in July, 17 in August and 4 in September. Western encephalitis virus was isolated from the brains of two gray tree squirrels which died in Butte County 8/7/67 and 9/23/67, a Citellus beecheyi ground squirrel which died in Yolo County 8/2/67 and an unidentified squirrel which died near Lake Mendocino 7/16/67.

There were eight confirmed cases of St. Louis encephalitis in man in California during 1967. There were two cases in August, three in September

and three in October. There were two cases of St. Louis encephalitis in horses during 1967 confirmed by serological studies. Both occurred in September.

There were 21 cases of Colorado tick fever in 1967, all confirmed by isolation of the virus from the blood; a specific diagnosis was made on each case by examination of the blood smears by the FA technique.

Rio Bravo virus was isolated from the salivary gland tissue of a Tadarida bat collected in Berkeley 1/6/67. Three other isolations of Rio Bravo virus were made from salivary gland-lung-kidney pools of Tadarida bats, one from Berkeley 3/22/67 and two from Sacramento 4/6/67.

A strain of Modoc virus was isolated during 1967 from a salivary gland-lung-kidney pool of three young Peromyscus maniculatus mice trapped 6/25/65 in the Sierra Mountains of Mono County at an elevation of 9, 600 feet ASL.

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

In the March, 1967 issue of The Information Exchange, we summarized our arbovirus surveillance project for the period covering January, 1961 through June of 1966, and reported some preliminary findings from the fall of 1966. We have subsequently completed the identification of the 12 suspected virus isolates from mosquitoes which were collected in August and September of 1966. Nine of the isolates were identified as strains of SLE virus and the other three as WE-like viruses. This represented our first direct evidence of WE virus activity in the Tucson area since the beginning of the project in 1961.

During 1967 we were unable to undertake any additional virus isolation studies. However, serological studies with a limited number of sentinel chickens suggested that there was no SLE or WE virus activity in the late summer and early fall of 1967 in the Tucson area.

REPORT FROM THE ROCKY MOUNTAIN LABORATORY, U.S.P.H.S., HAMILTON, MONTANA

Attempts to isolate WEE virus from frogs

Studies to determine the role cold-blooded vertebrates may play in the ecology of WEE virus have been continued. A total of approximately 160 frogs collected in Saskatchewan, Canada, and the states of Montana, Idaho, Washington, and North Dakota have been tested one or more times for WEE virus the past year. "Wet" chicks were used in attempting to isolate virus. WEE virus was not isolated from any of the frogs. Additional field and laboratory studies are planned for the coming season.

(L. A. Thomas)

WEE and SLE HAI antibody survey in human sera from Oregon

A study to determine the yearly antibody conversion rate in the human population in the Vale, Oregon, encephalitis study area was inititated in 1967 and continued in 1968. Of the 212 sera collected in the spring of 1967, 20 (9%) were positive for WEE antibodies and 8 (4%) were positive for SLE antibodies. Of 153 sera collected in the winter of 1967, 10 (6%) were positive for WEE antibodies and 12 (8%) were positive for SLE antibodies. Paired sera were obtained from 147 individuals. It appears that within the past year one individual developed WEE antibodies and four developed SLE antibodies. The lower rates of WEE antibody in 1968 and the higher rate for SLE were associated with corresponding changes in mosquito isolation rates and the conversion ratio of 4:1 (SLE:WEE) is comparable to the isolation ratio (summer 1967) of 3.5:1. Selected sera will be tested both for CF and neutralizing antibodies.

(L. A. Thomas and W. A. Rush)

Mosquito-borne encephalitis

Findings at other laboratories suggested nestling birds as possible virus reservoirs, and these were investigated during April and May in the Oregon study area. Bleedings were made of 84 nestlings of English sparrow, starling, redwing blackbird, Brewer's blackbird and pigeon, as well as 21 adult birds of a number of species. In addition, 57 eggs from 18 nests of redwing blackbirds, Brewer's blackbird, meadowlark, killdeer, and mourning dove were collected for isolation attempt in suckling mice. Four hundred ninty *Culex tarsalis* of the overwintered generation were collected before mosquito reproduction began. Eggs of both Brewer's and redwing

blackbirds were beginning to hatch just when the earliest spring generation of C. tarsalis became apparent. This suggests that continued consideration be given to nestlings. All virus tests on avian material were negative, however. The mosquitoes of early spring have not yet been processed.

During spring C. tarsalis ingest a clear fluid of unknown origin and composition. To gain understanding of this material the intestinal contents of 120 fluid-containing C. tarsalis were dried on glass. Analysis indicated a composition of 100% carbohydrate.

C. tarsalis were collected throughout the summer in pigeon-baited traps at two sites. The mosquitoes were tested for virus content (suckling mouse inoculation) and the pigeons were bled periodically for HAI antibody determination. At present virus identification is only tentative, based on incubation periods in mice, but patterns leave little doubt as to identity of strains. At Site 1, located in irrigated farmland at the edge of uncultivated semi-arid land, 4,401 C. tarsalis yielded 10 isolations of SLE virus and 4 of WEE. At Site 2, a natural "oasis" site in arid terrain, 2,187 C. tarsalis yielded two isolations of SLE and one of WEE. This incidence of SLE virus (1.8/1,000 C. tarsalis) was unusually high for that region, and this virus appeared exceptionally early in the season (July 10). The higher incidence of SLE relative to WEE virus is the reverse of the usual pattern.

At Site 1, three of six sentinel pigeons converted to WEE, the earliest in the bleeding of July 22, and all six converted to SLE, all also appearing in the bleeding of July 22. At Site 2, two of six pigeons converted to WEE and 4/6 to SLE, first in the bleedings of August 12 and September 1, respectively. This pattern, like the mosquito isolations, indicates high rate and early appearance of SLE virus. It is apparent that some pigeons were exposed to both viruses. These included three at Site 1 and two at Site 2.

(W. A. Rush)

Tick-borne diseases in the northwestern U.S.

A project recently undertaken to study tick-borne diseases in the northwestern U.S. suffered a set-back due to the untimely death of Dr. Gordon Clark, one of the principal investigators. The project was re-assessed and priorities were re-assigned. Work on this project centered in the State of Oregon. This was mainly due to the excellent cooperation obtained from Mr. Robert Gresbrink of the Oregon State Department of Health. In all, over 136 pools of ticks were tested. The following isolations were

made from these pools: five isolations of Colorado tick fever virus from Dermacentor andersoni collected at various locations in Oregon; one of CTF from D. occidentalis from Douglas Co., Oregon; and one of tularemia from D. occidentalis from near Gold Beach, Oregon; and one isolation of a rickettsia in the RMSF group from Ixodes pacificus collected in the area near Tillamook, Oregon. This last isolation is of note as it is the first isolation of RMSF rickettsiae from this species of tick.

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

Soft Tick Viruses

Mr. G. Hughes has obtained a virus from Argas cooleyi from swallow nests in Granite Co. in western Montana. He also isolated a strain of the same virus from Ixodes howelli collected at the same time in the same area.

Work has continued on other isolates. The virus isolated from Ornithodoros capensis from Midway Island was identified in neutralization tests as Nyamanini virus or one closely related to it. This relationship was also demonstrated in CF tests by Dr. Thomas. Other isolates are currently under study.

Three dozen juvenile sooty and noddy tern were received from Pacific Islands for viremia studies with soft tick isolates. In an initial experiment 0.5 ml of a 10^{-1} suspension of Hughes (titer 4.87 SM LD_{50}/ .02 ml) and Johnston Atoll (titer 7.40) viruses were inoculated subcutaneously into the two species of birds. Virus was detected in the blood of both species 48 to 240 hours following inoculation with Hughes virus (titers ranged from $10^{0.75}$ to $10^{3.4}$ sm LD_{50}). Only slight amounts (highest titer: $10^{1.15}$sm LD_{50}) of Johnston Atoll virus were detected in the blood of either species. The one surviving bird inoculated with Hughes virus had no detectable neutralizing antibodies, but all the birds that received inoculation of Johnston Atoll virus had detectable antibody.

(C. M. Clifford)

Release-recapture of radiolabeled ticks

Field trials were undertaken in 1967 to determine the feasibility of recapture of radiolabeled ticks. In early July, 30, 600 larval Dermacentor andersoni, tagged with Carbon-14 labeled glycine, were released in a 1.8 acre study area in Mill Canyon, following a release-recapture plan devised by

Dr. Daniel Sonenshine, Old Dominion College, Norfolk, Virginia. Recapture of most marked larvae from small-mammal hosts occurred during the first trapping week and ceased by the end of the third week. Of 1,168 ticks collected on 55 animals, 22 (1.7%) were radioactive. Approximately 172,000 larvae per acre was a resulting crude estimate of tick population density. Recaptured ticks were distinctively marked and easily separated from natural populations. This study was done in collaboration with Dr. Sonenshine, who indicates that recapture success and infestation rate were high in comparison with similar studies done by him in Virginia. This study will be continued on a larger scale in 1968, with the objectives of 1) estimating population size of larval and nymphal D. andersoni, 2) measuring tick dispersal, and 3) detecting overwinter survival of tagged individuals and/or transstadial transfer of label.

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

Host-preferences for pre-imaginal ticks

In early 1967, an attempt was made to identify, in unfed adult ticks taken by flagging, the host-origin of blood meals consumed in the nymphal stage. A model system was devised wherein as little as 10^{-6} ml of host serum could be detected by precipitation reaction of blood-meal extract and antiserum in immunodiffusion plates. Preliminary trials allowed the detection of rabbit serum proteins in the guts of adult D. andersoni and Rhipicephalus appendiculatus 44 days after the nymphal feeding and 17 and 22 days, respectively, after emergence of the adult from the nymphal skin. In subsequent tests, host-proteins were identified in both sexes of D. andersoni 129 days after nymphal feeding. However, retention of host proteins was affected by sex and species of tick and by species of hosts. Antisera have been prepared against serum proteins of most mammal species endemic to the Mill Canyon study area. Blood meals from 156 wild-caught flat adult D. andersoni were prepared for precipitin-testing and were freeze-stored. The ticks were then ground individually and inoculated into suckling mice for virus isolation. Nine isolations of Colorado tick fever virus, subsequently confirmed in CF test by Dr. L. Thomas, were made. After precipitin antisera has been cross-absorbed, the blood meals will be thawed and tested in the hope of identifying hosts which donated virus to the ticks.

(C. E. Yunker and J. A. Rudbach)

Arthropod tissue culture

Ten of 34 virus strains tested showed significant growth or were sustained in the established cell line derived from the insect Antheraea eucalypti and adapted to grow in absence of insect hemolymph. All were arboviruses; Japanese encephalitis (two strains), yellow fever, and St. Louis encephalitis (Group B); Cache Valley and Bunyamwera (Bunyamwera group); Tahyna, Snowshoe hare, and California (California group); Indiana vesicular stomatitis (ungrouped). Bunyamwera virus and those of the California group merely survived or propagated only to low levels, but the remaining six increased 3.7 - 6.0 logs beyond amounts inoculated. Cytopathogenic effect was not seen.

Viruses failing to propagate in these cultures, or which deteriorated commensurately with cell-free controls, were all members tested of groups A, C and Tacaribe, six of 10 members of the B group, eight ungrouped arboviruses and three non-arboviruses.

These results indicate that the Antheraea cell line may be a selective substrate for certain viruses, but possible differences among morphologically different cell types, with respect to the pattern of selectivity, remain to be studied in detail.

(C. E. Yunker)

Ecological studies of Colorado tick fever (CTF) virus in Mill Creek Canyon, Bitterroot Valley, Montana

During a three-year field study 774 small mammals of eight genera were captured. Population levels of these and their tick parasites were estimated from capture-recapture data. Tick populations were also studied by collection of unattached forms. The percentage of rodents infested ranged from 16 (Clethrionomys) to 100 (Citellus lateralis). Adult and nymphal ticks were active during May, June, and July; larvae during July and August. Virus isolations were made from 63 pools of Dermacentor andersoni nymphs and larvae which were removed from rodents. The percentage of total pools positive for virus ranged from 11 (1964) to 29 (1966). The greatest number of positive pools (30%-40%) were taken from C. lateralis and Neotoma cinerea. Isolations were also made from the blood of Peromyscus, Citellus, and Neotoma. The ancillary role of such species as Peromyscus maniculatus and N. cinerea in the maintenance of CTF virus in this biocoenose was demonstrated.

(G. M. Clark and C. M. Clifford)

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

Colorado: To date, five WE and one SLE human cases have been confirmed within the state during 1967. The acute serum specimens for these cases were obtained during the period August 16 through September 25, 1967. Isolations of WE virus from *Culex tarsalis* and SLE virus from *Culex pipiens* were obtained in the Weld and Larimer County study areas only during the period from July 30 through September 9, 1967. The highest weekly *C. tarsalis* WE infection rates of 8.9 per 1,000 occurred during the weeks of August 13 and 27 and the highest weekly *C. pipiens* SLE infection rate of 15.9 per 1,000 occurred during the week of August 20. The last isolations of both viruses from mosquitoes were detected the week of September 3. *C. tarsalis* population indices were higher during the months of July and August 1967 than they were during the same months in 1964 and 1966. However, they were lower than the population indices of the WE epidemic year 1965 until mid-August, and the indices rapidly declined after mid-August 1967.

Texas: In 1967, no confirmed clinical case of arboviral encephalitis occurred. However, serologic studies among approximately 200 school children, ages 7-13, in each of three Hale County towns provided evidence of inapparent infection during the period March-October 1967. There were five WE and seven SLE inapparent infections detected on the bases of antibody conversions in HI and plaque reduction neutralization tests. These represent inapparent attack rates of 28.4 per 1,000 for WE and 24.2 per 1,000 for SLE.

The use of repeated ultra low volume (ULV) applications, three ounces per acre, of technical grade malathion was studied to evaluate its effect upon vector mosquito populations and upon arbovirus activity. Two towns were treated weekly from mid-June through August and twice biweekly in August. One town was treated twice biweekly in July, another town and two rural study sites were utilized for untreated comparisons. Varying degrees of success were achieved with respect to reducing adult *C. tarsalis* populations in the treated towns. The return of the mosquito population indices to pre-treated levels before the next weekly treatment in the largest of the treated towns, was indicative of continuing mosquito production within the treated areas or of infiltration of adults from adjacent untreated breeding areas. No statistically significant reduction in the seasonal *C. tarsalis* infection rates was noted in the treated towns when compared with mosquito infection rates at the untreated town or at two untreated rural study sites.

Hawaii: Mosquito host preference studies have been conducted to obtain data on blood feeding habits of Aedes aegypti, A. albopictus, A. vexans and Culex quinquefasciatus. Capillary precipitin tests indicate that A. aegypti and A. albopictus generally fed on mammals, but that they occasionally took blood from avian hosts; whereas, A. vexans only rarely fed birds. Approximately two-thirds of the blood meals taken by C. quinquefasciatus were from birds and the balance was from mammals. Previous studies have shown that conclusions regarding mosquito host preferences may be erroneous if based upon the percentages of blood meals obtained from various hosts without regard to the availability of the various types of host animals.

Therefore, several sites have been selected and data are being obtained on the populations of available host animals. These data are being used in conjunction with the precipitin test data in calculating forage rations for each mosquito species.

REPORT FROM SOUTH DAKOTA STATE UNIVERSITY, BROOKINGS, SOUTH DAKOTA

Study of Virus Epizotic Hemorrhagic Disease (EHD) of Deer in South Dakota

In the past 17 years, 35 major epizootics of virus hemorrhagic fever (HF) have occurred in deer populations of 37 South Dakota counties. EHD like Bolivian (human) HF occurs during July to September in similar macro and micro habitat, i.e., in prairies and rural areas where corn is raised. Four EHD strains were isolated from spleen and liver of field-infected deer and adapted to grow in one-day-old mice by three to five intracerebral passages. Experimentally, 23 Black Hills deer were inoculated intramuscularly with EHD. Five deer died 8 to 10 days post-inoculation (PI) with typical hemorrhagic symptoms. Six deer were sacrificed 16 to 21 days PI, showing slight hemorrhagic symptoms. After six months, the remaining deer were sacrificed, showing no visible hemorrhagic symptoms. Complement-fixing (CF) and precipitiating viral antigens were prepared from six infected deer (liver and spleen) and sucrose-acetone extract from 5,000 mouse brains. CF and precipitating antisera were produced in 40 rabbits and six horses. Immune sera and experimentally infected deer sera gave CF titers from 1:4 to 1:32 and showed one precipitating line in immunodiffusion tests. Organs from 26 wild deer from 12 counties were collected and tested for EHD antigen by the CF test, showing 18 deer positive. South Dakota #10 EHD strain has been grown successfully by us in vero, BHK 21 and deer kidney cell lines. The virus titres at present are lower than 10^5 PFU per ml.

Analysis and Relationship of Climatic Factors with the Epidemiology of Virus Epizootics for Epizootic Hemorrhagic Disease

In the past the relationship of temperature and precipitation to EHD has not been analyzed systematically. Does the occurrance of the majority of the epizootics on alternate years have any relationship with hot and dry environmental conditions in disease transmission? The South Dakota game biologists working with epizootic hemorrhagic disease (EHD) of deer for the last fifteen years have observed hot and dry environmental conditions during the 35 epizootics with the majority occurring on alternate years.

EHD epizootics occur from late July to the first part of October with the majority occurring in September. Precipitation and temperature data covering a 17 year period and the months July through September were collected from 30 weather stations located (within five miles on the average) near 35 EHD epizootic sites. Average maximum temperatures for July, August, and September during the period of the outbreaks were found to be 89.6, 86.4, and 77.7°F respectively. Thirty out of 35 epizootics showed a temperature range from 75° to 85° F during the outbreak. Maximum temperature for September was 6° F higher during the year of an outbreak than at that site the preceeding year. Seventy-five percent of the epizootics occurred on alternate years (1952, 1962, 1964, 1966). From July through November precipitation was 2.5 inches higher at each site the year before the outbreak occurred. The number of deer involved in each outbreak (intensity factor) when compared with temperature and precipitation further supports that climatic factors do play a role at the outbreak site. A complex method of ranking is devised to show correlation of outbreak intensity factors to temperature and precipitation recorded in the area of the outbreak.

Conclusions

The following inferences could be drawn from the above discussed weather data and its relationship with the disease occurrance, its intensity and location of the eipzootics:

1. T. max of July and September was hotter the years of the outbreaks than the years before the outbreaks.

2. A relationship was found between the larger number of animals involved in an outbreak (i.e. intensity) and higher four monthly (July-October) ave T max of the 27/35 EHD epizootics.

3. The T max for July, August and September for the years of the outbreaks was 89.6, 86.4, and 77.7 respectively. The T max range for

the three months mentioned was 70° to 95°.

4. During the outbreak years the precipitation was found to be lower than the year before the outbreak. Hence, during the year of the years of the epizootics hot and dry conditions exist which confirms the field observations by game biologists.

5. Intensity in 27/35 EHD epizootics shows correlation with total precipitation of the years of and the years before the outbreaks.

6. Ave temperature for the years of and the years before the outbreaks showed very little difference.

(G. C. Parikh)

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

The 1967 reported incidence of arboviral infections was lowest in many years in Texas. There were two laboratory confirmed and three presumptive cases of WE and one confirmed and two presumptive cases of SLE infection. One of the latter was considered as aseptic meningitis until enterovirus culture failed and a high HI titer for SLE was found. In 32 cases of coxsackievirus B5, echovirus 9 and other enterovirus-related infection, mostly classified as aseptic meningitis, one-fourth had signs or symptons of encephalitis. These were predominantly serious or alarming illnesses of infants or young children, and coxsackie B virus was recovered from brain and other tissues of a fatal case.

The six confirmed cases of equine encephalomyelitis also were lowest in recent years. The overall WE reactor rate in 185 horses was nearly 15%.

One hundred pigeon, 300 sparrow and 600 chicken sera principally from Dallas county showed a reactor rate less than 5%. This was in contrast with 35% of rabbit sera from Lubbock and 50% of chicken sera from Wichita Falls in 1966 which were reactive for WE and both WE and SLE respectively.

From more than 100,000 mosquitoes, principally Culex quinquefasciatus, in nearly 300 pools, predominantly from Dallas county, four different virus types were identified. These were 90 Hart-Park, two WEE, and one each Turlock and Bunyamwera group. WE virus was found in Culex quinquefasciatus from Wichita Falls in September and in a Culex tarsalis

pool from the Boca Chica area near Brownsville in November.

A CEV virus which the Houston City Public Health Laboratory and the Harris County Mosquito Control District recovered from a mixed pool of Aedes atlanticus and A. infirmatus appears to be a new member of the CEV group. Both we and Sather and Hammon are conducting further study of this virus.

(Tom Guedea, Manuel Guerra and J. V. Irons)

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

In a survey of over 1,000 Mexican free-tailed bats (Tadarida b. mexicana) netted in the southwestern United States the incidence of natural infection with Rio Bravo virus varied from 0 to 7% in groups of 50 to 275 bats captured at various locations during summer and fall months, with an overall infection rate of approximately 2% (Constantine and Woodall, 1964). In studies with free-tails held captive for 1 to 2 years Rio Bravo virus was recovered from saliva at intervals throughout the observation periods, indicating a carrier state in the naturally infected bat (Constantine and Woodall, 1964; Baer and Woodall, 1966). Aware that bat populations in south Texas could be persistently infected with Rio Bravo virus at a rate equal to that which might be expected for any virus in a reservoir host population, we become concerned with the problems which might arise in attempting to evaluate the role of Mexican free-tailed bats in the ecology of SLE virus in Texas during epidemics caused by this agent in 1964 and 1966 (Information Exchange # 15, March 1967).

The identification of a virus isolated from bat tissues as SLE virus rather than a strain of Rio Bravo virus was not difficult since, although these two agents are closely related serologically, they are distinguishable by the serum neutralization test. Our concern was with the possibility that bats naturally infected with Rio Bravo virus would not be susceptible to SLE virus. Also, assuming that dual infection could occur, the question arose as to whether in the assay of a tissue infected with both viruses, Rio Bravo virus might interfere with the growth of SLE virus and prevent its isolation. The initial phase of studies designed to answer these questions was concerned with determining the susceptibility of Mexican free-tailed bats to experimental infection with Rio Bravo virus so that the interrelationship of these two viruses in producing infection in vivo could be studied using experimentally infected bats as models of what might occur in nature.

In the preliminary experiments two bat species, Tadarida b. mexicana and Eptesicus f. fuscus (big brown bats) received subcutaneously approximately 1,000 weanling mouse intracerebral LD_{50} ($MICLD_{50}$) of a strain of Rio Bravo virus isolated from a naturally infected Mexican free-tailed bat by Dr. George M. Baer. Groups of bats were sacrificed 3, 7, 12, and 18 days post-inoculation and blood, brown fat, brains, salivary glands and spleens were obtained and assayed for virus by the intracerebral inoculation of weanling mice. Rio Bravo virus was demonstrated in one or more of the tissues obtained from bats of both species at each harvest interval.

Virus was not detected in salivary glands with any greater frequency than in the other tissues tested. This is of interest since, in the survey by Constantine and Woodall, brains, lungs, kidneys, mammary glands, and fetuses, in addition to salivary glands, were assayed and, with one exception (lung), Rio Bravo virus was recovered only from salivary glands. In other studies, Rio Bravo virus has been isolated from the blood and brown fat of naturally infected bats.

While additional experiments following the course of experimental Rio Bravo virus infection in bats over prolonged periods of time are in progress, studies to determine methods which can be used in detecting interference between or dual infection with Rio Bravo and SLE viruses have been initiated. There is some controversy in the literature as to the degree of cross-reactivity between these two agents in the serum neutralization test. Previous studies from this laboratory, as well as those of certain other investigators, have shown a one-way cross between SLE and Rio Bravo viruses in which anti-SLE virus serum neutralizes Rio Bravo virus but anti-Rio Bravo virus serum does not neutralize SLE virus.

In a recent study describing the identification of a virus strain isolated from a fox as SLE virus, Emmons and Lennette (1967) found little or no cross-reactivity between these two arboviruses and suggested that the demonstrable relationship between the two may depend on the strains of viruses studied and the test systems used. Currently we are studying cross reactions between several strains of SLE and Rio Bravo viruses in serum neutralization tests in weanling mice, in plaque reduction tests in VERO Cells and by the fluorescent antibody technique. Results obtained to date indicate that the degree of relationship between these two agents does vary when different strains are compared in different test systems.

(S. Edward Sulkin and Rae Allen)

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

Arbovirus surveillance at selected sites in 1967

The NCDC Arbovirus Infections Unit provided assistance in arbovirus surveillance, particularly for SLE virus, to health departments at Corpus Christi and Houston, Texas; Memphis, Tennessee, and at the NASA Test Facility at Bay St. Louis, Mississippi. The service consisted of testing suspected vector mosquito species for virus and, in the case of Texas stations, blood samples from young-but-flighted house sparrows.

Although no SLE virus was isolated, a number of strains of other viruses were obtained. Table 1 shows the isolations made from mosquitoes collected at the various surveillance sites. The California Group isolates were of special interest, with two from Corpus Christi being the first made from *Psorophora discolor*. Four of this virus group were obtained from *Aedes atlanticus* collected at the Mississippi site. All other isolates were of Flanders and a member of the Bunyamwera group, presumably Tensaw or Cache Valley virus.

The results of HI tests on 879 sera from juvenile house sparrows collected at Corpus Christi, May-August, 1967, are shown in Table 2. About 7% of the earliest May sera gave HI reactions against SLE antigen, but in low titer. During late May and early June, only four percent were positive. This low grade reaction might be explained on the basis of maternal antibody, since the previous year had been one of epidemic SLE activity. Only 4/135 sera collected in July and 1/112 collected in August and September were positive. The latter serum, collected on September 1, reacted in relatively high titer, however (1:160), indicating that SLE virus was present later in the season, although scarce.

Arbovirus studies at Brus Laguna, Republic of Honduras

More than 21,000 mosquitoes, with 40 species represented, were collected at Brus Laguna in April, 1967. A Group C virus was isolated from *Culex* (*Melanoconion*) *educator* and is being studied further.

A total of 885 bird sera was collected and tested, with HI results shown in Table 3. Companion collections of 1,356 birds were made concurrently at Pilottown, Louisiana. (Table 4). The antibody rates for EE, WE and SLE were very low in the migratory species taken in both areas.

Blood samples were also obtained from 39 horses and 140 human residents in the vicinity of Brus Laguna. The HI results (Table 5) indicate that VEE was common, that WEE, SLE and YF viruses had been active. A member of the Group C-Guama-Patois complex also appeared to be active, and a few individuals seem to have had experience with a member of the California Group. The SLE HI positives in the human sera were confirmed by neutralization tests.

Production of California (LaCrosse) HA in tissue cultures

Following the lead of Halonen et. al. (Ann. Med. Exp. Fenn. 45: 182-185, 1967), who produced rubella HA in serum-free suspension cultures of BHK-21 cells, production of LaCrosse HA is being investigated in a similar system. The basic procedure is as follows:

1. Grow BHK-21/13s cell monolayers in 32oz. prescription bottles, using Wistar growth medium containing 10% fetal calf serum.

2. Pool cell sheets from eight bottles, trypsinize and suspend the cells in 800 ml of the Wistar growth medium-10% fetal calf serum.

3. Place on a magnetic stirrer at 35°C for 24 hours.

4. Centrifuge and resuspend in 100 ml of Wistar medium containing 0.4% bovine plasma albumin instead of calf serum. Use a one-liter bottle.

5. Inoculate the suspension with virus and incubate, with occasional shaking, at 35°C for two hours.

6. Add 700 ml of Wistar medium containing 0.4% bovine plasma albumin.

7. Place on magnetic stirrer at 35°C and test samples of fluid periodically for HA activity.

8. When a satisfactory HA titer is reached, harvest fluid and use as is or extract with Tween-80 and ether as described by Norrby (Proc. Soc. Exp. Biol. & Med. 111: 812, 1962). This treatment usually increases the HA titer at least twofold and destroys infectivity.

The effect of virus dose on antigen production is shown in Table 6. When 1 and 10 PFU per cell were used, somewhat higher titers were obtained with the lesser inoculum. Peak HA titers were reached by 22 hours. A medium change at 22 hours (consistent with the Halonen et. al. procedure for producing rubella antigen) caused a drastic and immediate drop in HA titer. Table 7 demonstrates more clearly the detrimental effect of medium change. Parallel cultures were inoculated with a multiplicity of 1.

The HA titer in both cultures reached 1:128 by the 24th hour. With medium change at this time, the HA titer was reduced to 1:4; without medium change, it remained high. The infectivity titer was also reduced by the medium change.

The same spinner culture method has also been used successfully to produce HA antigens for BFS-283, Trivittatus, SLE, Marituba, Shark River and Guama viruses. It has been possible also to produce usable HA antigens of LaCrosse, BFS-283, Trivittatus, Keystone and Pahayokee viruses in monolayer cultures of BHK-21/13s.

Studies are in progress to find ways of increasing the HA titers obtained, and to extend and modify the method to successfully include other arboviruses which produce hemagglutinins poorly in animals.

Replication kinetics of selected arboviruses in suckling mice, and application of findings to their rapid identification

The viruses of California encephalitis (LaCrosse), SLE, Flanders and Tensaw were inoculated intracerebrally into groups of suckling mice; and the CF, HA and agar gel precipitating antigens and infectivity studied at different periods of incubation. The results are shown in Figures I-IV. By sacrificing the mice blindly prior to appearance of symptoms and checking for presence of antigen, the viruses could be identified two or more days earlier than by waiting for symptoms to appear.

When this system was applied to identification of virus in infected mosquito pools, a significant reduction in time required was demonstrated. Since these four viruses are among those most frequently isolated in association with each other in the United States during encephalitis outbreaks, such a time saving could be quite important.

Application of an agar gel precipitin test to diagnostic arbovirology

Seventy-one arboviruses representing Groups A, B, C, California, Bunyamwera, Guama, Capim, Turlock, Simbu, VSV, Tacaribe and Ungrouped, as well as Herpes, were tested in a modified Ouchterlony agar gel precipitin system similar to that described by Murphy and Coleman (Jour. Immunol. 99: 276-284, 1967). The test generally was found to be satisfactory for the identification of these viruses; but for some, not as satisfactory as the CF test. Of the Group A viruses tested, EEE and WEE gave unreliable results; however, Sindbis reacted well. Bunyamwera Group viruses could not be differentiated from one another but did give group reactions (Guaroa reacted with Bunyamwera but not California). The dif-

ferentiation of Group B, California Group and Simbu Group viruses was accomplished. Studies to improve the system are underway; preliminary results suggest that substitution of Agarose for Ionagar improves delineation and allows demonstration of lines which previously were not readily apparent. Within certain groups, the test promises to be especially useful for rapid identification.

Socioeconomic level and SLE attack rate, Corpus Christi, 1966

The epidemics of SLE which occurred in Dallas and Corpus Christi, Texas, 1966, differed in socioeconomic levels of people affected. The Dallas cases occurred predominantly in the lower socioeconomic strata. The Corpus Christi cases, on the other hand, were primarily in the higher income districts; the low-income areas were relatively spared.

Differences in distribution of the vector mosquito, *Culex quinquefasciatus*, in the two cities were apparent. Vector breeding was heaviest in the low-income areas of Dallas. In Corpus Christi, on the other had, open ditches which served as storm drainage for the low-income census tracts had dried up prior to the epidemic; vector production was highest in storm sewers found in the high-income districts.

However, differences in immunity of the various human populations were also suspected of affecting the epidemic patterns. Preliminary results of serologic surveys in Dallas and Corpus Christi tend to confirm this suspicion. Little evidence was found in Dallas to indicate that widescale infection with SLE had occurred prior to the 1966 epidemic. In Corpus Christi, however, a high proportion of individuals in the lower socioeconomic groups were immune, and past records revealed laboratory confirmed cases as far back as 1956. The SLE immunity rate in the higher socioeconomic districts, on the other hand, was low, probably attributable to the 1966 epidemic alone. Therefore, the scarcity of cases in the lower socioeconomic groups in 1966 appears to have been partly due to their high level of protective immunity.

Table 1

Summary of Virus Isolations made from Mosquitoes
Processed for Surveillance and Special Study, 1967

	Corpus Christi, Texas			Houston, Texas		Memphis, Tenn.	NASA Test Facility	
Mosquito species	Calif.	Flanders	Tensaw	Flanders	Tensaw	Flanders	Calif.	Total
Aedes atlanticus-tormentor							2	2
sollicitans	2		2					4
taeniorhynchus					2			2
vexans							1	1
species							1	1
Culex quinquefasciatus		7		4				11
quinquefasciatus-pipiens						2		2
Psorophora confinnis	1		2					3
discolor	2							2
Total	5	7	4	4	2	2	4	28

Table 2

SLE Surveillance Through Immature House Sparrows

Corpus Christi, Texas 1967

Date of Collection	No. Tested	No. HI SLE Reactors	Rate
5/8/67 - 5/19/67	135	10	7.40%
5/24/67 - 6/9/67	164	7	4.26%
6/13/67 - 6/28/67	165	0	0
7/10/67 - 7/20/67	135	4	2.80%
7/27/67 - 8/15/67	168	0	0
8/17/67 - 9/1/67	112	1	0.89%
	879	22	

Table 3

Serological Results on Bird Sera Taken in April, 1967 at Various Points around the Gulf of Mexico

Location	No. Tested	No. and Percent (in parentheses) of HI Reactors			
		EEE	WEE	VEE	SLE
Brus Laguna, Honduras					
Total	885	13(1.47)	10(1.13)	7(0.79)	7(0.79)
Migratory Species	453	3(0.66)	3(0.66)	3(0.66)	3(0.66)
Endemic Species	432	10(2.32)	7(1.64)	4(0.93)	4(0.93)
Pilottown, Louisiana	1356	7(0.52)	4(0.30)	no test	1(0.06)
Galveston Island, Texas	430	2(0.47)	0(-)	no test	6(1.40)
Ingleside, Texas	99	0(-)	1(1.10)	no test	3(3.04)

All HI reactors reacted at a dilution of 1:20 or greater.

Table 4

HI Results on Migrating Bird Sera taken at Pilottown, Louisiana

Year	Number Tested	Number and Percent (in parentheses) HI Reactors SLE	EEE	WEE
1965	1,009	134 (13.3)	15 (1.5)	5 (0.50)
1966	1,821	163 (9.00)	8 (0.40)	8 (0.40)
1967	1,356	1 (0.06)	7 (0.52)	4 (0.30)

Table 5

HI Results on 39 Horse Sera and 140 Human Sera from Honduras, April - May, 1967.

Antigen	Number of Positive Sera							
	Human Sera				Horse Sera			
	1:10	1:20	≥1:40	Total	1:10	1:20	≥1:40	Total
EEE	4	0	0	4	2	0	0	2
WEE	24	3	1	28	28	0	0	28
Mayaro	0	0	0	0	0	0	0	0
VEE	5	14	49	68	17	11	7	35
SLE	8	5	2	15	13	1	1	15
MVE	11	1	2	14	24	1	0	25
Dengue II	10	2	0	12	7	0	0	7
YF	22	9	7	38	6	0	0	6
Ilheus	6	4	1	11	0	1	0	1
Cowbone	0	1	0	1	0	0	0	0
Bussaquara	5	1	1	7	0	1	0	1
Powassan	1	1	0	2	0	0	0	0
Cache Valley	0	0	0	0	0	0	0	0
Guaroa	0	0	0	0	1	0	1	2
Itaqui	3	2	0	5	0	0	0	0
Marituba	21	4	5	30	22	0	1	23
Guama	5	1	0	6	0	0	0	0
Shark River	4	5	0	9	0	0	0	0
VSV (NJ)	0	0	0	0	0	0	0	0
VSV (Ind)	0	0	0	0	0	0	0	0
LaCrosse	8	0	1	9	19	1	1	21

Table 6

Effect of Dose on La Crosse Virus
and Antigen Production in BHK-21/13s

Sample	Hours After Inoculation	Spinner TC-65383 Titers (1)			Spinner TC-65384 Titers (2)		
		HA (Recip.)	CF (Recip.)	PFU/ml (-log)	HA (Recip.)	CF (Recip.)	PFU/ml (-log)
B	2	<2	<2	5.9	<2	4	6.9
C (3)	22	64	32	7.9	32	16	7.5
D	23.5	<2	32	6.8	<2	16	6.6
E	47.5	16	16	6.2	8	8	5.4
F	71.5	16	16	5.3	4	16	3.5
G	95.5	8	16	4.0	2	16	<3.0
H	119.5	8	16	<3.0	4	16	<3.0

(1) Inoculum = 1 PFU per 10 cells
(2) Inoculum = 1 PFU per 1 cell
(3) Medium was changed in both cultures after C samples were collected.

Table 7

Effect of Medium Change on La Crosse Virus and Hemagglutinin Production in $BHK_{21}/13s$

Sample	Hours After Inoculation	Spinner 66480 (2)		Spinner 66481 (3)	
		HA Titer (Reciprocal)	PFU Titer/ml (-log)	HA Titer (Reciprocal)	PFU Titer/ml (-log)
(1)					
A					
B	2	0	5.9	0	5.8
C	4	0	5.6	0	5.8
D	6	0	6.1	0	6.3
E	8	4	7.3	4	7.4
F	10	32	7.6	16	7.7
G	12	32	7.7	32	7.7
H	14	32	7.8	64	7.8
I	16	64	7.9	64	7.9
J	22	128	7.7	64	7.8
K	24	128 (2)	7.6 (2)	128	7.6
L	25	4	6.6	128	7.8
M	26	2	6.3	128	7.7
N	28	8	6.7	128	7.8
O	30	4	7.0	128	7.9
P	48	8	6.2	64	7.4
Q	54	8	6.0	64	7.3

(1) Multiplicity = 1.0
(2) Medium Change
(3) No Medium Change

Figure I

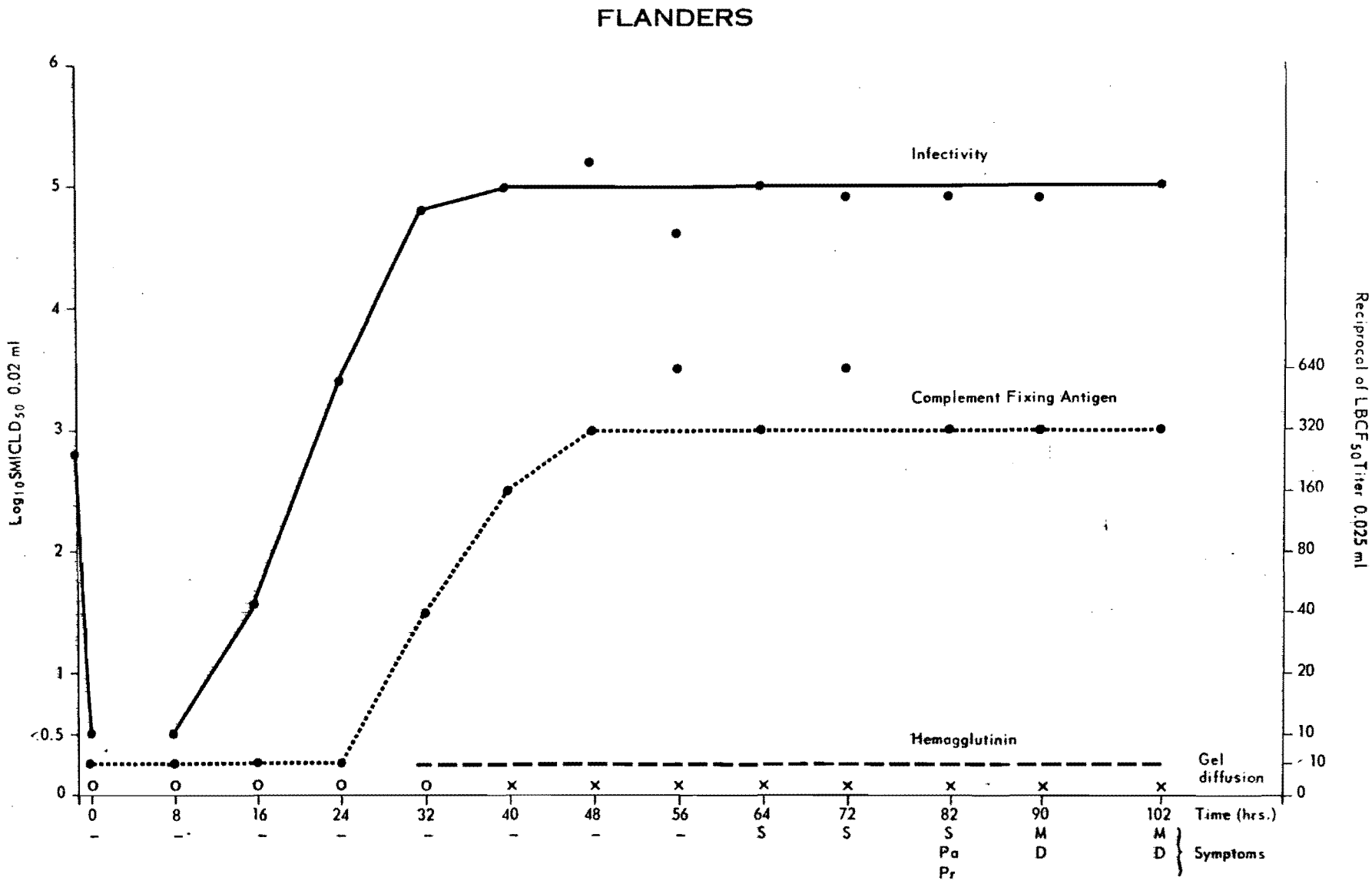

FLANDERS
Infectivity
Complement Fixing Antigen
Hemagglutinin
Gel diffusion
Log10SMICLD50 0.02 ml
Reciprocal of LBCF50 Titer 0.025 ml
6
5
4
3
2
1
<0.5
0
640
320
160
80
40
20
10
<10
0
0 8 16 24 32 40 48 56 64 72 82 90 102
Time (hrs.)
S S
Pa
Pr
M M
D D
Symptoms

Figure II

CALIFORNIA (LA CROSSE)

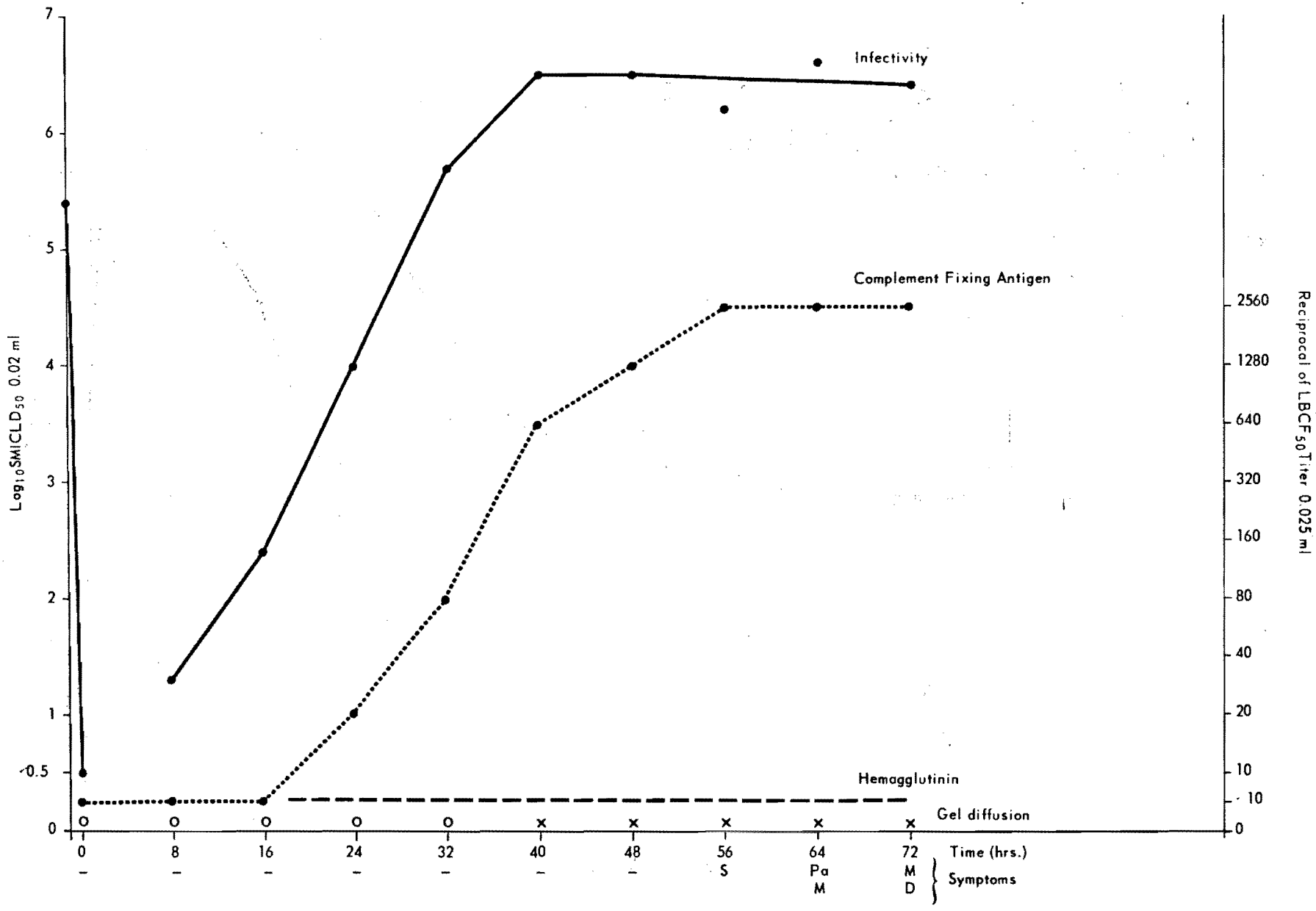


Figure III

ST. LOUIS ENCEPHALITIS

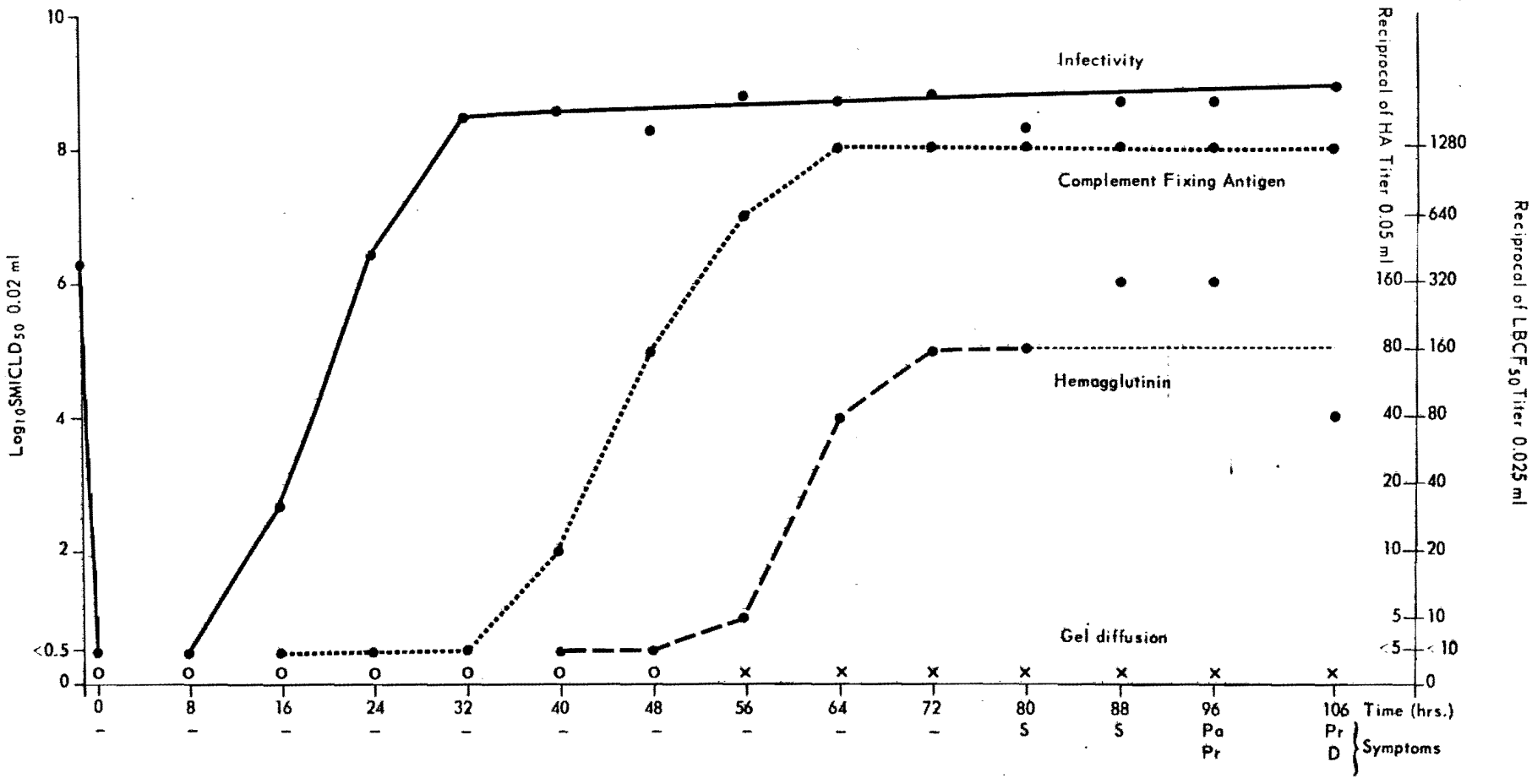
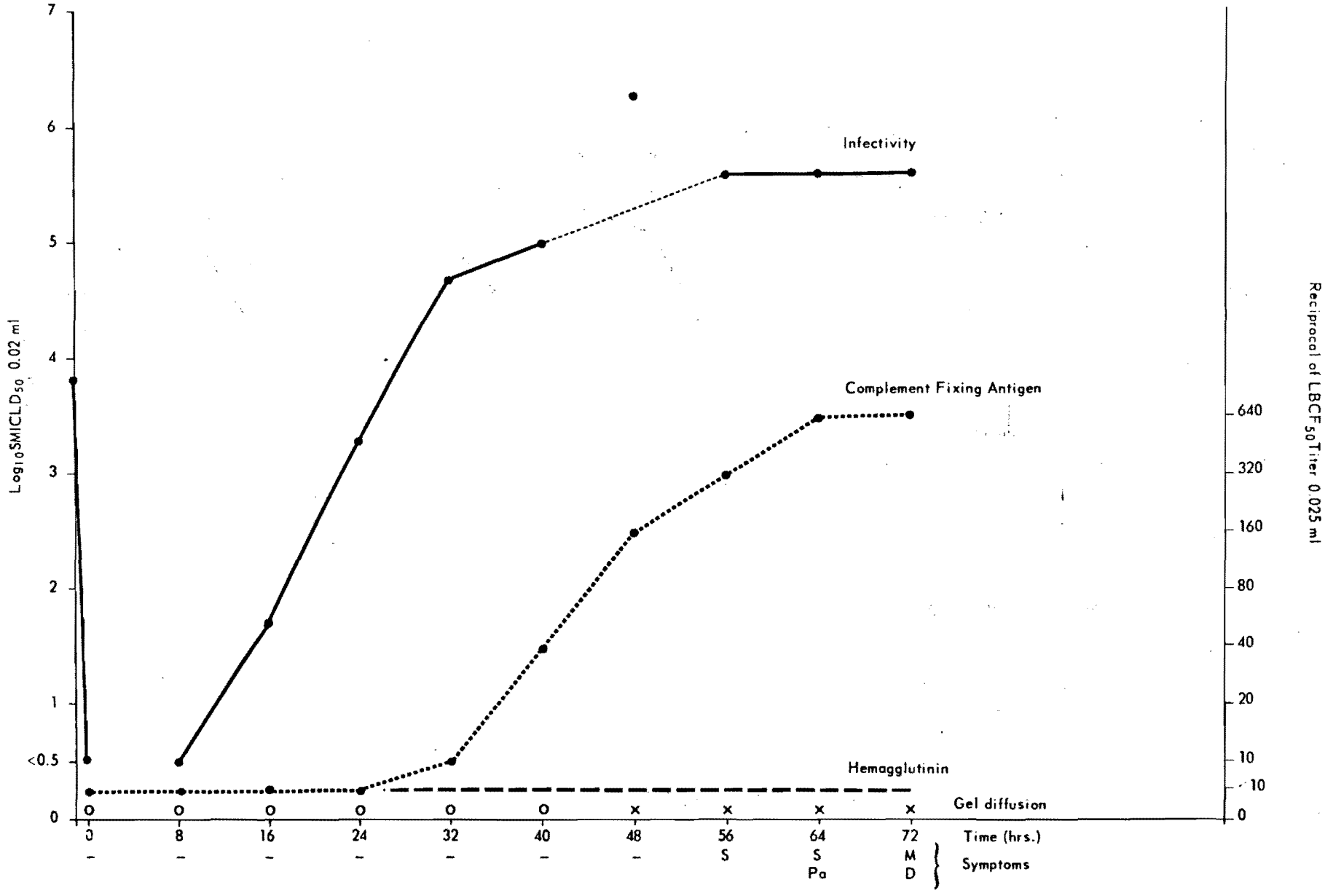


Figure IV

TENSAW



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

Tamiami Virus in the Tampa Bay Area

Beginning in December, 1963, the Encephalitis Research Center staff has made 19 recoveries of a viral agent from Tampa Bay area cotton rats, seven of these during 1967. One of the early prototype strains was intensively studied at the University of Pittsburgh, the Yale-Rockefeller Laboratories, and at the National Communicable Disease Center. In 1967, it was identified by the latter laboratory as a member of the Tacaribe group of viruses and almost identical to an agent recovered from rodents in the Everglades region of Florida and given the name of "Tamiami" virus by the N. C. D. C. investigators. Serological identifications were carried out with the CF test, using either Tacaribe group, immune mouse ascitic fluid or immune mouse ascitic fluid prepared against the N. C. D. C. prototype Tamiami virus strain. Using mouse immune sera prepared in the Tampa laboratory against one of the 1965 cotton rat isolates, all of the Tampa Bay area isolates were shown to be identical by the CF test.

The 19 isolates in the ERC laboratory came from 308 cotton rats examined over a four year period. This total recovery rate of six percent varied according to the weight of the animal, which is presumed to be a rough indicator of age. In the very young animals, the recovery rate was 10 to 13 percent, and in the older animals, two percent. All of the isolations were made from animals collected in one small geographic site, which provided a very favorable ecologic environment for cotton rats. A definite seasonal relationship was observed between density of cotton rat populations and recovery of the viral agent.

One of the isolates was sent to Dr. Herbert S. Hurlbut (Southwest Foundation for Research and Education, San Antonio, Texas) for attempted propagation in arthropods. He did not detect replication in either Culex pipiens mosquitoes of larvae of the black carpet beetle after experimental inoculation.

Studies on Marsh Rabbits

The suspected importance of rabbits in the ecology of California encephalitis viruses in the Tampa Bay area, led project biologists to institute special studies on the local marsh rabbit. A technique was developed for capture of this animal and approximately 50 were obtained for virological studies. These wild-caught marsh rabbits have been used in experimental

infections with both Keystone and Sawgrass viruses. Results are incomplete; however, it appears that the wild marsh rabbit has a much longer and higher titered viremia with Keystone virus than the domestic rabbit. No viremia was detected following experimental inoculation with Sawgrass virus (tick agent recovered from both the rabbit and dog tick in the Tampa Bay area). However, suckling mouse neutralization tests on the preinoculation rabbit sera, suggested that these wild marsh rabbits possessed neutralizing substances prior to their experimental inoculation in the laboratory.

Experimental Infection of Aedes Mosquitoes with California Encephalitis Viruses

A series of experiments have been performed attempting to infect either Aedes infirmatus or Aedes atlanticus mosquitoes on viremic domestic rabbits 48 hours after inoculation with different strains of California encephalitis virus. To date, these attempts have been unsuccessful in infecting the mosquitoes. However, project entomologists were successful in infecting Aedes infirmatus by feeding them upon cotton pledgets containing defibrinated rabbit blood with Keystone virus titering 3.2 logs MLD_{50}'s. Each day after the blood meal, the engorged specimens were dissected into three portions; head, thorax and abdomen, and each tested for the presence of virus by suckling mouse inoculation. The virus was found in the abdomen only on day one, two, and three. No virus was isolated on the fourth day, but again was taken from the abdomen on the fifth day. On day six, seven and eight, isolations were made from all three sections of the body. On the 14th day, all mosquitoes that were exposed but did not feed, were tested, but no virus was obtained.

Special Laboratory Projects

The ERC laboratory has repeatedly encountered the problem of non-specific inhibitors in HI tests of avian sera with both Group A and Group B antigens. This inhibitor was apparently related to the ovulatory cycle of the wild birds being examined. In 1967, a special study evaluated the protamine sulfate extraction technique of Holden, et al., in removing these non-specific inhibitors. The results of the comparative study were completely satisfactory and were confirmed by the N.C.D.C. laboratories in Fort Collins, Colorado. Our laboratory has, therefore, developed as a routine the protamine sulfate-double acetone extraction procedure for all avian sera being subjected to the HI test.

Special studies during the year also evaluated the technical feasibility of using plain filter papers discs (No. 740-E Schleicher and Schuell Company,

Keene, New Hampshire) in collecting field specimens for arbovirus isolation and serology. Using rabbit blood collected from known viremic animals, experimentally inoculated with Keystone virus, we demonstrated that Keystone virus was not viable when collected and stored on the filter paper discs under conditions that would be involved in the transport and storage of field specimens.

In a separate study, sera from humans was obtained, using both filter paper discs and standard vacutainer tubes. We demonstrated that SLE-HI antibody could be readily detected in the eluate from the filter paper discs. The HI titers on the disc collected sera were, in general, one dilution lower than those obtained with the standard collection procedure.

Special Monograph on SLE Studies in Florida Over the Period 1962 to 1967

A monograph is in preparation covering the investigations on the epidemiology and control of St. Louis encephalitis in Florida. Contents will include chapters on : (1) SLE Virus in Human Populations of the Tampa Bay area, (2) Virological Studies of *Culex nigripalpus*, including Epidemic Observations and Laboratory Transmission Experiments, (3) a chapter on The Basic Biology of *Culex nigripalpus* Mosquitoes, (4) a section on The Control of SLE Vectors in Florida, including both Observations and Research, (5) a section on The Studies of Vertebrate Reservoirs of SLE Virus in Florida, and (6) an Appendix containing manuscripts on Measurement of Bird Densities and Bird Populations, which have previously not been published elsewhere. The monograph should be available by early summer of 1968, and can be obtained free-of-charge by addressing a request to the Florida State Board of Health, Encephalitis Research Center, 4001 Tampa Bay Boulevard, Tampa, Florida 33614.

(James O. Bond, William L. Jennings, D. J. Taylor and A. L. Lewis)

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

The activities on birds as reservoirs of arboviruses conducted by D. E. Davis at Pennsylvania State University from 1962-67 is being continued at North Carolina State University. The data for 1962-67 have been put on computer tape. The serological results will be analyzed by Dr. William Hann now at Bowling Green State University. D. E. Davis expects to initiate studies of birds as winter reservoirs of arboviruses in the piedmont of North Carolina, an area that may be very important in the migratory path of birds.

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

Since our last report this laboratory has been busy with a research program on many different viruses. Of particular interest to this group are our studies on the simian hemorrhagic fever (SHF) and yellow fever viruses.

In the fall of 1964 a new febrile hemorrhagic disease caused the death of 223 monkeys in the NIH quarantine colony of 1,050 animals. Three Macaca species were affected. The disease affected animals in 16 of 18 rooms housing the colony. Manner of spread of the disease through the colony is not known but several possible ways have been considered. Clinical features of the disease included rapid onset, early fever, facial edema, anorexia, adipsia, dehydration, proteinuria, cyanosis, skin petechiae, melena, epistaxis, and occasionally retrobulbar hemorrhages. Therapeutic measures, (broad spectrum antibiotics, vitamins, forced feeding and oral or parenteral electrolyte administration) were ineffective. Mortality among infected animals was thought to be 100%. However, experimental transmission studies indicated that some animals survived clinical illness. A similar outbreak had occurred among rhesus monkeys newly imported to a primate colony in the U.S.S.R. shortly before the NIH outbreak; this suggests a common source for this disease, since both colonies received animals from the same Indian supplier.

The lesions of simian hemorrhagic fever included capillary-venous hemorrhages in the intestine, lung, nasal mucosa, dermis, spleen, perirenal and lumbar subperitoneum, adrenal gland, liver and periocular connective tissue. Evidence of vasodilation, stasis, and venous thrombosis often was found in association with the hemorrhages. Shock was therefore suspected as an underlying causative factor. Vascular fragility, blood clotting defects, and trauma appeared to be associated factors, particularly in connection with certain of the skin petechiae, the hemorrhages under the renal capsule, and those occurring around lung-mite lesions. The direct effects of virus invasion of tissues were not determined. Degenerative changes in the liver, kidney, brain, lymphatic tissue, and bone marrow were believed to be due to blood stasis and hypoxia. The peculiar splenomegaly of SHF evidently was caused by follicular hemorrhage and engorgement of the cords with plasma and fibrin.

Transmission studies revealed that the disease was due to an infectious agent pathogenic only for monkeys. A virus, designated simian hemorrhagic fever (SHF) virus, was isolated in MA-104 cell line of embryonic

rhesus monkey kidney. Of the 13 cell cultures (primary and continuous) tried, the virus produced CPE only in MA-104 cells, grew without CPE in BS-C-1 cells and could not be propagated in the other 11 cell cultures. The characteristic CPE began as foci of refractile spindle-shaped cells which remained connected to each other by protoplasmic projections forming a network. Biochemical and biophysical studies revealed that the virus contains RNA and is less than 50 mμ in size, chloroform sensitive, pH 3.0 labile and relatively heat stable. Divalent cations, however, enhanced inactivation at 50° C. The virus reproduced the typical hemorrhagic disease in rhesus monkeys. CF antibodies were demonstrated. No relationship could be found between SHF virus and some 85 known RNA viruses.

In July, 1964, prior to our epizootic, there was another outbreak of simian hemorrhagic fever at the Sukhumi Institute of Experimental Pathology and Therapy, U.S.S.R. Although the Soviet workers showed that the disease could be transmitted from monkey to monkey they have not yet isolated the causative agent in either cell culture or small laboratory animals. After isolating SHF virus we tested sera from monkeys involved in the Sukhumi epizootic by a complement-fixation (CF) test with a 20 X concentrated virus-infected cell culture antigen. Professor V. D. Soloviev (Moscow) and Professor B. A. Lapin (Sukhumi) were kind in supplying us (by way of Dr. Robert J. Huebner, NIH) with 36 sera from monkeys involved in their epizootic. We were able to isolate a viral agent from two of these sera and this virus was indistinguishable from the strains isolated from the NIH epizootic.

The occurrence of these two epizootics of a heretofore unknown disease points out again the potential hazard involved in working with monkeys that have not yet been quarantined. Although the Sukhumi serum samples traveled half-way around the world, we had no difficulty in isolating the virus from them. The fact that two epizootics of SHF resulted from the importation of rhesus monkeys from the same area in India into two distantly separated places in the world emphasizes again the known potential dangers involved in the transport and introduction of animals from one area to another. The more recent experience in Germany with a fatal human disease resulting from contact with monkeys (Cercopithecus aethiops) recently imported from Africa is a dramatic and sad illustration of what can happen when the infectious agent is also communicable to man.

Concerning our studies on yellow fever we have developed an avian leukosis virus-free, primary yellow fever virus vaccine seed by ridding the 17D virus vaccine seed of its avian leukosis virus contaminant. By differential filtration through Millipore membrane filters of different pore sizes, yellow fever virus was separated from its contaminant by physical means. Avian leukosis viruses could not be detected in the new primary

and secondary vaccine seeds by RIF, COFAL and FAB tests. The need for a yellow fever vaccine free from contaminating leukosis viruses is obvious.

(Acknowledgement: Dr. Amos E. Palmer was the senior investigator in the clinical and epizootiological aspects of the outbreak of SHF which occurred among the quarantined monkeys here at NIH. Dr. Anton M. Allen conducted the pathological studies. We thank both for allowing us to report summaries of their work).

(Nicola M. Tauraso and Alexis Shelokov)

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

Arbovirus Indicator Study of 1967

The indicator rabbit study was continued for the summer of 1967 at two sites. Site I was in the Helderbergs in Albany County where the elevation was about 1,000 feet. The area had hemlock trees which were close by a running brook and a swamp. A dog-proof pen was constructed under the hemlock branches. Site II was across the Hudson River in Rensselaer County. A second dog-proof pen was constructed among hardwood trees, the underbrush was light and swamps and a pond were close by. Six laboratory rabbits were set out in each pen May 22 and the the experiment terminated September 11, 1967. Bi-weekly bleedings were taken and the serum and clots separated; the sera were stored at -20° C, the clots at -70° C for attempted virus isolation. One set of bleedings a week were examined in a micro HI test with test arbovirus antigens of EE, WE, group A; POW, SLE, group B; MA of BUN group and BFS-283 of the CEV group. Neutralization tests in suckling mice were also done; the test arboviruses were Cache Valley of the BUN group and 65-8569, the New York State isolate of the CEV group. Rabbit 372 showed CEV antibody conversion by July 11, 1967 when the serum had a complement-fixation titer of four with CEV antigen. Neutralizing antibodies were detected the following week, the serum neutralized 2.3 logs of 65-8569 virus. The animal was brought to the laboratory and is still under observation. The CF titer has ranged from 4 to 8 from July 11, 1967 to February 11, 1968, except for October 11, when it was 16. The neutralizing antibodies rose to 3.8 logs September 5 and 11 and then gradually decreased to 2.3 logs by February 13, 1968.

The blood clots collected from Rabbit 372 from May 39 through July 18, 1967 yielded no infectious agent when inoculated into suckling mice by two routes of inoculation - intraperitoneal and intracerebral. Seven days later blind passages were done.

Bunyamwera and California Encephalitis Virus Group Antibodies in Deer Sera from Seneca County, New York

As in previous serologic surveys of deer sera from four other New York State counties, no group A or B reactions were noted by hemagglutination-inhibition (HI) of 50 deer from Seneca County in the last week of December 1967 and first week of January 1968. A number of these specimens, however, had reproducible HI titers with antigens of the Bunyamwera (BUN) group and California encephalitis virus (CEV) group. Neutralization (NEUT) tests in suckling mice were also done with these 50 sera; the test viruses were Cache Valley (CV) of the BUN group, and 65-8569, the New York isolate of the CEV group. In the following table the results of the two serologic examinations are shown and the age groups of the deer are noted.

(Elinor Whitney)

Bunyamwera and California encephalitis virus group antibodies in Seneca County deer sera

Age	No. Tested	Bunyamwera Group				Calif. Encephalitis Virus Group			
		HI		NEUT		HI		NEUT	
		Maguari		Cache Valley		BFS-283*		65-8569**	
		No. React.	%	No. React.	%	No. React.	%	No. React.	%
6 mos.	14	3	21.4	3	21.4	0		0	
1 yr. 7 mos.	20	14	70.0	15	75.0	3	15	10	50
2 yrs. 6 mos.	7	5	71.4	7	100.0	2		5	
3 yrs. 6 mos.	3	2		2		2		2	
4 yrs. 6 mos.	3	1		1		0		3	
6 yrs. 6 mos.	2	1		1		0		1	
7 yrs. 6 mos.	1	0		1		0		0	
TOTAL	50	26	52	30	60	7	14	21	42

HI = Hemagglutination-inhibition
NEUT = Neutralization test in suckling mice
* = Prototype strain
** = New York State isolate

REPORT FROM THE YALE ARBOVIRUS LABORATORIES, YALE UNIVERSITY, NEW HAVEN, CONNECTICUT

Primary and Secondary Response in Monkeys Infected with Dengue Viruses

As part of an investigation on the pathogenic effect of sequential inoculations of dengue viruses into Rhesus monkeys (M. mulatta) a detailed study of the concomitant circulating antibody response was carried out.

The results summarized here derive from tests on about 20 animals thus far studied and do not comprise any serum samples taken later than 8 days after the secondary challenge.

Circulating antibodies have been determined by HI and CF microtests, using a total volume of reagents of four and six drops, or 0.1 ml and 0.15 ml, respectively. However, due to the fact that the CF tests have not progressed as yet far enough, only the HI test results are reported at this time.

In order to remove non-specific inhibitors from the sera used in the HI tests, the kaolin treatment method was used throughout. With sera from a number of animals, a comparison was made between kaolin and acetone treatments; no substantial or systematic difference in titers of antibodies was noticed between the two procedures.

All sera were tested in increasing 2-fold dilutions beginning at 1:10 against 8 units of the following antigens: dengue types 1, 2, 3 and 4, yellow fever, Zika, St. Louis, West Nile and Wesselsbron. The monkeys used in the experiments had been pretested by HI and neutralization plaque tests; they were devoid of circulating antibodies.

Primary response

Four monkeys infected with dengue 1, 6 with dengue 2, 4 with dengue 3 and 6 with dengue 4 have thus far been studied. Samples of serum were taken almost daily during the first 10 days after infection, at wider intervals thereafter.

As of the 10th day after infection, no animal had detectable circulating antibodies except two, which were positive for the first time on that day. One, following inoculation of dengue 1 reacted with a titer of 1:10 against Zika antigen; the other, after injection of dengue 2, reacted at dilutions 1:20 with Zika and SLE.

The next blood sample after the 10th day was taken on the 16th day from monkeys with dengues 1, 2 and 3; on the 12th day, from monkeys with dengue 4.

For the sake of brevity, the essential results of the HI tests have been summarized and are given in Table 1. The table shows composite results; this procedure seemed justified in view of the fact that individual deviations from the mean values given were not out of line.

The main conclusions to derive from the results shown in Table 1 concerning the primary immune response following dengue infection of monkeys, under the experimental conditions stated, are: a) Cross-reactive titers with non-dengue antigens, particularly SLE, Wesselsbron and Zika, were often as high as homologous titers in monkeys infected with dengues 1 and 2. b) Within the dengue systems there was in the mean values as well as in each individual instance a difference between homologous and heterologous titers sufficient to identify the dengue type present in the inoculum. c) During the period covered by the observations, from 0 to 50 days post-challenge, the highest mean titers, whether homologous or heterologous, with all antigens were: between 6 and 7 (titer from 1:320 to 1:1280) for dengue 2; between 3 and 3.5 for dengue 3 (titers were 1:40 or 1:80); and 5 for dengue 4 (titer spread between 1:80 and 1:640).

Secondary response

The number of combinations possible when taking into account the succession of dengue types and the three time intervals planned between challenges, 1 1/2, 3-4 and 6 months, is 48; it is not certain that all combinations can be tested. At this time, HI tests have been completed with serum samples taken daily for 8 or 9 days after the secondary infection from monkeys challenged as shown here:

Primary	Interval, months	Secondary
dengue 1	4	dengue 2
dengue 1	4	dengue 3
dengue 2	4	dengue 1
dengue 2	4	dengue 3
dengue 3	4	dengue 1
dengue 3	4	dengue 2
dengue 3	1 1/2	dengue 3
dengue 4	1 1/2	dengue 2
dengue 4	4	dengue 4

Certain discernible patterns of the secondary response in its earliest period, up to 8-9 days from challenge, are illustrated by Tables 2, 3 and 4.

a) No measurable response, or hardly any, to the second virus. This occurred when the first virus was dengue 2 and the second, dengue 1 or 3.

b) Moderate secondary response. This took place when the first and second viruses were both dengue 3 or dengue 4; and when dengue 3 followed dengue 1.

c) Marked secondary response. It was noted in all the cases tested when dengue 2 was the second virus, the first one being dengue 1, 3 or 4. This marked response also occurred with dengue 3 followed by dengue 1.

It is still too early in the course of this work to derive general conclusions; it would seem, however, that some are more successful than others in bringing about an accelerated and marked antibody rise. It may well be that if dengue 2 or 1 is given first it prevents multiplcation of dengue 3 or 4, given subsequently; as a result the secondary response is due to the mass of the actual antigen in the inoculum, consequently weak. On the other hand, perhaps neither dengue 3 or 4 can effectively prevent multiplication of dengue 2, at least to a point; so that the latter multiplying in a sensitized animal, brings about a sharp increase in the circulating antibodies.

The titers of circulating antibodies 8 days after the secondary infection, in the responsive combinations, were not excessively higher than those that resulted, at any time, after primary inoculation of either dengue 1 or 2. The striking feature was that in these responsive combinations, signs of an immune response were seen in many animals by the 5th day after secondary challenge, and in all by the 8th day; this is in sharp contrast with the primary response in animals after primary inoculation of either dengue 1 or 2 when (see above) no immune response was detectable by the 10th day.

(S. B. Halstead and J. Casals)

Connecticut Encephalitis Survey, June-September 1967

Mr. Arne Saslow, working out of Yale Arbovirus Research Unit, carried out a special encephalitis study in Connecticut in the summer months of 1967. The Virus Diagnostic Laboratory of the State tests material from encephalitis suspect cases with antigens of influenza A and B, adenovirus, psittacosis, respiratory syncytial, EE, WE, SLE, mumps, LCM, herpes simplex, Q, Mycoplasma pneumoniae, and where indicated, rickettsial antigens. The YARU study was concentrated on cases not diagnosed with above antigens, and brought to bear an extended battery of eleven arbovirus antigens, in complement fixation test. These included EE, WE, SLE, Powassan, Modoc, Montana Myotis Leukoencephalitis, California, Cache Valley, Hart Park, Silverwater and Colorado Tick Fever. The intent was to include all arbovirus antigens for which there might be even a remote possibility of occurrence in the region.

A state wide program of case finding, involving local hospitals, local directors of health, summer camp physicians and the epidemiologic services of the State Health Department, served to locate cases and to get necessary serum specimens early in the course of illness and during convalescence. After the State Laboratory screening, there remained 54 cases, with presumed encephalitis but without specific etiologic diagnosis for extended arbovirus investigation. No evidence of complement fixation by any of the 11 antigens was observed in any of the patients.

Remarks

The negative findings with this group of patients are reminiscent of similar findings in similar series of cases investigated elsewhere. If there is arbovirus activity in humans in Connecticut in summer months, it was not detected in this particular season, or in these particular cases, with the battery of antigens used. Historically, both EE and California viruses have been recovered in the State, but not from human beings.

Note: This survey was the result of a joint effort between YARU, Department of Epidemiology and Public Health, Yale University School of Medicine and of the State of Connecticut, Department of Health, Division of Communicable Diseases. The study was supported by Training Grant 5T1-GM-5-11 from the United States Public Health Service, and by Rockefeller Foundation funds.

(W. G. Downs)

Table 1

Primary Antibody Response by Hemagglutination-inhibition.
Monkeys Subcutaneously Inoculated with Dengue Viruses.
Combined Results and Mean Titer Values

Virus	Days After Inoculation	Antigen, 8 units								
		D 1	D 2	D 3	D 4	YF	Zika	SLE	WN	WESS
Dengue 1	16	*5	3	3.5	3.5	3	5	1	-	4
	19	5.5	3.5	3.5	5	3	6	4	5	5
4 monkeys	30	5	3.5	3.5	4	2.5	5	3	-	4
	41	5	3	3.5	4	2.5	5	3.5	5	5
	52	5	3	3.5	3	2	4	2	-	3
Dengue 2	16	1	5	2.5	3	1.5	4	4	-	3.5
	19	3.8	7	4.3	6.8	4.3	6.5	6.3	6.0	6.5
6 monkeys	30	1.5	5	2	3	1	4	4	-	3.5
	41	4.5	6.8	4	6.3	4	6	6	5	6.5
	52	2	6.5	3	3	2	5	4.5	-	4.5
	16	0	0.5	3.5	1	0.5	1.5	2	0.5	2
Dengue 3	19	0	0	2	0	0	0	0.5	0	0
	30	0.5	0.5	3	1	0	2	2	0.5	2
4 monkeys	41	0	0	2	0	0	1	1	0	0.5
	52	0.5	0.5	3	1	0.5	2	2	0.5	2
Dengue 4	12	0	0	0	0.8	0	0.3	0.3	0.3	0.3
6 monkeys	21	1.5	2	1.7	5	2	3	2	1	3.3
	42	0.7	1.2	1	3.9	1.2	3.2	3.2	2	2.9

* For each day an antigen is given the mean titer of the sera tested. The titers are expressed as: 1 for 1:10, 2 for 1:20, 3 for 1:40, 4 for 1:80, etc.

Table 2

Infection with Dengue Viruses. Hemagglutination-inhibition.
Challenge by second virus resulting in no or hardly
detectable response within 8 days

Antigen 8 units	Sequence of dengue viruses and days after second inoculation							
	Type 2 followed by 1				Type 2 followed by 3			
	0	5	8	*change	0	5	8	change
Dengue 1	#3	3	3	0	5	5	6	1
Dengue 2	7	7	7	0	7	7	7	0
Dengue 3	4	4	4	0	4	4	4	0
Dengue 4	7	7	7	0	5	5	5	0
Yellow Fever	2	2	3	1	4	5	5	1
Zika	6	6	6	0	7	7	7	0
SLE	5	5	6	1	7	7	7	0
WN	8	8	8	0	4	5	5	1
Wesselsbron	6	6	6	0	7	7	7	0

* Change: increase in titer between 0 and 8th day expressed as number of 2-fold dilutions.

\# For titers, see Table 1.

Table 3

Infection with Dengue Viruses. Hemagglutination-inhibition.
Challenge by second virus resulting in a moderate response within 8 days

Antigen 8 units	Sequence of dengue viruses and days after second inoculation											
	Type 1 followed by 3				Type 3 followed by 3				Type 4 followed by 4			
	0	5	8	change	0	5	8	change	0	5	8	change
Dengue 1	6	6	7	1	0	0	1	1	1	1	2	1
Dengue 2	4	4	5	1	0	0	1	1	1	2	2	1
Dengue 3	4	4	6	2	2	4	4	2	2	2	3	1
Dengue 4	4	4	5	1	0	0	1	1	4	5	6	2
Yellow Fever	3	4	4	1	0	1	1	1	2	2	3	1
Zika	5	5	6	1	0	2	2	2	3	5	5	2
SLE	3	4	4	1	1	2	3	2	3	4	4	1
WN	2	2	3	1	0	1	2	2	3	4	4	1
Wesselsbron	5	5	6	1	1	2	3	2	3	4	4	1

See Table 2

Table 4

Infection with Dengue Viruses. Hemagglutination-inhibition.
Challenge by second virus resulting in marked
response within 8 days

Antigen 8 units	Sequence of dengue viruses and days after second inoculation															
	Type 1, then 2				Type 3, then 1				Type 3, then 2				Type 4, then 2			
	0	5	8	change	0	5	7	change	0	5	8	change	0	5	8	change
D 1	6	6	8	2	0	0	2	2	0	0	1	1	0	0	3	3
D 2	4	4	8	4	0	0	2	2	0	0	2	2	0	0	3	3
D 3	4	4	6	2	1	2	4	3	2	2	3	1	0	0	3	3
D 4	4	4	6	2	0	0	3	3	0	0	1	1	3	3	4	1
YF	3	3	5	2	0	0	2	2	0	0	2	2	0	0	2	2
Zika	6	6	8	2	0	2	4	4	0	1	4	4	2	3	5	3
SLE	3	3	5	2	1	3	5	4	0	2	4	4	2	2	5	3
WN	5	5	7	2	0	0	2	2	0	0	1	1	0	0	3	3
Wess	5	5	7	2	0	2	5	5	0	1	3	3	1	2	5	4

See Table 2.

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

Presence of Virus in Anal Discharge of Sindbis-infected Aedes aegypti

Virus was discharged from the anus of approximately 60% of 156 female A. aegypti infected with Sindbis virus while the mosquitoes were feeding on infant mice. The virus was recovered from the anal discharge during feeding and for a period of 10 minutes thereafter. The quantity ranged from 1-180 PFU per mosquito. This virus is identical with Sindbis virus stock (Strain MP-684) in its pathogenicity for infant mice, plaque development in chick embryo cell culture, and neutralization by Sindbis antiserum. The presence of arbovirus in the anal discharge of infected mosquitoes has been reported solely by Aragão and Costa Lima who worked with A. aegypti infected with yellow fever virus (Mem. Inst. Cruz, Suppl. #8, 1929, 105).

Virus that was expelled from the anus did not derive from ingested salivary fluid. This was established by demonstration of virus in the anal discharge of infected mosquitoes in which the "food" (labrum) and "salivary" (hypopharynx) channels were held in separate capillary tubes. Further studies suggested that the malpighian tubules might be the immediate source of the "anal" virus.

The "Posterior Route" of infection appeared not to be important in the infection of infant mice. Sealing the anus of infected mosquitoes did not reduce the rate of transmission. Surprisingly, females in which the salivary ducts had been cut, continued to expel fluid-containing virus through the hypopharynx. Thus it was not possible to directly test whether transmission would occur with the blocking of the "Anterior Route".

However, the presence of virus in the anal fluids of infected mosquitoes may be of epidemiological significance. A larger quantity of virus was detected in fluids collected from the anus than from the mouthparts of the infected females. Infant mice could be infected by topical application of virus to the site of the mosquito bites, to freshly abraded skin, and to the nasal mucosa. Moreover, freshly engorged mosquitoes were observed to remain in contact with their experimental hosts for several minutes after feeding. During this time, coarse droplets of fluid were discharged from the anus and, frequently, a fine spray was released into the air. The role of such "Aerosols" in the transmission of arbovirus in nature merits definition.

REPORT FROM THE VIROLOGY AND WILDLIFE DIVISIONS, UNIVERSITY OF GUELPH, GUELPH, ONTARIO, CANADA

Mechanical and Biological Transmission of Cocal Virus from Bats (*Myotis l. lucifugus*) to Suckling Mice by *Aedes aegypti* Mosquitoes

Twenty-two hiberanting little brown bats (*Myotis l. lucifugus*) were taken from a local hibernaculum and kept at 24° C (relative humidity 60-80%) for one week before being inoculated subcutaneously with 0.2 ml each of Cocal virus (9th passage BHK-21 cells, titer $10^{-7.5}$ $TCID_{50}$/1.0 ml).

Two bats were killed daily for 11 days beginning 24 hours after virus inoculation and blood, brown fat, kidneys and spleen were tested for virus by inoculation into BHK-21 cells. Cocal virus isolates were confirmed by serum neutralization with specific hyperimmune guinea pig serum.

Bats were viremic for the duration of the experiment (11 days).

Two viremic bats (11 days post inoculation) were physically restrained and exposed to 30 *Aedes aegypti* mosquitoes for 10 hours. Commencing 12 hours after the bats had been removed from contact with the mosquitoes litters of six suckling mice (2-4 days of age) were exposed to the mosquitoes for 4 hours each day. Deaths of suckling mice are recorded in Table 1.

Table 1

Transmission of Cocal virus from Bats to suckling mice by *A. aegypti* mosquitoes

Days after infection of mosquitoes	1	2	3	4	5	6	7	8
Ratio, mice which died of Cocal infection: mice exposed to mosquitoes	1/5	0/5	0/5	3/5	4/5	1/5	2/5	0

Mice were not exposed to *A. aegypti* after 8 days as the mosquitoes had died off at this stage.

The brains of dead mice were removed and Cocal virus isolates were confirmed either by serum neutralization or direct fluorescent antibody testing.

The 11 day period of viremia for bats infected with Cocal virus is the longest which has so far been reported for an animal infected with V.S.V.

The above experimental work is presently being repeated and the persistence of Cocal virus in bats kept at lower temperatures is being studied.

(Alex I. Donaldson and Lars Karstad)

Editorial Note:

Tables to accompany the Report of the Belem Virus Laboratory in Issue #16 of the Arbovirus Information Exchange were inadvertently ommitted. The entire report - with tables - is reproduced in this subsequent issue.

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

The 242 viruses isolated during the first half of 1967 at the Belem laboratory are summarized by type and source in Table 1.

Of particular interest are the 59 isolations of Amapari virus from Oryzomys goeldii (25) and Neacomys guianae (34) trapped at Serra do Navio, Amapá Territory. This Brazilian member of the Tacaribe Group has been the subject of a special study since its first isolation in July 1964. Amapari isolation since 1964 are presented in Table II. It will be seen that the rate of positive Oryzomys and Neacomys has risen from 4% and 2% respectively to 20% in early 1967. This rise may partly be explained as due to the introduction in July 1966 of a different technique for detecting Amapari infected mice. The method consists in sacrificing an inoculated baby mouse between the 10th and 14th day, regardless of its apparent state of health and testing the brain for CF antigen. Results of comparative tests run in 1966 are as follows:

No. of specimens	CF Positive	Pos. by clin. signs or death
410	32	5

Table III details the isolations made by source of materials tested. During the first half of 1967, 22% of 36 Oryzomys urine samples were positive as as were 10% of 61 Neacomys urine samples. In this characteristic, Amapari virus shows a strong resemblance to Machupo virus. Also noteworthy are the isolations from embryos and newly born infants.

Early in March, the second known outbreak of Oropouche virus involving humans was uncovered when a laboratory worker, on holiday near Bragança (130 miles east of Belem), suffered a mild illness and Oropouche virus was isolated on the third day from his blood. A subsequent investigation resulted in the recovery of virus from nine additional people hospitalized in Bragança. CF studies indicated the existence of additional subclinical cases. Virus was also isolated on one occasion from blood-engorged Culex pipiens quinquefasciatus collected in the Bragança hospital. The first and much larger outbreak of Oropouche virus occurred in Belem in 1961.

Of particular note are 14 isolates from sentinel mice exposed in the upper levels of the forest. A climber was used to attach pulleys to branches high in trees. A nylon cord was then strung through these as well as through two other pulleys fixed at ground level and then tied top and bottom to cage or trap. By means of a system of counter weights, the cage could be raised to any desired height. One such device was used for hoisting and exposing a caged sentinel chicken and a cage of sentinel mice to a height of 12 meters. A second was used for hoisting a blower trap to 16 meters. A third unit was used for hoisting to 30 meters alternately either a mouse-baited blower trap or a light trap which had a blower mechanism attached to it.

The 14 viruses isolated from sentinel mice in these aerial traps are as follows:

12 Meters	16 Meters	17 1/2 Meters
Marituba (1)	Marituba (1)	Marituba (1)
Oriboca (2)	Apeú (1)	AN 109303 (2)
EE (1)		
AN 109303 (4)		
AN 116382 (1)		

It is significant that the dominant mosquito caught in the traps at these heights was Culex (Melanoconion) portesi - a species perhaps responsible for the majority of virus transmission in the experimental area.

(Staff of the Belem Virus Laboratory)

Table I

Virus isolations at Belem Virus Laboratory for period January-June, 1967

Virus	Human	Sentinel mice	Sentinel monkey	Wild animals	Arthropods	Total
EE		6			1	7
Bussuquara				1		1
Oriboca		12			1	13
Marituba	1	4				5
Apeú		2				2
Caraparú		35	1	1		37
Itaquí		21				21
Nepuyo		2				2
Guamá		11	1	2		14
Catú		11		1	1	13
Mojú		9		3	1	13
AN 109303		7		1		8
AN 116382		2				2
AN 117565					1	1
Capim		4		1	1	6
Guajará		6			1	7
Bushbush		2				2
AN 84381		1				1
Mirim		6				6
Wyeomyia		1				1
Oropouche	10				1	11
Itaporanga		2				2
Amapari				59		59
Acará		3				3
?		1				1
EMC				3	1	4
Total	11	148	2	72	9	242

Note:
Multiple isolations of same virus from same sentinel group or animal counted only once.

Table II

Isolations of Amapari virus from Oryzomys & Neacomys
1964-1967 (January-June)

Year	Oryzomys			Neacomys		
	Positive	Tested	% pos.	Positive	Tested	% pos.
1964	2	45	4	1	56	2
1965	5	97	5	6	124	6
1966	14	173	8	33	179	18
Jan-Jun'67	25	128	20	34	153	20

Table III

Amapari isolations by virus source from Oryzomys & Neacomys
during 1966 and 1967 (January-June)

Materials tested	Oryzomys				Neacomys			
	Reactors		% Pos.		Reactors		% Pos.	
	1966	1967	1966	1967	1966	1967	1966	1967
Blood	9/162	§17/125	6	14	13/161	14/132	8	11
Viscera	12/167	21/130	7	16	30/175	24/153	17	16
Urine	0/42	8/36	-	22	3/17	6/61	12	10
Embryos	0/10	2/18	-	11	2/9	3/12	22	25
Babies	1/5	1/6	20	17	0/0	0/1	-	-

§ Numerator = nº positive, denominator = nº tested.

EDITORIAL NOTE

In the last issue of the Arthropod-borne Virus Information Exchange announcement was made of the forthcoming VIIIth International Congresses of Tropical Medicine and Malaria to be held in Teheran, Iran, from 7 - 15 September 1968. Since the 1953 Congresses in Istanbul, the number and proportion of papers being presented on arboviruses has steadily increased, reflecting the growing importance of these diseases in tropical medicine.

The 1958 meeting in Lisbon brought together the largest number of arbovirologists ever assembled in one place. The two days of meetings organized by Dr. Arturo Saenz launched the World Health Organization program in Arbovirus Diseases. In the past decade the W.H.O. has organized special study groups on pertinent problems in arbovirology, two of which resulted in publication of reports in the W.H.O. Technical Report Series (#219 in 1961, #369 in 1967). Initiation of Dr. Saenz' special W.H.O. Arbovirus Program was encouraged by Dr. Anthony Payne as W.H.O. Chief of Virus Diseases, and since 1961 continuously supported by Dr. Charles Cockburn, who succeeded Dr. Payne.

It was in later years that an active and responsive global network of W.H.O. Regional Reference and participating Arbovirus Laboratories was established. Particular interest in yellow fever began with W.H.O. support of investigations of the Ethiopian yellow fever epidemic in 1961. This has now developed into long term participation in the East African Virus Research Institute in Entebbe, Uganda, and in an extensive yellow fever surveillance effort which has been developed in West Africa.

The arbovirus sessions at the 1963 International Congresses in Rio de Janeiro focused on the ecology of arboviruses, with special attention by the Pan American Health Organization (Regional Office of W.H.O. for the Americas) on potential arbovirus disease problems in the great river basins and continent of South America.

A decade after the outstanding Lisbon Congresses, it appears time again to assess arbovirus disease problems on a global basis. This is the theme of the program for Section A.5.1 Arbovirus Infections and Diseases at the Congresses in Teheran in September. Because the program will again bring together arbovirologists from all over the world and because it reflects again the increasing importance of arbovirus diseases, the final program is included in this issue of the Arbovirus Information Exchange.

It should be noted that the hemorrhagic fevers and dengue have such expanded concern the world over that a separate Section under Chairmen Karl Johnson of Panama, M.P. Chumakov of the U.S.S.R., and Lim Kok Ann of Singapore has been scheduled as a separate series of sessions. The program for these was not available in time for this issue.

The deadline for the next issue #18 of the Arthropod-borne Virus Information Exchange will be 1 October 1968.

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FINAL PROGRAM
FOR
SECTION A.5.1 ARBOVIRUS INFECTIONS AND DISEASES
OF THE
VIIIth INTERNATIONAL CONGRESSES OF TROPICAL MEDICINE & MALARIA

Teheran, Iran September 7 - 15, 1968

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Section A.5.1 Arbovirus Infections

Chairman Professor T. H. Work
Division of Infectious
and Tropical Diseases
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A.5.1.1 Pathobiological Aspects

Vice Chairman :	O. R. Causey	Nigeria/USA
Organizer :	M. Mussgay	Fed. Rep. of Germany

SESSION 1

Structure of Horse Sickness Virus Y. Ozawa (Iran)	10 minutes
Structure of Tick-borne Encephalitis Virus I. Slavik (Czechoslovakia)	10 minutes
Neutralization of Arboviruses by Antibodies P. R. Osterrieth (Belgium)	10 minutes
Biochemical Aspects of Arbovirus Multiplication R. M. Friedman (USA)	10 minutes
Photodynamic Effects on Infectivity of Purified Group A Arboviruses D. W. Hill (USA)	10 minutes

Genetic Marks of Tick-borne Encephalitis Virus V. Mayer (Czechoslovakia)	10 minutes
Genetic Characteristics and Immunogenic Activity in Attenuated Varieties of Tick Encephalitis and Malayan Langat Viruses E. N. Levkovich and L. G. Karpovich (USSR)	10 minutes
Study of Some Genetic Symptoms of Japanese B Encephalitis Virus N. V. Logvinova (USSR)	10 minutes
Characters of Attenuated Strains of Japanese Encephalitis Virus in Respect to Its Genetic Stability and Cultural and Immunogenic Particularities V. V. Pogodina (USSR)	10 minutes

SESSION II

Detection of TBE Virus Proteins by Immunofluorescence Staining C. Kunz (Austria)	10 minutes
Studies on the Pathogenesis of Tick-Borne Encephalitis Virus D. Malakova (Czechoslovakia)	10 minutes
Propagation of Arboviruses in Mosquito Cell Cultures K. R. P. Singh (India)	10 minutes
Behaviour of Plague Variants of Group A Arboviruses in Aedes aegypti mosquitoes S. R. Pattyn (Belgium)	10 minutes

Free Communications

A.5.1.2 - 3 Clinical, Pathological, Therapeutic, Epidemiological and Control Aspects of Arbovirus Infections

Vice Chairman :	T. R. Rao	India
Organizers :	C. Serie	French Guiana/France
	W. G. Downs	U.S.A.

SESSION I

Yellow Fever (Introduction) with Special Notes on the 1961-62 Yellow Fever Epidemic in Ethiopia C. Serie (French Guiana/France)	10 minutes
Clinical and Epidemiological Significance of Recent Yellow Fever in Ethiopia: The Recent Outbreak in Sidamo, Ethiopia B. Teoume-Lessan (Ethiopia)	10 minutes
Epidemiological Considerations of the 1965 Yellow Fever Epidemic in Senegal P. Brès (presented by L. Chambon, Senegal/France)	10 minutes
Pathological Findings in the Yellow Fever Fatalities in Senegal Camain (Senegal/France)	5 minutes
Biochemical Investigations Yellow Fever M. Rey (Senegal/France)	5 minutes
Ethnic Groups as Potential Disseminators of Yellow Fever in West Africa G. Pichon, J. Hamon and J. Mouchet (France)	5 minutes
Yellow Fever in Portuguese Guinea M. R. Pinto (Portugal)	5 minutes
Yellow Fever in East Africa B. Henderson (Uganda/USA)	5 minutes
Yellow Fever in Brazil J. P. Woodall (Brazil/UK)	5 minutes

International Implications of the Recent Resurgence of Yellow Fever — 10 minutes
A. C. Saenz (W.H.O /Argentina)

Japanese B Encephalitis

Recent Epidemic of JBE in Japan — 10 minutes
A. Oya (Japan)

Recent Epidemics of JBE in Korea — 5 minutes
H. W. Lee (Korea)

Extent of Japanese B Encephalitis in Thailand — 5 minutes
T. Yuill (Thailand/USA)

SESSION II Epidemic Arbovirus Diseases of Timely Importance

Venezuelan Equine Encephalitis

Epidemic VEE in Venezuela — 15 minutes
J. Avilan (Venezuela)

The Virological Nature of the VEE Complex — 10 minutes
R. Shope (USA)

St. Louis Encephalitis

St. Louis Encephalitis in the Caribbean (A.R.) — 10 minutes
J. Bond (USA)

St. Louis Encephalitis in Texas — 10 minutes
B. F. Hollinger and
T. H. Work (USA)

St. Louis Encephalitis in the Mississippi Valley — 10 minutes
R. Kokernot (USA)

Expansion of St. Louis Encephalitis in U.S.A. — 10 minutes
M. Goldfield (USA)

California Encephalitis

Emergence of California Encephalitis as a Rural Disease in North America — 10 minutes
W. H. Thompson (USA)

Implications of Antigenic Differences in the California Complex — 10 minutes
G. E. Sather (USA)

SESSION III Arbovirus Diseases of Continental Significance

Arbovirus Disease in Australia — 15 minutes
R. L. Doherty (Australia)

Arboviruses Recently Isolated from the Sepik District of New Guinea — 5 minutes
I. Marshall (Australia)

Zika Virus in Malaya — 5 minutes
N. J. Marchette (Malaysia)

Arbovirus Infections in Indonesia — 5 minutes
S. Hotta (Japan)

Eruption of New Arbovirus Diseases in Asia — 15 minutes
T. Rao (India)

Practical Aspects of Serological Epidemiology of Arbovirus In India — 5 minutes
K. Pavri (India)

Mechanical Transmission of Chikungunya Virus — 5 minutes
S. Paul (India)

Chandipura Virus Infection in India — 5 minutes
P. N. Bhatt (India)

Kyasanur Forest Disease in India — 10 minutes
P. K. Rajagopalan (India)

Arbovirus Infections in Ceylon — 10 minutes
J. Vesenjak-Hirjan (Yugoslavia)

West Nile Fever in the Middle East — 10 minutes
N. Goldblum (Israel)

SESSION IV Arbovirus Diseases of Continental Significance

Current Arbovirus Disease Problems in Africa — 15 minutes
B. McIntosh (South Africa)

The Role of Arboviruses in the Etiology of Exanthemic Fevers in Central Africa — 5 minutes
L. Chambon (Senegal/France)

West African Tick-borne Viruses O. R. Causey (Nigeria/USA)	5 minutes
Simbu Group Viruses in Nigeria C. Causey (Nigeria /USA)	5 minutes
Investigations of the Animal Reservoirs of Arboviruses in the Region of Yaounde' A. Poirier (Cameroun/France)	5 minutes
Vectors of Arboviruses in Senegal R. Taufflieb (Senegal/France)	5 minutes
Arbovirus Diseases Emerging in Europe M. Greshikova (Czechoslovakia)	15 minutes
West Nile Fever in the Rhone Delta C. Hannoun (France)	5 minutes
Arbovirus Infections in Corsica B. Corniou (France)	5 minutes
Experimental Verification of Theoretical Prognosis of Distribution of Mosquito-Borne Viruses in Some Parts of the USSR D. Lvov (USSR)	5 minutes
Mosquito-Borne Viruses in the USSR S. Ya. Gaidamovich (USSR)	5 minutes
Vectors of Arbovirus Infections in the Focus of Equine Encephalitis in Eastern Kazakhstan V. Anufrieva (USSR)	
Tick-Borne Encephalitis in the USSR B. Semenov (USSR)	5 minutes
Tick-Borne Encephalitis in Central Europe C. Kunz (Austria)	5 minutes
Arboviruses and Diseases in Scandinavia N. Oker-Blom (Finland)	5 minutes

SESSION V Arbovirus Diseases of Continental Significance

Dynamics of Epidemic Western Equine Encephalitis R. Hayes (USA)	10 minutes

Dynamics of Epizootic Eastern Equine Encephalitis R. Chamberlain (USA)	10 minutes
Arbovirus Diseases of Central America K. Johnson (Panama/USA)	10 minutes
The Sandfly Fevers J. Schmidt (Ethiopia/USA)	5 minutes
Epidemic Oropouche Virus Disease F. Pinheiro (Brazil)	5 minutes
Arbovirus Infections in Residents of British Honduras D. Bertram (UK)	5 minutes
Free Communications	

SESSION VI Timely Topics on Arbovirus Vectors and Reservoirs

Arboviruses and Ticks J. Casals (USA)	10 minutes
Arboviruses in Bats N. Karabatsos (USA)	10 minutes
Bats as Reservoir Hosts for Arboviruses (A.R) S. Sulkin (USA)	5 minutes
Avian Hosts of Arboviruses in Europe D. Blaskovic (Czechoslovakia)	10 minutes
Arboviruses in Poikilotherms J. McLintock (Canada)	10 minutes
Arboviruses from Sentinel Animals W. Scherer (USA)	5 minutes
Non-Culicene Vectors of Arboviruses G. De Foliart and R.P. Hanson (USA)	10 minutes
Free Communications	

SESSION VII Arbovirus and Associated Zoonotic Disease: Open Session

A Yellow Fever Vaccine Free from Contaminant Viruses C. Draper (UK)	5 minutes

Attenuated Japanese B Encephalitis Vaccines K. Inoue (Japan)	5 minutes
Prophylaxis with Inactivated JBE Vaccine in Japan M. Kitaoka (Japan)	5 minutes
Attenuated Western Equine Encephalitis Vaccine H. Johnson (USA)	5 minutes
Low Virulence TBE Viruses as Living Vaccine A. Dubov (USSR)	5 minutes

Open Discussion

Propagation of Mount Elgon Bat Virus in *Aedes aegypti* Mosquitoes D. Metselaar (Netherlands)	5 minutes
Jamshedpur Fever J. John (India)	5 minutes

Free Communications

SPECIAL REPORT FROM THE YALE ARBOVIRUS RESEARCH UNIT

New Approaches to the Preparation of Hemagglutinating Antigens for the California Encephalitis Group of Viruses

Despite the fact that some members of the California encephalitis group of viruses exhibit low but workable hemagglutinating properties after sucrose-acetone extraction, several of them still have not yielded satisfactory HA antigens. Therefore we believed that it could be of interest to apply sonication and trypsin treatment to the production of effective antigens for this group of viruses to permit cross-antigenic comparison by hemagglutination-inhibition test (HI).

The source of antigen was infected baby mouse brains sucrose-acetone extracted, according to the technique described by Clarke and Casals (Amer. Jour. Trop. Med. & Hyg., 7: 561, 1958). Sonication* periods were of two minutes for four times with one minute cooling period between each burst. Effective cooling was achieved by the use of an NaCl-ice-water bath with magnetic stirring in which the tube containing the antigen was immersed. The sonicated antigen was then centrifuged for 10 minutes at 800 G. Antigens were then tested for HA activity over a large pH range. Tests were incubated after adding cells at 37° C, room temperature and 4° C.

If sonication did not give a satisfactory HA titer, subsequent trypsin treatment was undertaken. To 1 volume of sonicated antigen, 1 volume of a 512 μg per ml solution of Difco trypsin (1:250) in borate saline pH 9.0 was added. The final concentration of Difco trypsin was thus 256 μg per ml. The antigen-trypsin mixture was incubated 50 minutes at room temperature (approximately 22° C) and the action of trypsin promptly terminated at the end of the 50 minutes incubation by addition of soybean trypsin inhibitor (Worthington crystallized inhibitor). The inhibitor was added at a weight equal to total weight of Difco trypsin present. Such treated antigens were tested for HA activity and checked by HI for specificity against homologous, heterologous and normal mouse antisera. The trypsin treated antigens were then left overnight at 4° C and a final titration was done at that point. This "development" period at 4° C very often leads to an increase in HA titer.

At this point it is important to introduce a note of caution about the use of trypsin. Higher trypsin concentration, incubation at 37° C or prolonged

*Branson Sonifier, model W-185-C, distributed by Heat Systems Company, Melville, Long Island, New York.

action of the trypsin can sometimes lead to a non specific HA, giving a pattern that produced by an arbovirus. Its presence is suspected by the occurrence of a rather wide pH range and it can be proven by the fact that it is inhibited by all immune or normal antisera. For this reason, trypsin treatment is carried out at room temperature with moderate quantities of the enzyme and its action stopped promptly by addition of soybean inhibitor.

HA results obtained with the California group of viruses are shown in the table.

Tahyna, California, San Angelo, La Crosse and Snowshoe Hare antigens had their HA titer significantly increased by sonication alone. The increase in titer varied from 32 to 512 fold.

Of interest were the results obtained with two different pools of Tahyna strain Caspius D. One showed an increase in titer of 128 to 256 fold by sonication alone. The other pool required sonication plus trypsin treatment to unmask its hemagglutinin properties to a workable titer. We do not yet have any valid explanation for this, but we suspect a difference in the quality of the sucrose-acetone extraction step which rendered the unmasking of HA properties more difficult.

The results obtained with Jamestown Canyon and Simsbury California antigens were quite unexpected. Both gave a low but workable antigen by sonication, but their HA activities were inactivated by trypsin treatment. It is of interest that they have been found closely related if not identical by preliminary HI crossing test. Such low titered HA antigens were concentrated 2-fold by centrifugation at 81,000 G for one hour, the pellet being resuspended by sonication in 0.4% Bovalbumin in borate saline pH 9.0 .

Trivittatus, Lumbo and Keystone antigens required both sonication and trypsin treatment to unmask their HA properties. Trivittatus had to be concentrated two-fold by centrifugation at 81,000 G.

Melao virus antigen had an intermediate reaction to the sonication and trypsin treatment. It responded to sonication to a certain extent but trypsin had no effect on the hemagglutinin obtained by sonication.

The last two viruses, Be Ar 103645 and Sp An 5881 did not show any trace of HA activity either after sonication nor after trypsin treatment.

It has been found that very often better titers are obtained if the HA tests are incubated at 37° C after the cells are added.

It is of some interest to note how viruses within the same antigenically related group vary greatly in the ease with which satisfactory hemagglutinins may be obtained. In addition, and may be more striking, are the different way in which sonicated hemagglutinins react to trypsin treatment within the California group of viruses. They can fall into three categories:

1) Those having a positive reaction to trypsin. This is those requiring trypsin to realize a satisfactory hemagglutinin.
2) Melao sonicated hemagglutinin which is not affected in either way by trypsin.
3) Those having a negative reaction to trypsin, their sonicated hemagglutinin being inactivated by trypsin treatment.

HI cross antigenic comparison of the California group of viruses is now in progress.

(Dr. Pierre Ardoin, Dr. Bernard Corniou, and Dr. Delphine Clarke)

HA Results

	Sucrose Acetone		Sonication		Sonication + Trypsin		Concentration by centrifugation		pH
incubation temp.	RT	37°C	RT	37°C	RT	37°C	RT	37°C	
Tahyna	64-128	64	2048	2048					5.8
California	16	16	256-512	512					5.8
Tahyna Caspius D	0*	0	128-256	128-256					5.8-6.0
Tahyna Caspius D	0	0	32		256-512				5.8-6.0
Trivittatus	0	0	4-8	8	64-128		512-1024		6.0
Lumbo	0	0	4-8	8-16	512-1024	512			6.0
Jamestown Canyon	0	0	32	32-64	inactivated by trypsin		128		6.2
San Angelo	0	0	512-1024	512					5.8
La Crosse	4-8	8	512-1024	1024					6.0
Keystone	0	0	4-8	8-16	64	256-512			6.0
Melao	0	0	32	64	unchanged by trypsin				6.0
Snowshoe Hare	8-16	8-16	256-512	512-1024					6.0
Simsbury Calif.	0	0	16	16-32	inactivated by trypsin			32-64	6.0
Be Ar 103645	0	0	0	0	0	0			
Sp An 5881	0	0	0	0	0	0			

*<4