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

ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

Number 16 January 1967

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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

Dr. Hammon, acting as Chairman of the ACAV at the open meeting in San Juan, Puerto Rico, on November 1, 1966, read the following as a suggestion for a new committee activity.

A Subcommittee of ACAV on Acceptance of Names for New Viruses in the Arbovirus Catalogue

"It has for a long time been recognized as a problem in respect to new catalogue entries, that no one individual or any committee accepted the responsibility for saying that a new virus was sufficiently different from another one in the catalogue to be given a name as a new virus rather than to be considered as a strain of a previously named virus. The Committee on the Catalogue and Information Exchange has carefully avoided this responsibility. As a result, there are probably numerous instances of cards in the catalogue which many would consider strains of another virus, yet they bear another name. I would like to suggest that the chairman of the ACAV appoint Dr. Jordi Casals as chairman of a subcommittee with a suitable name to be chosen, to which these problems would be referred. They would systematically review the present cards in the catalogue and make decisions.

I discussed this with a number of arbovirologists attending the Pacific Science Congress in Tokyo, and all approved of this heartily. The Americans consulted agreed that at first this was probably going to have to be an American Committee, preferably one that could meet from time to time, and certainly correspond readily and quickly. Subsequently, when the Committee on Arthropod-borne Viruses is more thoroughly internationalized, this may require an international subcommittee, even though the difficulties of getting together for a long enough period of time and at frequent enough intervals to handle the job satisfactorily would be many. Perhaps even at the beginning, one or two international referees might be added as unofficial members of the committee, and abstracts of some of the discussions and copies of the recommended decisions be sent to them for comment and consideration before final action was taken."

After a short period of discussion, Dr. Hammon's suggestion was voted upon by the membership present and overwhelmingly endorsed (one negative vote).

Dr. Hammon and Dr. Casals then selected the committee with the

following membership, Miss Gladys E. Sather, Dr. William F. Scherer, Col. E.L. Buescher, and Dr. Charles L. Wisseman, Jr.. An appropriate name was then selected, which is Subcommittee on Immunologic Relationship Among Catalogued Arboviruses, and abbreviated to SIRACA. The committee has held two meetings, the first on 20 March 1967 and the second on 14 June 1967 at Cornell Medical School, New York. The first portion of the report for the June 14th meeting follows:

Report of the SIRACA Meeting on June 14, 1967

For various practical considerations the Subcommittee had decided to take up first the viruses in group B. It was very clear to us, however, that regardless of the viruses to be examined, the first working session would be mainly dedicated to try to evolve a set of guidelines on which our conclusions were to be generally based.

1. Mandate

There is hardly need to repeat here that the mandate from the Chairman, ACAV, to SIRACA was clearly stated: "To examine the cards of the viruses registered in the Catalogue and decide on immunological grounds only which constitute repeated entries."

2. Sources

It soon became apparent to the Subcommittee that in many instances, with group B viruses, the cards did not contain enough immunological data to justify a registration, if the cards alone were considered. Obviously the Subcommittee could not be guided only by statements in the cards; results reported in the literature, whether referred to in the cards or not, were taken into consideration. This applies particularly to the "older" viruses; it stands to reason that, for example, viruses such as yellow fever, RSSE or JBE should have a card in the Catalogue even if the card were to have no immunological data whatever, for the reason that accumulated general knowledge justifies it. It is, however, up to the registrant of a newly submitted agent to supply evidence showing that the new agent is not yellow fever, RSSE, JBE, etc., if he intends to have it accepted.

In addition to the data given in the cards and in the literature, the Subcommittee members have looked, to the best of their ability, into sources not usually considered references, such as annual reports from various laboratories, unpublished reports from individuals, etc., in an effort to gather data to help them. The Subcommittee felt, however, that it could accept an opinion only when some sort of a document existed to back it up.

3. Criteria

Reduced to bare essentials the question before the Subcommittee is: given 2 strains, X and Y, is the immunological evidence available sufficient to consider them as different viruses or not?

The crucial matter in guiding the decisions of the Subcommittee is that of the criteria to follow. These criteria can be considered at two levels: A, the type of immunological tests that are to be taken into consideration, B, interpretation of the tests' results.

A. Tests - Immunological data can, obviously, be gathered by a number of different tests. Some tests tend to accentuate differences between related viruses, others tend to minimize them; some tests have been extensively used in the past, others have come into use or application to the arboviruses in recent years; other tests have been used only for special studies. The Subcommittee agreed that the main basis for its decisions would be the results of tests generally and extensively used up to now; in addition we agreed that it was definitely not within our jurisdiction, nor indeed that we had the knowledge, to advise or tell investigators what sort of serological tests they are to perform with their viral strains. Investigators submit results, as recorded in the cards or other sources, and the Subcommittee's function is to do its best to give an estimate as to whether these results make two registered viruses distinct or not.

The tests whose results the Subcommittee mainly considered were neutralization, complement-fixation and hemagglutination-inhibition. This does not mean that the results of other tests, cross-challenge, fluorescent antibody technique, immuno-diffusion, immunoelectrophoresis, etc., are unacceptable; it simply means that results with neutralization, CF and HI are generally available, while with other tests they are not.

Within group B, the neutralization test by intracerebral route of inoculation in mice gives, generally, the greater specificity, followed by the complement-fixation test, with the hemagglutination-inhibition giving the higher overlap; in this group, therefore, the results of the neutralization test are essential in defining serotypes.

The acceptance of the neutralization test as the touchstone in group B, and probably in other groups as well, greatly complicates the task of evaluation, because the next question that arises is what type of neutralization test is to be considered. As is well known the choice is great, and so is the number of reported results: intracerebral and intraperitoneal tests in mice; tests in tissue cultures either in fluid or in overlaid cultures;

in cultures with overlay, plaque neutralization, suppression, reduction or inhibition; use of virus dilutions or serum dilutions; addition or not of accessory factors and kind; type of serum (or when applicable, ascitic fluid), convalescent, two-injection or 4-or more injection sera; animal species supplying the serum, monkey, mouse, man, rabbit, etc..

At first one is tempted to say that all types of neutralization tests should be considered equally important and therefore all taken into consideration in solving the problem before the Subcommittee. However, if this is done one is soon faced with contradictory sets of results. This is not a theoretical situation but one that occurred repeatedly in the course of the Subcommittee's session, namely certain viruses were nearly indistinguishable in a set of published neutralization tests in mice, while the same viruses were reported distinct in a plaque inhibition test; other sets of viruses have been reported almost identical using hyperimmune mouse sera in neutralization tests, while being entirely distinct when human sera were used in the same test.

If a special type of neutralization test shows a moderate but reproducible difference between viral strains, even though no significant differences can be observed by other types of neutralization tests, the logical conclusion is that the two strains are not identical, therefore different. Following this approach to the extreme, however, might end up in a situation in which every strain of a serotype may be uniquely identifiable; this has been noted with serotypes in viral families other than the arboviruses. Immunologically unique identification of strains is not, in the opinion of the Subcommittee, the problem at hand.

The Subcommittee repeats here that it had to work with the results and data that are available, not the ones that it would have liked to have. Everything considered,the last resort generally used in the Subcommittee's decisions were the results of neutralization tests by intracerebral route in mice, preferably using simple immune sera (i.e., either convalescent sera or 2-injection sera, in contrast to 4- or 5- injection sera); the sera being derived mainly from mice and monkeys (pretested); virus used in dilutions against constant serum and, as far as we could ascertain, in the absence of accessory factor added.

B. Interpretations- Even if ideal sets of reported results were available, the matter of interpreting observed immunological differences between strains represents an almost unsurmountable problem.

Let us assume that viral isolates X and Y have been compared reciprocally by neutralization test and that antiserum X has log neutralization indices of

4 against virus X and 2 against virus Y; and that antiserum Y has a log neutralization index of 5 against virus Y and 3 against virus X; are X and Y significantly different?

In the first place, it can be asked whether the above are the results of a single observation or have such differences been noted repeatedly, for after all, the criterion for validity is consistency.

The second comment is that in the above test viruses X and Y may have been represented by strains X_1 and Y_1, respectively; what would have been the reported values of the neutralization indices if other strains, X_2 and Y_2, had been compared? Often characterization of a virus has been done using a strain that may have had many passages in an experimental test.

Even assuming that the reproducibility of differences between viruses is as good as can be desired, and that strain variation plays no important role in our theoretical case, there still remains the final and decisive question, what is to be considered a significant difference in terms of neutralization index values; if, for example, a difference of log 2 between neutralization indices is considered to be significant, why not log 1.9? Obviously, one is dealing with a variable, neutralization index, the value of which can be said to vary in a continuous way; any dividing point that is chosen to separate significant from non-significant values, or dissimilar from similar strains, is arbitrary. To expect this Subcommittee, in a set form of words, to lay down a rule that is universally applicable, is completely unrealistic and lacking in the appreciation of the true nature of the problem.

It so happened that in the comparison of log neutralization indices among group B viruses, in instances in which repeated values were given either in the cards or in the literature, differences between homologous and heterologous values with the agents that this Subcommittee considered distinct, were of the order of 1.7-2.0, or higher; but, evidently this is only a loose criterion subject to reappraisal.

Acceptance of small differences as a basis for consideration of strains as distinct, can lead to extreme individualization, which is no good for taxonomy; ignoring as too small real differences results in the opposite error, as a consequence of which one might end by considering all viruses in an immunological sub-group or complex as a single agent, an erroneous conclusion.

Whatever recommendations this Subcommittee makes on this or subsequent occasions, they should be considered in the light of the fact that the immunologic characterization of the arboviruses, both old and new, is a flexible, changing situation. In addition, the best criterion to determine whether a given recommendation is valid, is the test by time and usage; if, let us say, 5 years from now said recommendation is still useful and accepted, it may have some merit; if not, a new one will have to be drawn.

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 (No. 15) of the Information Exchange, three "new" viruses have been registered, making a total of 207 now registered in the Catalogue.

As of December 1967, 136 Catalogues including current information files have been distributed: 55 within continental U.S.A. and 81 to overseas addresses representing 44 different countries.

Including the third quarterly issue of this year, 4,136 3" x 5" current information slips have been distributed to Catalogue recipients, comprising 2,290 abstracts and references from Biological Abstracts and Bioresearch Index, 1,624 from Bulletin of Hygiene and Tropical Diseases Bulletin, and 222 personal communications.

Accompanying the July (second quarter) issue of Catalogue material, a "Special Report" or statistical synopsis of the 204 viruses registered as of April 1967 was sent to all recipients of the restricted or working Catalogue. This Special Report is herewith being sent to all participants in the Information Exchange (Info Exch) who do not receive the Catalogue.

Reference to publication of the Catalogue was made in the No. 15 issue of the Information Exchange and as stated it will represent essentially a facsimile of the cards in the active Catalogue as of February 1, 1967. This includes cards issued in April and comprises a total of 204 viruses.

The publication is being sponsored and aided by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, of the U.S. Department of Health, Education and Welfare, and will be printed by the U.S. Government Printing Office.

The final draft of the text and the virus registration cards, including modifications requested by those submitting the registrations, has been approved and is ready for printing. It may now be considered to be "in press."

ANNOUNCEMENTS REGARDING THE EIGHTH INTERNATIONAL CONGRESSES OF TROPICAL MEDICINE AND MALARIA

The Eighth International Congresses of Tropical Medicine and Malaria will be held in Teheran, Iran from 7 through 15 September 1968.

The organization of the program has been initiated by Dr. Chamseddine M. H. Mofidi, Secretary-General of the Congresses and of the Iranian National Organizing Committee. The overall plans for the Congresses are the responsibility of the International Interim Committee's International Executive Group, with the following membership:

PRESIDENT:	J. H. Saleh (Iran)
VICE-PRESIDENTS:	
Division A - Tropical Medicine:	B. G. Maegraith (United Kingdom)
Division B - Malaria:	A. Gabaldon (Venezuela)
SECRETARY:	M. K. Afridi (Pakistan)
TREASURER:	J. C. Edozien (Nigeria)
MEMBERS:	J. Rodriguez da Silve (Brazil) (Past President)
	D. J. Davis (U. S. A.)

Secretariat of the Congresses: P. O. Box 1310, Teheran, Iran. Telegraphic Address: Healthstate, Teheran.

Division A of Tropical Medicine, Subdivision A. 5 encompasses Viral and Rickettsial Infections. Organization of Section A. 5. 1 on Arbovirus Infections has been assigned to Dr. Telford H. Work, Division of Infectious and Tropical Diseases, The Center for the Health Sciences, University of California, U. S. A., 90024.

Section A. 5. 1 has been divided into three subsections; Subsection A. 5. 1. 1 on Pathological Aspects is under Chairmanship of Dr. M. Mussgay.

Subsection A. 5. 1. 2 on Clinical Pathological and Therapeutic Aspects and Subsection A. 5. 1. 3 on Epidemiology and Control are under joint Chairmanship of:

Dr. Charles Serie
Pasteur Institute
Cayenne, French Guiana

and

Dr. Wilbur G. Downs
Yale Arbovirus Research Unit
Yale University School of Medicine
60 College Street, New Haven,
Connecticut, U.S.A. 06510

A tentative program for Subsections A.5.1.2 and A.5.1.3 has been planned and inquiries sent to suggested participants. Time has been allowed in several of the Arbovirus Sessions for free communications.

Article 28 of Section IX of the Rules of Procedure for the Congresses outlines the regulations governing the Submission of papers.

a) Papers may be requested for presentation either in regular sessions or in Seminars;

b) Each paper shall be sent in duplicate and accompanied by an abstract of not more than 300 words; only one paper should be submitted by each author in one session;

c) Papers presented shall be limited to 3,000 words and the time of presentation shall not exceed 15 minutes;

d) Uninvited papers may be presented in special meetings devoted to free communications, and will be limited to five minutes;

e) The abstracts shall be in the hands of the Publications Committee of the National Organizing Committee not later than four months before, and copies of the papers, not later than 3 months before the date of the Congresses. Authors not complying with these requirements sacrifice their right to have their abstracts printed;

f) Papers and abstracts shall be submitted in an official language;

g) Authors desiring to revise their papers subsequent to the Congresses, must submit their revised papers not later than 30 days after the conclusion of the Congresses;

h) Papers may be accompanied by illustrations and tabular material. The Publications Committee may place a limitation on such material;

i) Papers submitted should have special reference to recent development pertaining to the subjects concerned;

j) The Chairman of the Section and the Organizer of each Sub-Section in close and advance agreement with the National Organizing Committee will determine the number of papers to be read and discussed at a single session.

Article 31 of Section XII contains rules governing Discussions.

a) The discussion of the papers shall take place at the end of each session and shall be limited to not more than 30 minutes. No one may speak from the floor for more than three minutes, and no speaker may speak more than once in the discussion of any paper unless given permission by the presiding officer;

b) Authors may have three minutes at the end of the final discussion to answer the remarks about their papers;

c) All speakers will hand a written resume of their remarks to the Secretary of the Section or Sub-Section. The resume shall be in one of the official languages and should not exceed 300 words;

d) The Chairman of any session may give the floor to persons who are not delegates or members but who are particularly qualified to discuss the subject under consideration.

Those who will attend the Congresses and wish to present a paper should send the request and title now to the Secretariat in Teheran with copies to Dr. Work at U. C. L. A.

Dr. Dorland J. Davis, Director of the National Institute for Allergy and Infectious Diseases is the American member of the International Organizing Committee. Dr. Davis announced that the American Society of Tropical Medicine and Hygiene has been awarded a grant by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, for the purpose of supporting the travel of scientists to participate in the Eighth International Congresses on Tropical Medicine and Malaria in Teheran, Iran, September 7 - 15, 1968.

Monies granted the American Society of Tropical Medicine and Hygiene for this purpose will be administered by a committee appointed by the Council of the Society and will be awarded to applicants for the expenses of travel from the United States to the Congresses in Teheran. Support will be provided in the amount required to cover the cost of a minimum rate round trip air ticket from the home of the applicant to Teheran, Iran; an additional

amount of $50 will be included for other expenses incident to travel, registration and attendance. Conditions of application and award are as follows:

1) Applicants will be asked to provide information on their official relationship to the Congresses (i.e. officer, invited participant, etc.) and to state their estimate of the benefits which will accrue to themselves and their institutions through their participation.

2) Federal Government employees will not be eligible.

3) Applicants must be United States citizens, or residents of the U.S. or Canada.

4) Members of the American Society of Tropical Medicine and Hygiene will not receive preferential consideration over non-members.

5) Successful applicants will not be allowed to utilize funds derived from other U.S. Government sources to supplement their awards.

6) Awards will be made to approximately 75 qualified applicants.

7) U.S. flag carriers must be used wherever possible.

One of the stipulations of the National Institutes of Health grant to the American Society of Tropical Medicine and Hygiene is that no other source of N.I.H. funds may be used for the purpose of attending the Congresses in Teheran.

Applications will be required 6 months in advance of the Congresses. A deadline of March 1, 1968, has been set for their receipt, but earlier applications may result in an earlier decision. Decisions on the awards will be made not later than 3 months prior to the date of the Congresses, and earlier where possible. It is suggested that application by letter be made as soon as possible after the applicant has become aware of his relationship to the organization of the Congresses (officer, invited participant, etc.). When an applicant receives later word of additional functions in the Congresses, this information should immediately be provided to the Committee in a supplemental letter.

All inquiries and letters of application should be mailed to the following address:

Executive Secretary, Travel Grants
American Society of Tropical Medicine and Hygiene
P.O. Box 295
Kensington, Maryland 20795

EDITORIAL NOTE

Sometimes preoccupation with the isolation and description of new arboviruses and elucidation of the clinical aspects and epidemiology of the diseases they cause ignores the importance of the laboratory techniques on which such progress is based. Development of sound laboratory technology proceeds slowly. The significance of past accomplishment of those who have developed principles and techniques in the laboratory have not been appropriately recognized.

In this issue of the Arbovirus Information Exchange are what appear to be three significant advances:

1) Improved methods for the preparation of hemagglutinating antigen for arboviruses from Dr. Delphine Clarke of the Yale Arbovirus Research Unit.

2) Establishment of cell cultures derived from larvae of Aedes albopictus and Aedes aegypti from the Virus Research Centre in Poona, India.

3) Beta propriolactone inactivation of highly infectious types of arbovirus hemagglutinins from Dr. Arthur Gorelick of Fort Detrick, Maryland.

Attention is drawn to them not only because of their immediate importance in helping the entire fraternity of arbovirus investigators in their work, but in appreciation for the previous technical advances on which they are based.

Delay in production of the 16th issue of the Information Exchange stems from temporary loss of the plates for the color masthead, further development of resources for the new system for producing the typescript at U. C. L. A. for printing and distribution at the National Communicable Disease Center, and the again increasing time consuming problem of having to recast tables because they are not properly prepared before submission. A number of contributions arrived as carbon copies, the originals having possibly been submitted elsewhere for other purposes. Please take a little more time to send us 1) A double-spaced original plus one carbon copy of your manuscripts, and 2) Original typescripts of your tables on white paper so they can be reproduced directly.

Deadline for Issue Number 17 will be April 1st.

Telford H. Work, M.D.
Editor
Division of Infectious and
Tropical Diseases
The Center for the Health Sciences
University of California
Los Angeles, California 90024

REPORT FROM THE WHO INTERNATIONAL ARBOVIRUS
REFERENCE CENTER
YALE ARBOVIRUS RESEARCH UNIT
NEW HAVEN, CONNECTICUT

IMPROVED METHODS FOR THE PREPARATION OF HEMAGGLUTINATING ANTIGEN FOR ARBOVIRUSES

It is to be emphasized that this descriptive outline should be considered preliminary in nature since the studies are early in their development. Most of the viruses successfully treated belong to the Bunyamwera supergroup.

I. Usual sucrose-acetone extraction method followed by drying and rehydration in 0.15 M NaCl overnight at 4° C. (Clarke and Casals, Amer. Jour. Trop. Med. & Hyg. 7, 561, 1958). Do not centrifuge this antigen but treat it whole.

II. Sonication

1. Apparatus is the Branson Sonifier, Model W-185-C, distributed by Heat Systems Co., Melville, L.I., N.Y.

    a) Micro tip needed for smaller volumes.
    b) Rosett cooling cells valuable for good heat exchange.
    c) Sealed atmosphere treatment chamber important for hazardous viruses.

2. Thin-walled containers should be used, either glass or plastic, to insure good heat exchange. The temperature should be monitored at the end of each sonication burst to determine that proper control is being maintained.

3. The most effective cooling is achieved by use of a NaCl-ice-water bath with magnetic stirring. Too high a salt concentration can prove a nuisance since antigen freezing may occur between sonication bursts. This is probably not harmful to the antigen but delays the procedure.

4. Container size must be adjusted to antigen volume. Too small a volume per container leads to foaming and poor processing.

5. Probe must be centered and deeply immersed to avoid foaming.

6. Low intensity sonication has been used throughout most of our work, with good antigen yields. It is not yet clear whether higher intensities may give better (or worse) results. Sonication periods of 1-2 minutes with 1-2 minute cooling periods have been used. These are repeated 2-3 times.

III. Centrifugation for 10 minutes at 2000 rpm. Pellet should be small and well packed.

IV. Check supernatant fluid for HA over an extended pH range (with emphasis on pH 5.8-6.6 for Bunyamwera supergroup). If a satisfactory HA has not been achieved at this point continue with subsequent steps.

V. Trypsin treatment

1. Two preparations have been used:

   Difco 1:250 crude at a final concentration of 256 microgm/ml
   Worthington 2X crystallized at a final concentration of 16 microgm/ml

   Difco appears to give slightly better results (other trace enzymes?).

2. The trypsin preparation to be used should be assayed to be sure of the approximate potency. We use an assay kit marketed by Worthington (Determatube TRY) which permits measurement of the rate of splitting of the ester of N-benzoyl-L-arginine. Based on this assay we use approximately 140 units/ml in antigen treatment.

3. Treat at pH 9 and room temperature for 40 minutes.

4. Promptly terminate the trypsin action by addition of soybean trypsin inhibitor. We use Worthington crystallized inhibitor added at a weight equal to the total weight of Difco trypsin present.

5. Centrifuge for 10 minutes at 2000 rpm if turbid.

6. Test for HA over a broad pH range.

VI. Problems of trypsin treatment

1. With larger amounts of trypsin a nonspecific HA, giving a pattern like an arbovirus, has been produced from both normal and infected suckling mouse brain. Its presence is suspected by the occurrence of rather a wide pH range and it can be proven by the fact that it is inhibited by all immune or normal ascitic fluids or sera. The production of this hemagglutinin seems to be favored by incubation at 37°C as well as by higher trypsin concentrations.

2. We are not able, at present, to tell whether or not lesser amounts of this nonspecific HA are present in other of our antigens. If present they may act to decrease sensitivity in HI tests.

3. Because of the phenomenon of nonspecific HA it is vital to test all antigens by HI as soon as possible, using homologous, heterologous and normal fluids.

VII. Resonication

1. Repeat sonication 4 X 2 minutes at minimal intensity.

2. This step usually improves the post-trypsin titer.

3. Check HA.

VIII. Development

Some antigens on standing at room temperature for a few hours or overnight for 1-2 days show further titer increase.

IX. Storage stability

1. Antigens store well at 4°C and in the Revco.

2. A few antigens have been lyophilized after adding a little bovalbumin. No significant titer loss has been observed.

X. Sensitivity

It is not clear whether antigens produced by these procedures are optimally sensitive by HI since no points of reference are

presently available. However, since antigen sensitivity is somewhat of a problem with other arboviruses, work is now beginning on this problem. The first approach will utilize calcium phosphate treatment along the lines described by Ardoin and Clarke, Amer. Jour. Trop. Med. & Hyg. 16, 357 (1967), for increasing the sensitivity of group C virus antigens.

(Pierre Ardoin and Delphine Clarke)

REPORT FROM THE AEDES RESEARCH UNIT OF THE WORLD HEALTH ORGANIZATION, BANGKOK, THAILAND

The Aedes Research Unit (ARU) was established in cooperation with the Government of Thailand to conduct research on Aedes (Stegomyia) mosquitoes that are, or may be capable of transmitting dengue or dengue-like fever, i.e. haemorrhagic fever. Of particular interest is the development of techniques to determine absolute populations of all the life history stages of the mosquitoes.

The first step in this direction was a Mark-Release-Recapture Study in a Wat in Bangkok. This study allowed estimates of absolute numbers of male and female A. aegypti for a period of a year, estimations of life expectancies during the seasonal changes, and mapping of movement of the recaptured mosquitoes within the Wat.

The second step was the start of a life budget study which took into account each developmental step of the mosquito. This study will continue for at least another year. The study should be the foundation for developing of more exacting methods of insecticide evaluation as well as a starting point for future biological-genetical control should break throughs occur in these fields.

A number of larval surveys have been done in Bangkok. The results indicate that A. aegypti is the predominent receptacle breeding mosquito and that it is found throughout the city. These surveys have now been expanded to include all the provinces.

Work has begun on the inter-relationship of A. aegypti and A. albopictus in a rural area. The significance of A. albopictus in disease transmission has not been ascertained.

Insecticide susceptibility of A. aegypti is being carried out. Field Trials have begun on OMS 786. These trials will be followed by a Pilot study and then an operational one. In addition investigations to provide emergency control measures of these mosquitoes in case of an epidemic of haemorrhagic fevers have started.

REPORT FROM DEPARTMENT OF IMMUNOLOGY STATE RESEARCH INSTITUTE OF INFLUENZA IN LENINGRAD, U.S.S.R.

A prospective approach to development of live vaccine against tick-borne encephalitis is the search for naturally attenuated strains among the specific viruses isolated directly on tissue cultures from the ticks Ixodes persulcatus or Ixodes ricinus. A group of such strains was found in West Siberia, in Kirgiz Republic, others were isolated in European regions of the USSR where the disease occurred quite rarely but where circulation of virus between ticks and humans was very intensive according to serological survey of the human population.

Seventy-seven strains of tick-borne and biphasic meningoencephalitis viruses, isolated in different geographical zones, were tested in monkeys. A group of strains not pathogenic by intracerebral inoculation has been found. As a result of analysis of the biological properties of the tick-borne encephalitis viruses isolated in Western Siberia it was possible to classify these viruses into three main groups according to their virulence for monkeys (Table 1).

Group One included viruses which provoked in monkeys a lethal outcome of the disease with the development of paralysis.

The strains of Group Two provoked a milder disease in monkeys with a peculiar clinical course indicative of primary affection of the cerebellum. About a half of the infected monkeys recovered completely or with mild sequelae (ataxia, pareses of the limbs). The strains of this group possess also a moderate reproduction in tissue cultures of chick embryo fibroblasts at 40°.

The strains of Group Three were characterized by a complete nonpathogenecity or a mild pathogenicity for monkeys on intracerebral inoculation.

The virulence for monkeys decreases distinctly with transition from Group 1 to 3 .

Among the viruses of tick-borne encephalitis which we were able to classify according to the degree of their virulence for Macacus rhesus monkeys, the strains of Group 1 were isolated chiefly from foci with a high tick-borne encephalitis morbidity and the strains of the groups 2 and 3 from foci with rare sporadic cases. It is, however, highly probable that in any tick-borne encephalitis foci rather than one there may circulate several variants of tick-borne encephalitis virus differeing in virulence.

For the strains of Groups 1 and 3 there was some correlation although not very regular between sensitivity for monkeys and their virulence for mice on subcutaneous inoculation and also for reproduction rate in tissue cultures of chick embryo fibroblasts at 40^{o}. For Group 1 strains high virulence for mice on subcutaneous inoculation was more characteristic than for Group 3 strains. Quite often there was reduced extraneural activity for mice and less intensive reproduction rate at 40^{o}.

According to our recent experience with human volunteers, the strains of Group 3 demonstrated the elaboration of a safe and effective live vaccine against tick-borne encephalitis, and possibly against many other arbovirus diseases. It would appear advisable to use naturally occuring strains of virus isolated from arthropods in areas of low actual disease incidence. This approach looks more prospective than usual attempts to develop attenuante from highly virulent strains.

(A.A. Smorodintsev, V.I. Ilyenko, A.V. Dubov)

Table 1

CLASSIFICATION AND BASIC PROPERTIES OF THE VARIOUS STRAINS OF TICK-BORNE ENCEPHALITIS VIRUS ISOLATED IN WEST SIBERIA

Group of strains and region of isolation	The code of strain	The susceptibility of monkeys to intracerebral inoculation		
		Clinics	Viremia	The termination of illness
1 (Kemerovo)	K 1-1	paralysis	+	death
	K 1-2	"	+	"
	K 1-3	"	+	"
	N 1-1	"	+	"
	The average for the group 1	"	+	"
2 (Tyumen)	T 2-1	ataxy	+	death (unregularly)
	T 2-2	"		"
	T 2-3	"	+	"
	T 2-4	ataxy, paresis	+	"
	T 2-5	"	±	
	The average for the group 2	ataxy	+	"
3 (Tyumen)	T 3-1 (Elantsev)	no	-	No illness
	T 3-2 (Isetsk-237)	"	-	"
	T 3-3 (Isetsk-9[a])	"	-	"
		"	-	"
	The average for the group 3	"	-	"

REPORT FROM THE DEPARTMENT OF ARBOVIRUSES OF IVANOVSKY INSTITUTE OF VIROLOGY, MOSCOW, U.S.S.R.
GROUP "A" ARBOVIRUS ANTIGENS FOR HI TEST FROM TISSUE CULTURE

It was shown by previous investigations that potent specific antigens for HI and CF tests were obtained from tissue culture fluid infected by tick-borne and Japanese encephalitis viruses, and Venezuelan and western equine encephalomyelitis viruses.

In 1966-1967 these investigations were carried on with other arboviruses from A group: Aura, Pixuna, Una, Geta, Mayaro, Bebaru, chikungunya, Sindbis, Middelburg.

Primary tissue culture of chick embryo fibroblasts and stable lines BHK-21 and PS were infected with above mentioned viruses, using as inoculum 10^{-2} -10^{-5} dilutions. At intervals 24, 48 and 72 hours after inoculation, the samples of tissue culture fluid were taken and HA activity was measured. HA tests were done at pH range from 5,8 to 7,0 at 4° C.

The macromethod was used employing plastic trays and 0.25% of goose erythrocytes.

The results are shown in the following table.

Virus	Tissue Culture	Harvesting	Titre	Optimum pH in HA-test
Aura	BHK-21	48-72	256	6,0-6,2
Pixuna	BHK-21	48	512	6,0
EEE	BHK-21	48	128	6,4
Mayaro	BHK-21	48	128	5,8-6,0
Getha	BHK-21	48	128	6,0
Una	BHK-21	48	64	5,8-6,0
Chikungunya	BHK-21	48	1024	5,8-6,0
Middelburg	BHI-21	48	256	6,0

The development of hemagglutinins depends to some extent on the dose of inoculated virus. Dilutions 10^{-2} - 10^{-3} are the best for use. The HA-titre was in some cases not higher than 1:128 but even these antigens were suitable for routine work. The advantage of tissue culture antigens is the very simple technique of preparation. For serological investigations non-infectious antigens are more convenient than infectious. Two methods of inactivation were used: thermoinactivation at 37 during 7-10 days or treatment with 0,06-0,1% β-propiolactone /BPL/.

To prevent the drop of titres of haemaglutinin as a result of oxidation which follows BPL action, the pH was adjusted all the time of treatment. The non-infectious antigens were as specific as the infectious antigens.

(S. Ya. Gaidamovich, A. I. Lwova, Ie. E. Melnikova).

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

In contrast to the results of studies with Ťahyňa virus in suckling mice, a lesser susceptibility of young white mice to the peripheral inoculation of small and large doses of this virus was demonstrated. Specific viral antigen was not visualized by a fluorescent antibody method in any tissues of subcutaneously inoculated mice. By biological titration of infectious virus in blood and in some organs of mice, low titres were detected. Young white mice survived without signs of illness in all experiments. The presence of specific virusneutralizing antibodies in sera of the inoculated mice on 5 and 7 days after administration of virus ranged from 1:16 to 1:64.

After intracerebral inoculation of Ťahyňa virus viral antigen was demonstrated in the organs of young white mice by the fluorescent antibody method, but only in the cells of the central nervous system. By the histological examination the degenerative changes in the brain cells of tissues were also detected.

The course of Ťahyňa virus infection in "specific pathogen free" mice under the conventional conditions was compared in mice placed in special isolators (filtered air, sterilized diet). No influence of these special conditions on the course of experimental infection was observed.

The adsorption ability of the Tahyňa virus on some human embryo tissues "in vitro" was investigated, using the method described in a previous report. As in previous experiments in which the mouse tissues were used, the high adsorption capacity of the human skin-muscle tissue homogenates was observed. Optimal temperature for the adsorption was 37°C at optimal pH of 7, 2.

A Symposium on Arboviruses of the California and the Bunyamwera groups was organized on October 18-21, 1966, in Smolenice near Bratislava by the Research Institute of Epidemiology and Microbiology, Bratislava, together with the Institute of Virology of the Czechoslovak Academy of Sciences, Bratislava, and the Institute of Parasitology of the Czechoslovak Academy of Sciences, Prague. Seventy workers from 21 countries participated in this meeting.

The programme included the topics: biology and ecology of viruses of the California and Bunyamwera groups, and the pathogenesis and medical importance of these infections. In addition, a seminar held after these sections offered opportunity to present the results of recent investigations on various mosquito-borne viruses belonging to other arbovirus groups. Altogether 53 communications, including 7 introductory lectures, were presented.

The Proceedings of the Symposium will be available at ARTIA Foreign Trade Corporation, Ve Smečkách 30, Prague, Czechoslovakia.

(Wallnerová, Z., Schwanzer, V., Šimková, A.)

REPORT FROM THE FEDERAL RESEARCH INSTITUTE FOR ANIMAL VIRUS DISEASES, TUEBINGEN, W-GERMANY

a) Purification of Sindbis-virus by column chromatography.

It was demonstrated that chromatography on hydroxylapatite columns can serve as a procedure for the purification of Sindbis-virus. The following method was employed:

Virus: Sindbis-virus grown in chick embryo cell cultures was concentrated by ultracentrifugation. The concentrated virus was suspended in borate-buffer, pH 9.0, and then dialyzed against 0.001 M phosphate buffer, pH 6.8.

Column: Hydroxylapatite columns were prepared with dimensions 14.0 cm x 1.4 cm. These columns were equilibrated with 0.001 M phosphate buffer, pH 6.8.

Fractionation: 10 ml of the dialyzed virus concentrate was floated on a column. The effluent was collected in 5 ml samples. Stepwise elution was performed by using 10 ml of phosphate buffer solutions, all adjusted to pH 6.8; the molarity of the buffer solutions was 0.001, 0.005, 0.01, 0.05, 0.1, 0.5 and 1.0, respectively. The course of the chromatographic test was followed by recording the optical density at 256 mμ, the electric conductivity, and by titration of hemagglutinating and infectious activity present in the various samples. In addition, the complement-fixing activity of each sample was determined by using anti-Sindbis-hyperimmune serum. For the CF-reaction, the samples had to be dialyzed against the buffer solution used in the CF-test. All experiments were run at room temperature.

Results: Usually, most of the viral activities (infectivity, hemagglutinating and complement-fixing activity) were detected in one sample, namely in fraction No. 15. The electric conductivity in this sample was determined to be in the range from 13 - 20 mS, which corresponds to a molarity of about 0.2.

b) Serological specificity of viral subunits.

The serological specificity of four subunits of Sindbis and WEE-virus was investigated in cross reactions. The four subunits were: a) "complete" virus particles; b) the hemagglutinin obtained after Tween 80 and ether treatment and purified by density gradient centrifugation; c) and d) the so-called 1.300 and 1.345 "cores" obtained by treating the "complete" virus with cobra venom and isolated by subsequent density gradient centrifugation. In neutralization test, a one-way relationship between Sindbis and WEE-virus was demonstrated in which Sindbis antibodies neutralized Sindbis as well as WEE-virus, whereas WEE-antibodies reacted with homologous virus only. An analogous relationship was also found in the CF-reaction with 1.300 and 1.345 "cores", especially with the latter. On the other hand, "complete" virus particles and "hemagglutinin" showed clear cross reactions when used in CF-reactions and HI tests. These results shall be published in Arch. ges. Virusforschg.

(M. Mussgay)

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

The behaviour of large and small plaque variants of Middelburg virus was studied in Aedes aegypti mosquitoes reared in our laboratory and maintained at 26° C. Viruses were fed in different concentrations and suspensions of individual mosquitoes were plated on CETC after 1-2-3-4- and 5 weeks.

It was found that MB-1 virus was more infectious for A. aegypti than the small plaque virus (see table).

In high concentrations MB-1 virus stocks mostly contain some small plaque virus. In the few instances where MB-1 virus had been fed to the mosquitoes, these gave rise to pure or almost pure small plaque virus.

One such virus strain-designated as Qq virus has been studied further. When it was passed in CETC and fed in various concentrations to A. aegypti it was found to be as infectious as or even slightly more infectious than MB-1 virus. In vitro, Qq strain behaved as MBs virus: increased plaque size through the addition of DEAE dextran to agar and immediate partial inactivation by agar extract.

It is concluded from these observations that MB-1 virus gives rise at least in vitro, to small plaque variants of at least two types, one being as infectious for mosquitoes as MB-1, the other much less infectious for mosquitoes.

Other characters of the Qq and similar virus strains are under study.

(S.R. Pattyn)

Table

Sensitivity of A. Aegypti for MB-1 and MB s and MB-q^9 Virus Strains

(% of mosquitoes positive after 4-5 weeks after adsorption of infected meal containing indicated amounts of virus)				
Virus concentration fed to the mosq.		MB-1	MB s	MB-Qq
5.5	10^8		50%	
3	10^8	100%		77-100%
9	10^7	100%		
1.5	10^7		30-33%	81-87%
	10^7	54-62%		
2	10^6		4%	41-50%
	10^6	0		
2	10^5			7-18%
2	10^4		0	

REPORT FROM THE MICROBIOLOGICAL RESEARCH ESTABLISHMENT PORTON, SALISBURY, UNITED KINGDOM
PRODUCTION OF SEMLIKI FOREST VIRUS IN CONCENTRATED SUSPENSIONS OF CHICK EMBRYO CELLS

High titre Semliki Forest Virus (SFV) has been obtained from concentrated suspensions of chick embryo cells. The cells, freshly prepared from 12 day whole embryo, have a much lower glycolysis rate than any cultured tissue cells. This is an important advantage since the usable cell concentration is limited by the amounts of acid produced and glucose utilized by the cells. The CE cells could be used at 10^7/ml. with 0.1% glucose, 0.22% bicarbonate and 5% CO_2 in air to yield SFV at 10^{10} PFU/ml. A further 10 fold increase was obtained by using a culture vessel equipped with automatic pH control and 10^8 cells/ml. with 1% glucose produced 10^{11} PFU/ml. At this higher concentration, oxygen became limiting if air was used in the gas phase but this was remedied by using 100% oxygen.

Optimal virus yields were obtained after 20-24 hr. at 31° from the cells infected with 5 PFU/cell and suspended in Earle's Saline containing appropriate glucose and bicarbonate concentrations. No other nutrients or serum were required. Too vigorous agitation reduced the virus yield and adequate oxygenation was obtained by gently stirring a shallow layer of cell suspension.

The Enhancement of Neutralization by Anti-antibody

During the course of experiments on the accessory actions of fresh, normal monkey or guinea-pig serum it was observed that host specific anti-antibody was also effective in the amplification of the neutralizing activity of anti-viral sera.

Rabbit or chicken anti-SFV sera at appropriate dilutions were incubated for 2 hr. at 37° C with an equal volume of a serial dilution of Semliki Forest virus. Dilutions of goat anti-rabbit or rabbit anti-chicken sera were now added to these incubated mixtures and after a further incubation at 37° C for 2 hr. the samples were titrated for residual infectivity without further dilution in unweaned mice (0.025 ml. ip.) or in 10 ml. suspension plates of primary chick embryo fibroblasts (1 ml. sample & 8 x 10^7 cells). Table 1 shows the pattern of data obtained in a typical experiment. The depression of infectivity of about 1 log unit in the reaction mixtures B and C without anti-antibody was increased to about 2.7 log units when the host-homologous anti-antibody was added (D and G), but was unchanged

by the addition of the host-heterologous anti-antibody (E and F). Serum neutralization indices were increased from about 4.5 to 6.3; equivalent to almost a hundred fold amplification of anti-viral activity.

When chicken anti-SFV serum was used at other dilutions the data obtained were as shown in Table 2. The differences between the neutralizing activity in unweaned mice and CEF-suspensions are significantly reduced after the addition of anti-antibody. The enhancement of neutralization by anti-antibody is apparent in both assay systems but is greater for the initially less sensitive CEF-suspension. Similar data have been obtained with Langat virus as a representative of the tick-borne viruses of group B.

These findings are interpreted in terms of the blocking by the anti-antibody of the residual infectivity of the virus-antibody complexes formed in the primary reaction. The anti-antibody combines with the available host-homologous antibody sites at the surface of this virus-antibody complex and initiates the formation of even larger, more stable complexes or further impedes virus-cell interaction by steric hinderance in regions not blocked by specific antibody.

The anti-viral bodies are thus involved in reactions at both virus specific and host specific sites. Dilutions of the relatively stable anti-antisera may be much more convenient in some studies of anti-viral activity than the fresh, normal mammalian sera currently used as accessory factor.

Table 1

Reaction mixtures of 3ml.: 1ml. of each reactant	Infectivity in CEF suspension		Infectivity in unweaned mice	
	Log PFU/ml	Log Depression	Log LD50/ml	Log Depression
A. Virus only	8.25	=	9.2	=
B. Virus + chicken anti-SFV serum at 1/2000 overall	7.39	0.86	7.8	1.4
C. Virus + rabbit anti-SFV serum at 1/6000 overall	7.06	1.19	6.9	2.3
D. 2ml. B + rabbit anti-hen serum at 1/30 overall	5.41	2.84	6.6	2.6
E. 2ml. B + goat anti-rabbit serum at 1/30 overall	7.41	0.84	8.0	1.1
F. 2ml. C + rabbit anti-hen serum at 1/30 overall	7.21	1.04	7.3	1.9
G. 2ml. C + goat anti-rabbit serum at 1/30 overall	5.56	2.69	6.1	3.0

Table 2

Overall dilution of chicken anti-SFV serum in primary reaction with Semliki Forest Virus	Log Depression of Infectivity After			
	Incubation without anti-body		Incubation with rabbit anti-hen sera at 1/90 overall	
	CEF-Suspension	Unweaned mice	CEF-Suspension	Unweaned mice
1/2,000	0.93	1.5	2.69	2.8
1/6,000	0.59	1.6	1.53	2.0
1/20,000	0.02	0.6	0.63	0.7

Laboratory infections with louping ill

During the period October through December 1966 three cases of louping ill complicated by encephalitis occurred elsewhere as laboratory infections consequent upon handling infective material. One of the patients had previously received a course of inactivated mouse-brain vaccine; the other two had each had a course of inactivated tick-borne encephalitis vaccine. All were admitted to hospitals and have since made complete recoveries.

This laboratory received serial blood samples from each patient for testing. CSF samples were collected from only two patients. The results are shown in the Table and show HI serum antibody responses to louping ill in all three cases. Neutralizing antibody responses were found in the sera of cases A and B (Case C was not tested). Rising HI and neutralizing antibody levels were detected in the CSF specimens as well. Serum and CSF are routinely extracted with kaolin and although some CSF samples contained considerable amounts of neutralizing antibody no HI could be detected. It was thought that kaolin might be removing HI antibody as well as non-specific inhibitor since CSF contains only a low level of protein. Extraction was repeated after the addition of 0.5% bovine plasma albumin and on consequent testing, HI antibodies were readily demonstrated. Parallel extraction with acetone gave similar positive results.

TABLE

Patient	Nature of Specimen	Day of Disease	HI Antibody		Neutralizing Antibody
			No Bovine Albumin	+ Bovine Albumin	
A	Serum (Pre-infection)	-	<10		Negative
	Serum	24	160		>80
	"	31	160		>80
	"	38	>1280		>80
	"	40	>1280		>80
	"	68	>1280		>80
	CSF	24	<10	<10	Positive undiluted only
	"	40	<10	40	>80
	"	54	<10	160	NT*
	"	68	-	40	5
B	Serum	5	<10		NT
	"	21	10		NT
	"	22	20		20
	"	31	20		320
	"	35	40		320
	"	50	160		
	"	84	80		NT
	CSF	22	<10	<10	Negative
	"	35	<10	<10	20
	"	50	<10	20	NT
	"	84	<10	20	NT
C	Serum (Pre-infection)	-	<10		NT
	Serum	6	<10		"
	"	21	80		"
	"	32	320		"

*NT = not tested

Isolation of Langat virus from the original site after 10 years

A pool of four adult Ixodes granulatus ticks (1 male and 3 female) collected off rodents from the UIU Langat Forest in September 1966 by kindness of Mr. M. Nadchatram of the Institute for Medical Research Kuala Lumpur, Malaya. They were processed and inoculated into suckling mice. A virus, subsequently identified as Langat, was isolated, proof that Langat virus is still active in this forest reserve 10 years after its original isolation in 1956.

REPORT FROM THE MICROBIOLOGY AND PARASITOLOGY DEPARTMENTS, ISTITUTO SUPERIORE DI SANITA', ROME, ITALY

Field studies of arboviruses have been started in 1964 at the Istituto Superiore di Sanità, Rome. Two areas, one in Northern and one in Central Italy, were chosen because of their different ecological and climatic conditions.

1) NORTHERN ITALY (Region of Gorizia).

Gorizia province is at the north-eastern side of a flat plain that extends to the sea and lies in the first subsidiary ranges of Eastern Alps, where the highest altitude is over 600 m. Climatic conditions are in some aspects similar to the continental climate of Central Europe. The annual average temperature ranges between 10° and 15° C and between 0° and 1° C in the coldest months.

a) Serological surveys

Human sera: 166 human sera (about 1:250 of population) have been tested for HI antibodies against ten arbovirus antigens: Sindbis, WEE, TBE, WN, YF, Del, De2, Bunyamwera, Sicilian and Neapolitan Phlebotomus fevers. 58 sera (35%) reacted with one or more antigens 3.6 % to TBE, 10.8% to WN, 16.2% and 16.2% respectively to Sicilian and Neapolitan Phlebotomus fevers (nine sera being positive for both). Some of the HI positive sera were further tested with TBE, WN and Sicilian Phlebotomus fever viruses in the mouse neutralization test. Some of them were confirmed (1).

Wild animal sera: Sera from 26 wild captured Apodemus, grouped in pools of 2-6, were tested for HI antibodies: 1 pool was posi-

tive to WEE, 1 pool to TBE, 3 to WN and 2 to Bunyamwera antigens.

b) Sentinel pastured ovines.

Animals belonging to a migrating and non-migrating flock were bled twice (April and August 1967) and sera were tested for the presence of HI antibodies in an attempt to localize the activity of arboviruses in the area. In the non-migrating flock two animals were found positive to WEE (titer 1:80) and two positive to WN antigen. In the migrating flock only one animal was positive to Sindbis antigen.

c) Tick and mosquito collection

Ticks: In the periods April-November 1966 and April-July 1967, 7.499 unfed ticks (mostly nymphs of Ixodes ricinus) were collected from vegetation on a white blanket.

Ticks were processed in 138 separate samples according to the instars and inoculated intracerebrally into suckling mice.

Mosquitoes: Between July and October 1967, 4.740 mosquitoes belonging to the species Culex pipiens and Aedes vexans and A. caspius were captured using animal-traps and mosquito nets. Only 2.182 mosquitoes have been processed until today in 19 separate pools and inoculated intracerebrally into suckling mice.

d) Virus isolations:

Out of the total number of 1.897 Aedes mosquitoes inoculated, two pools containing 190 and 127 specimens of Aedes vexans and Ae. caspius, caught on 21/9/67 and 25/9/67, yielded strains of virus. The mosquito suspensions were inoculated in suckling mice on 24 October 1967 and after three mouse passages the strains were stabilized with an incubation period of 36-48 hours. They are lethal for young adult mice by intracerebral route and partially lethal by intraperitoneal route and sensitive to sodium desoxycholate. Antigen extracted by sucrose acetone method is not haemagglutinating. Preparation of immune serum and identification tests are in progress.

II) CENTRAL ITALY (Region of Fondi).

Fondi area lies between the sea and the Ausonii Mountains, where the highest altitude is over 1.300 m. and it includes a small alluvial plane flooded each year for about six months. The climate is mild and the annual average temperature ranges between 14° and 19° C and between 4° and 8° C in the coldest month.

a) Serological surveys

Human sera: 205 human sera (about 1:100 of population) have been tested by the HI test against the same ten antigens as in the survey of human sera from Northern Italy. 60 sera (29%) reacted with one or more antigens: 1.9% to TBE, 8.3% to WN, 2.9% to Bunyamwera, 20.5% to Sicilian Phlebotomus fever and 9.7% to Neapolitan Phlebotomus fever. Some of the HI positive sera were further tested with the corresponding viruses by mouse neutralization test. Only TBE positive sera were confirmed (2).

Domestic animal sera: 153 animal sera (bovine, ovine, chicken and rabbit) were tested by HI against the same ten antigens used for the human serological survey. In marked contrast to the human sera, HI antibodies against group A viruses were encountered in the animal sera tested: 24% of bovine sera, 32% of goat sera and 7% of sheep sera reacted with WEE antigen; 2% of bovine sera, 24% of goat sera and 7% of sheep sera reacted with Sindbis antigen. Regarding group B antigens it was remarkable to find so high a value as almost 44% of goat sera reacting by the HI test with TBE; 5% of bovine sera, 32% of goat sera and 11% of rabbit sera were positive to WN antigen. The NT finding with goat sera confirmed the TBE HI-positive reactions: several sera showed remarkable NI_{50} (i.e., $NI_{50} \geq 5.000$) (2).

Wild animal sera: Between the years 1965 and 1967, a total number of 369 wild animals were captured and sera tested by HI test against WEE, TBE, WN, Del, and Bunyamwera antigens.

The specimens included 95 Chiroptera, 126 Apodemus, 37 Rattus and 111 Erinaceus europaeus. Sera (or pool of sera) positive to WEE were found only among Apodemus; positives to TBE among Chiroptera, Apodemus and Erinaceus europaeus; positives to WN among Chiroptera, Apodemus, Rattus and Erinaceus europaeus; positives to Bunyamwera among Apodemus.

b) Sentinel pastured goats.

Because of the high incidence of TBE antibodies found in goat sera surveyed previously, during the year 1967 goats, belonging to one flock migrating during the summer to the Appennines highlands and one remaining on the hills of the region, were bled. The aim was to find the period and the geographic limits in which the presumptive TBE virus was active. The migrating flock bled on May 1967 had only low percentage of HI antibodies to Sindbis and WEE antigens. In the non-migrating flock 63% of goats were found

positive to TBE virus at the first bleeding (July 1967); 57% were positive at the second bleeding (September 1967), carried out only on the youngest goats of the flock (all younger than 18 months). Some sera were also positive to WEE and Sindbis antigens.

c) Tick and mosquito collection:

Ticks: In the year 1964 a total number of 1.402 ticks, collected partially engorged on domestic animals and partially unfed on the vegetation, were processed in 53 separate pools according to the species and the instars. In the year 1966 a total number of 1.587 unfed ticks were processed in 53 separate samples. In the year 1967, the collection was restricted to the area where TBE positive flock is pastured and a total number of 1.685 were processed in 56 separate pools.

Ticks most frequently found in nature belong to the following species: Ixodes, Haemaphisalis, Rhipicephalus, Hyalomma and Dermacentor.

Mosquitoes: A total number of 3.639 mosquitoes in 1965 and 24.853 in 1966 were collected, processed and inoculated intracerebrally to suckling-mice. Mosquitoes collected in 1967 (over 70.000) have not yet been processed. The species of mosquitoes with the highest incidence in the area are: Culex pipiens, Aedes vexans and Ae. caspius.

d) Virus isolations

In 1966 a virus was isolated from a pool of 129 Culex pipiens caught on 24 October 1966. The mosquito suspension was inoculated in suckling mice on 2nd February 1967 and after four mouse passages the strain was stabilized with an incubation period of 36-48 hours. The virus is lethal for young adult mice and for guinea-pigs by intracerebral and intraperitoneal routes and it is sensitive to sodium desoxycholate and to ether.

Antigen extracted by sucrose acetone method is not haemagglutinating (10th mouse passage). Final identification has not yet been achieved, although it seems to be related to WEE virus by N test and CF test.

In 1967 four viruses were isolated from pools of adults Haemaphysalis punctata. The ticks were collected on 6-12-15 and 19 September in the area where the flock of sentinel goats HI positive to TBE virus is pastured. The tick suspensions were inoculated in suckling mice respectively on 12, 15, 26, and 28 September 1967. After

three or four mouse passages the strains were stabilized with an incubation period of 4-6 days.

These viruses are almost completely lethal for young adult mice by intracerebral route, and not by intraperitoneal route and sensitive to sodium desoxycholate. Haemagglutinating antigens were extracted and optimal pH was 6.0-6.2. In the serological tests, carried out to identify the new isolates, we observed that postive TBE human and goat sera from the same area reacted by HI test against the new isolates.

References: (1) Balducci, M., Verani, P., Lopes, M.C., Gregorig, B. (1967): Survey for antibodies against arthropod-borne viruses in man and animals in Italy. II. Serological status of human beings in a northern Italian region (Gorizia province). Am. J. Trop. Med. & Hyg. 16: 211-215.

(2) Verani, P., Balducci, M., Lopes, M.C., Alemanno, A., Sacca, G. (1967): Survey for antibodies against arthropod-borne viruses in man and animals in Italy. I. Serological status of human beings and animals in a central Italian region (Fondi). Am. J. Trop. Med. & Hyg. 16: 203-210.

REPORT FROM THE ARBOVIRUS LABORATORY, PASTEUR INSTITUTE, DAKAR, REPUBLIC OF SENEGAL

Chikungunya Virus in Senegal

By the middle of November 1966, clinicians reported cases of "dengue like fever" in the city of Rufisque, thirty kilometers east of Dakar. A similar illiness was observed in Diakhao, between Fatick and Diourbel.

Investigations were initiated immediately. During the mission, five persons of the Entomological Team working in the Bandia forest and in the mangrove in Saboya near the Gambia border, contracted the disease.

While no definite data of the commencement of the outbreak could be determined, it was found that the cases were already being noticed in October. In Rufisque, some patients were contaminated attending a meeting on October, 25th.

The signs and symptoms of the disease were: sudden onset of fever

accompanied by very severe joint and muscular pains, mostly fingers, wrists, and ankles. Vomiting and restlessness were frequent and a maculo-papular rash has been observed in some cases. The acute stage lasted 4 to 6 days, but apyrexial joint pains recurred intermittently over a period of 5 months. No hemorrhagic symptoms were noticed. No deaths were reported.

Virus isolations

Nineteen isolations of chikungunya virus have been made in November. Sixteen are from human origin, from blood samples taken during the first two days of the disease.

Three are mosquitoes isolates: one from a pool of 6 Aedes aegypti collected in N'Dofene (near Diakhao) and two from pools of Aedes luteocephalus, caught on human bait in a mangrove forest, in the region of Saboya near the border with Gambia.

All the strains have similar titer in 3 day old mice (log $LD_{50} > 8, 5$) with AST of 2 days.

HA antigen was readily obtained by the sucrose-acetone technique, but was not improved by protamine sulphate treatment.

Serological studies

HI and CF tests were carried out on sera from human being, monkeys and other animals. For the HI tests, all sera were acetone treated.

1) Human sera: thirty-nine paired sera were collected.

In Rufisque, the samples were collected respectively the first and the twenty-first day after the onset. HI test showed crossing between chikungunya and o'Nyong nyong antigens but the CF test was specific for chikungunya.

In Diakhao, the second sample collected on the seventy-third day after onset showed crossing even in the CF test. Titers were higher with chikungunya that with o'Nyong nyong. On the 39 pairs, 19 showed seroconversion or significant rise in antibody titer and 7 a decrease in titer.

One hundred and thirteen sera have been collected in April 1967 from non-fibrile patients in Bandia village: 63% had HI antibodies and 38% CF antibodies for chikungunya virus.

2) Monkey sera: HI and CF antibodies for chikungunya were found in the sera from 15 monkeys captured in the Saboya region.

These findings with the isolation from Aedes luteocephalus point to the existence of a forest cycle of chikungunya virus in this area.

3) Other animals sera: numerous blood specimens have been collected from animals (birds, reptiles, mammals) using blood-saturated paper discs.

When serological comparison of paired sera of recaptured animals is complete, information will be available on activity of chikungunya virus in the wild animals population.

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

Seven virus strains, isolated in infant mice after inoculation with mosquito suspensions, were sent to the East African Virus Research Institute for identification (see the issue of March 1967 for details). After a few passages, six of the strains showed identical behaviour in mice, the seventh strain behaved differently. The latter was identified as closely related to or identical with An 5077 virus of the Simbu group. It was isolated from a pool of M. uniformis. An 5077 virus was first isolated from cattle in Nigeria. Our strain was isolated in an area where herds of cattle pass regularly on their way to the slaughter house in Mombasa.

Of the other six, the possibility of a mouse virus was considered, and one strain - MMP 15 - was sent to a laboratory in the U.S.A. for further investigations.

Preliminary results of the CFT, reported from the U.S.A. showed that MMP 15 antigen failed to react with several mouse virus antisera, but suggested relationship with agents of the psittacosis group.

In our laboratory Bedsonia-like organisms were demonstrated when impression preparations of infected mouse brain and spleen on slides were stained with Stamp's stain.

Histological examination of brains of infected baby mice, showed encephalitis. The strain was not transmitted by Aedes aegypti to healthy infant mice. The mosquitoes had been fed, through guinea pig colon wall, on a

suspension of infected mouse brain in defebrinated rabbit blood. After two weeks, virus could not be recovered from surviving mosquitoes.

Though there was not realy a random distribution of the infected mice in the mouse groups, inoculated with the mosquito suspensions, the possibility that we are dealing with Bedsonia infection of mice has to be considered.

From lst August till 20th September a virus isolation experiment was conducted on the shore of Lake Naivasha, situated at an altitude of 6, 200 feet in the Rift Valley. A very large bird population is associated with the lake. Most of the mosquitoes were collected on human baits, a small number on a horse. A total of 14 species and 1 group of related species were represented among the collections. Four virus strains have been isolated .

The four agents are ether sensitive. A transmission experiment with A. aegypti conducted with one of the isolates, was successful. The Table summarizes the results of the isolation experiments.

(D. Metselaar)

Table

(The Table Summarizes The Results of the Isolation Experiments)

Mosquito Species			No. Collected	No. of Pools	No. of isolations
Mansonia	(Mansonioides)	africana	4,926	18	1
-	-	uniformis	11,405	34	0
-	(Coquilletidia)	versicolor	3	1	0
Aedes	(Aedimozphus)	quasiunivittatus	717	3	0
-	-	dentatus group	2,024	6	0
-	-	cumminsi	22	1	0
-	(Neomelaniconion)	lineatopennis	111	2	0
-	-	circumluteolus	13	1	0
Culex	(Neoculex)	rubinotus	1,310	5	1
-	(Culex)	aurantapex	69	2	0
-	-	theileri	16	1	0
-	-	zombaeneis	364	2	0
-	-	nakuruenzis	2,295	9	2
Anopheles	(Anopheles)	coustani ziemanni	2,004	8	0
-	(Cellia)	squamosis	163	2	0
Total 14 Species - 1 Group			25,442	95	4

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

Antibody response to Sindbis virus by wild birds

Recent studies have indicated that although the antibody response to Sindbis virus by some birds is usually readily detectable and probably durable, the response by other birds, which seem to be largely restricted to certain families, is poor and transient. The table shows the results obtained with 10 masked weavers (Ploceus velatus), members of the family Ploceidae (weavers, sparrows, bishops), which apparently have a poor antibody response. The birds were inoculated twice at an interval of six months and the antibody response tested periodically with three serological tests. It will be seen that the response to the first inoculation was almost entirely negative with the NT and with the other two tests a positive response seemed dependent upon the bird having been viraemic. Even with the HI and PI tests, however, the response was weak as judged by HI titres and sometimes erratic, e.g., weaver 493 with HI and PI tests. By the 16th week after inoculation all birds were negative by any test. After the second inoculation of virus only one bird, 488, was observed to be viraemic. No viraemia had been detected in this bird after the first inoculation nor had any of the antibody tests been positive. It seems possible, therefore, that this bird had been unsuccessfully inoculated initially. The negative viraemias in the remaining birds seem to indicate that these birds were still immune at the second inoculation despite the completely negative antibody tests just prior to this inoculation. The antibody response of most birds to the second inoculation was slightly superior to the first, i.e., higher HI titres and more positive results with NT, even after 8 weeks. However, once again all birds within a few months reverted to a negative antibody status.

The correlation between the HI and PI tests was generally satisfactory with only three birds after the first inoculation and two birds after the second inoculation giving different results with the two tests. The conclusion seems justified that a positive result with any of the three tests is reliable and is indicative of recent infection whereas a negative test is not. Antibody surveys among wild populations of members of the Ploceidae have been in agreement with the above experimental results. For instance, of 1, 490 members of the Ploceidae tested for Sindbis HI antibody over a three-year period only nine were positive, despite the fact that this virus was isolated frequently from aviophilic mosquitoes and once from a masked weaver during this period at the same locality. It would seem that antibody surveys on certain bird species with Sindbis virus are largely meaningless.

RESPONSE OF MASKED WEAVERS TO INFECTION WITH SINDBIS VIRUS

Bird No.	Viraemia		ANTIBODY												Viraemia	ANTIBODY													
	Level. logs	Day	Pre-inoc.			Post-inoc. - weeks after 1st inoc.									2nd Inoc.	Post-inoc. - weeks after 2nd inoc.													
						4			7			16			28	6			6½			8			20			24		
			HI	PI	NT	HI	PI	NT	HI	PI	NT	HI	PI	NT		HI	PI	NT	HI	PI	NT	HI	PI	NT	HI	PI	NT	HI	PI	NT
487	1.5	2	N	N	N	2	P		1	N	N				N	6	P		3	P				P	N			N		
488	N	2.4	N	N	N	N	N	N	N	N	N				P	2	P		2	P				N	N			N		
489	1.5	2	N	N	N	1	P	N	1	N	N	All			N	N	N		N	N				P	N			N		
490	N	1.3	N	N	N	N	P		N	P	N	tests			N	2	P		4	P				N	N			N		
491	N	2.4	N	N	N	N	N	N	N	N	N	negative			N	N	N		N	N				N	N			N		
492	2.8	1	N	N	N	2	P	N	4	P	P				N	4	P		4	P				P	N			N		
493	4.5	1	N	N	N	2	N	N	N	P	N				N	4	P		2	P				P	N			N		
494	N	2.4	N	N	N	N	N	N	N	N					N	N	N		N	N				N	N			N		
495	1.8	1	N	N	N	2	P	N	N	P	N				N	1	N		N	N				N	N			N		
496	2.5	1	N	N	N	N	P	N	N	P					N	3	N		N	N				N	N			N		
Summary	6/4		0/10	0/10	0/10	5/5	6/4	0/8	3/7	5/5	1/7	0/10	0/10	0/10	1/9	7/3	5/5		5/5	5/5				4/6	0/10			0/10		

HI = haemagglutination-inhibition: PI= plague-inhibition: NT= neutralization test, IP in infant mice: N = negative;
P = positive. HI titres expressed as number of twofold serum dilutions: blank space = no test done.

REPORT FROM QUEENSLAND INSTITUTE OF MEDICAL RESEARCH, BRISBANE, AUSTRALIA

The following is a summary of relevant sections of the Institute's Annual Report for the year 1966-1967. Some copies of the full report are available from the Institute.

Several "new" arboviruses were isolated in the year: C5502 and C5581, from Ornithodorus ticks from a tern colony on the Great Barrier Reef and MRM4059 from the skink Ablepharus boutonii virgatus from Mitchell River Mission. In addition, Kunjin virus was isolated from a bird and MRM3929 from mosquitoes for the first time. Further studies confirmed that MRM3630 and MRM3929, isolated previously, were distinct from viruses previously known to occur in Australia.

Serological tests of man or sentinel fowls provided further information on the epidemiology of arboviruses: human sera from western Queensland were found to show an antibody pattern suggesting infection with the Murray Valley encephalitis-Kunjin-MRM3929 subgroup of group B, clearly distinct from that to the dengue subgroup found on the east coast; four cases of epidemic polyarthritis in Queensland, N. S. W. and Victoria had rising titres to Ross River virus; sentinel chickens at Charleville showed a low incidence of infection with Sindbis in and after January, 1967: neutralization tests on 130 sera selected on the basis of previous haemagglutination-inhibition tests gave no indication of the vertebrate hosts of the Koongol group viruses.

Field entomological studies at Mitchell River Mission in dry and wet seasons provided further data on fluctuations in mosquito populations. Evidence was found of seasonal changes in host preference for Culex annulirostris, the most important arbovirus vector in the area. Several newly-isolated viruses, including MRM4059 isolated from reptiles, were shown to multiply in experimentally inoculated mosquitoes and can therefore be accepted as arboviruses. A laboratory colony of the mosquito Aedes notoscriptus was established after a period of maintenance by forced-mating techniques.

Two group A viruses, Ross River virus and Sindbis, were shown to differ markedly in their ability to multiply in several vertebrates: Ross River virus multiplied to high titre in rabbits, rats, bandicoots and marsupial mice but not in adult fowls or pigeons; Sindbis multiplied in the two bird species but poorly in the four mammals. These results are compatible with serological evidence of antibody to Ross River virus in mammals and to Sindbis in birds.

Corriparta virus was confirmed to be sensitive to ether after careful purification of reagents. Although it is (by electron microscopy) structurally a reovirus, it has definite physico-chemical differences (as in ether sensitivity) from the classic reoviruses. Lipid inhibitors of arbovirus haemagglutination were found to inhibit infectivity also. Chemical studies showed that both cholesterol and phospholipid are necessary to produce potent inhibitors; the possibility was suggested that virus is inhibited nonspecifically by lipid micelles rather than by a specific receptor.

REPORT FROM THE SEATO MEDICAL RESEARCH LABORATORY AND THE SCHOOL OF PUBLIC HEALTH, BANGKOK, AND THE PASTEUR INSTITUTE (SAIGON, SOUTH VIETNAM)

Studies on the comparative dengue virus vector status of Aedes aegypti and A. albopictus are in progress. Daytime indoor and outdoor collections are being made in areas where recent cases of hemorrhagic fever have occurred on Koh Samui, Thailand, and Saigon, South Vietnam. The Saigon series is the larger, over 1,000 pools of 25 female mosquitoes each having been tested since 15 May. The results are given in Table 1. Of 23 dengue viruses recovered to date, all have been recovered by the direct and delayed plaque system and at least 5 of these would not have been detected by the use of 1-day-old suckling mice alone. Dengue viruses have been recovered from A. aegypti but not from A. albopictus. About 10 times more A. aegypti have been tested, however.

Recent laboratory studies with Aedes aegypti have led to the discovery of a virus inhibiting substance in normal, newly emerged, unfed females. Mosquitoes were triturated in various concentrations of local origin, low passage dengue and chikungunya viruses at the rate of 25 females per ml. The infectivity of dengue types 1 though 4 for LLC-MK_2 cells was reduced by 90% to over 99% (Table 2) but chikungunya virus was not. Mosquitoes were triturated in media containing a single dose of Ross strain chikungunya virus and prototype dengue virus type 1, 2 and 3 and then diluted and inoculated into mice and cell cultures. Viral inhibition was observed in cell cultures and was reversable on dilution (Table 3). There was some indication (slightly increased survival and longer incubation periods) of inhibition observed in suckling mice. When Culex tritaeniorhynchus and C. gelidus were triturated in various dilutions of Japanese encephalitis virus (JEV) inhibition was again seen in LLC-MK_2 cell culture (Table 4) but none occurred in suckling mice. The inhibitory substances in Aedes aegypti may account, in part, for the large number of strains detected by the delayed plaque method when no direct plaques were observed (Table 1) and

the difficulty in isolating these viruses in mice.

Field studies on arbovirus ecology are continuing at Bang Phra, Cholburi. In this area, year-round transmission of JEV by Culex gelidus and C. tritaeniorhynchus occurs. There are few large water birds and swine present in the area. Results of neutralizing antibody surveys are shown in Table 5. In addition to the species listed in Table 5, other species of reptiles, mammals and birds reacted in neutralization tests with JEV, but the number of individuals captured and tested was small. Some bird and mammal species have been inoculated with low passage JEV in the laboratory. Two sparrow species, Passer flaveolus and P. montanus responded with viremias of 5-6 days. Two bulbul species (Pycnonotus goiavier and P. blanfordi), bats (Cynopterus brachyotis), rats (Rattus rattus) and mice (Mus cervicolor) did not develop viremias. The bulbuls produced neutralizing antibody inconsistantly but all mammals responded well. In June, July and August, mosquitoes at Bang Phra were collected by means of (1) light traps, (2) horse biting collections, (3) traps baited with Passer flaveolus sparrows, Pycnonotus goiavier bulbuls and Cynopterus brachyotis bats. Each method resulted in the capture of different mosquito fauna (Tables 6, 7, 8). Most noteworthy of these differences is the attraction of Culex quinquefasciatus to sparrow, bulbul and bat bait. Most of these represent collections made in the canopy of rain trees, where sparrows roost in numbers.

(Philip K. Russell, Thomas M. Yuill and Joe T. Marshall, Jr. [Virology Department, S.E.A.T.O. Med. Res. Lab.], Douglas J. Gould [Entomology Department, S.E.A.T.O. Med. Res. Lab.], Charas Yamarat [School of Public Health, Faculty of Medicine] and Do Van Quy [Pasteur Institute]).

Table 1. Recovery of dengue viruses from Aedes aegypti from Saigon by LLC-MK_2 cell cultures and mice, 15 May - 30 Oct., 1967

Dengue Type	LLC-MK_2 Cells			Cells and Mice	Total
	Direct	1st Del.	2nd Del.		
1	1	2	0	2	3
2	2	5	2	7	9
3	0	1	0	0	1
4	0	1	0	0	1
Untyped	4	3	2	Incom*	9
Totals	7	12	4	9	23

* Incomplete, specimens in passage

Table 2. Inactivation of various concentrations of low-passage dengue virus by Aedes aegypti suspensions tested by LLC-MK_2 cell plaques

Dengue Type	Treatment	Undil.	10^{-1}	10^{-2}	10^{-3}	10^{-4}
D 1	Control	TN(87,000)*	TN(8,700)	TN(870)	87	
	Mosq.	35	19	0	0	
D 2	Control		TN(15,700)	TN(1,570)	157	20
	Mosq.		91	5	1	0
D 3	Control	TN(9,200)	TN(920)	92	30	
	Mosq.	TN	53	5	0	
D 4	Control	TN(800)	80	19	1	
	Mosq.	87	8	1	0	

* TN = too numerous to count (plaque count calculated)

Table 3. Inactivation of test doses of dengue and chikungunya viruses by Aedes aegypti suspension s and reactivation upon dilution as tested by LLC-MK_2 cell plaques

Prototype Virus	Treatment	Dose	$Dose^{-1}$	$Dose^{-2}$	$Dose^{-3}$
D 1	Control	TN(6,400)*	TN(640)	64	22
	Mosq.	17	4	1	0
D 2	Control	TN(80,000)	TN(8,000)	TN(800)	80
	Mosq.	TN	89	76	53
D 3	Control	TN(4,800)	TN(480)	48	14
	Mosq.	62	58	41	12
Chik	Control	TN(3,900)	TN(390)	39	2
	Mosq.	32	37	33	1

* TN = too numerous to count (plaque count calculated).

Table 4. Inhibition of Various Concentrations of Japanese Encephalitis Virus in LLC-MK_2 Cell Cultures by Suspensions of Normal *Culex tritaeniorhynchus* and *C. gelidus*.

	Plaque Numbers at Dilutions			
Mosquito	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Control	TN(1,900)*	190	26	3
C. gelidus	79	16	0	0
C. tritaeniorhynchus	72	3	0	0

* Plaques too numerous to count (plaque count calculated)

Table 5. Common to abundant species captured on the Bang Phra study area in 1966-1967 and tested for neutralizing antibody against 4 arboviruses

Species	JE*	Chik**	Sindbis	BKM 367
Birds				
Passer flaveolus	31/100(31)***	5/153(3)	3/169(2)	7/90 (7)
Copsychus saularis	18/66(27)	2/87(2)	2/92(2)	5/68(7)
Pycnonotus goiavier	41/230(18)	0/233(0)	0/215(0)	12/171(7)
Pycnonotus blanfordi	31/228(13)	4/200(2)	1/243(0.4)	15/203(7)
Rhipidura javanica	5/55(11)	1/56(2)	0/65(0)	1/50(2)
Mammals				
Rattus rattus	110/337(33)	10/300(3)	8/320(3)	47/292(16)
Cynopterus brachyotis	22/245(9)	0/269(0)	0/282(0)	2/210(1)
Reptiles				
Hemidactylus frenatus	7/16(44)	0/16(0)	0/16(0)	0/16(0)
Colates versicolor	8/15(53)	0/15(0)	2/15(13)	0/16(0)
Colates mystaceus	6/13(46)	0/13(0)	0/13(0)	2/13(0)

* JE = Japanese encephalitis virus

** Chik = chikungunya virus

*** Number of individuals reacting/total individuals tested (% positive).

Table 6. Mosquitoes collected in bait traps at Bang Phra, July, August, September 1967

Mosquito Species	Host Species		
	Passer flaveolus	Pycnonotus goiavier	Cynopterus brachyotis
Culex quinquefasciatus	995	78	44
Culex sitiens	48	1	1
Mansonia crassipes	7	0	0
Culex pseudovishnui	7	1	0
Culex bitaeniorhynchus	6	0	0
Culex tritaeniorhynchus	4	2	0
Culex fuscanus	3	0	0
Culex gelidus	3	0	0
Aedeomyia catastiota	2	0	0
Armigeres subalbatus	1	0	1
Culex annulus	1	0	1
Aedes albopictus	0	1	0
Aedes aegypti	0	1	1
Aedes vigilax	0	0	0

Table 7. Mosquitoes collected in light trap at Bang Phra. July, August, September 1967

Species	Number Collected
Culex tritaeniorhynchus	10290
Culex gelidus	8969
Culex fuscocephalus	2371
Aedes mediolineatus	442
Culex annulus	384
Anopheles philippinensis	203
Culex pseudovishnui	187
Mansonia uniformis	173
Aedes vexans	113
Mansonia annulifera	80
Culex sitiens	60
Anopheles vagus	49
Aedes lineatopennis	40
Culex quinquefasciatus	31
Anopheles subpictus	26
Anopheles indiensis	21
Aedes vigilax	16
Picalbia luzonensis	11
Anopheles annularis	9
Anopheles argyropus	9
Picalbia hybrida	5
Aedes albopictus	4
Aedes aegypti	1
Mansonia crassipes	1

Table 8. Mosquitoes collected biting horses at Bang Phra, July, August, September 1967

Species	Number Collected
Culex gelidus	496
Culex tritaeniorhynchus	394
Anopheles vagus	266
Aedes mediolineatus	238
Culex sitiens	81
Culex pseudovishnui	78
Mansonia uniformis	76
Anopheles subpictus	66
Culex annulus	61
Aedes vaxans	54
Aedes lineatopennis	52
Mansonia annulifera	24
Aedes vigilax	22
Culex quinquefasciatus	22
Culex fuscocephalus	9
Anopheles philippinensis	4
Armigeres subalbatus	4
Aedes albopictus	3
Culex bitaeniorhynchus	2
Anopheles tesselatus	1

REPORT FROM THE BUREAU OF RESEARCH AND LABORATORIES PHILIPPINE DEPARTMENT OF HEALTH, MANILA, PHILIPPINES

The year 1966 proved to be an inopportune year for our country with regards to Philippine hemorrhagic fever. The total admission for the period at San Lazaro Hospital alone, which is the national hospital for infectious diseases, was 5, 612 -- the highest since 1956. Of these admissions, 76.1% occurred during the months of July to October.

During this epidemic, 405 paired blood samples (acute and convalescent) were received from various hospitals, most of which came from San Lazaro Hospital and the Children's Memorial Hospital. Of these 405 paired samples, 299 or 73.8% had HI evidence for Group B arbovirus infection (at least a four-fold rise in titer or a persistent high titer for both sera of at least 640 but most were $\leqq$ 1280). 127 or 30.8% of the acute samples of these paired samples with evidence of Group B arbovirus infection had at least a titer of 320.

Acute single serum samples numbering 245 were likewise submitted by these hospitals. Of these, 76 or 31.1% had at least an HI titer of 640 but most were $\leqq$ 1280 for Dengue 2 antigen.

The prevalence of HI antibody was determined in 88 children seen at the Out-Patient Department of the Philippine General Hospital from October 1966 to February 1967. It was found that among those under 3 years age group, 43.7% (7/16) were positive for antibody for Dengue virus; 3-6 years group, 63.6% (14/22); 7-10 years group, 55.1% (16/29); 11-14 years group, 76.1% (16/21). Of the 88 children, 53 or 60.2% had HI antibody. A similar study was made in 1964 on 67 children from the same hospital (Philippine General Hospital) which showed 16 or 23.9% had HI antibody for Dengue virus.

615 acute serum samples were inoculated into BSC-1 cells and using the challenge virus resistance technic with Polio 3 as challenge virus, it was found out that 47 or 7.6% were positive.

474 of these sera were likewise inoculated into day-old suckling mice and using the challenge resistance technic with dengue 2 as challenge virus, 96 or 20.2% showed resistance to challenge with 14 or 14.5% showing 30-39% resistance, 13 or 13.5% with 40-49% resistance, and 69 or 71.8% with 50-100% resistance. Only 5 had produced illness in suckling mice.

Further work is being undertaken to identify these isolates but there seems to be great difficulty in adopting them to mice. So, more passages in both

tissue culture and mice are being done at the moment with the isolates.

It is quite apparent from our meager work in isolation that the suckling mice technic is more sensitive than the tissue culture work in routine isolation. This is quite significant especially when one considers the difficulties of an underdeveloped country like ours -- the cost of the sera and reagents for tissue culture, the fact that they are not locally available, the procurement time involved in acquiring these materials abroad, and the additional personnel, facilities and space needed in tissue culture work.

(Virginia Basaca-Sevilla)

REPORT FROM THE VIRUS RESEARCH CENTRE, POONA, INDIA

Establishment of Cell Cultures derived from larvae of Aedes albopictus and Aedes aegypti

Three cell lines of Aedes albopictus and two of Aedes aegypti have been successfully established using a culture medium without insect haemolymph. The culture medium used by Mitsuhasi and Maramorosch (Contrib. Boyce. Thompson Inst., 22: 435 (1964)) for leaf hopper tissue culture, was used in the present study. Studies on the susceptibility of these cell lines to different arboviruses are in progress.

Three cultures of cells of Aedes albopictus larvae were set up at different times and all the three cultures have undergone several passages. First culture is in 18th passage, the second is in 15th and the third in 11th passage.

Mainly three morphological types of cells were observed in the Aedes albopictus cultures. The predominant cell type was noted to be round and 6-20 u in diameter and the next common type was spindle shaped measuring 7-10 u wide and 15-90 u long. The third type of cell was binucleated, round and 37-53 u in diameter. Their exact relationship, if any, to each other is not known.

Four such cultures of cells of Aedes aegypti larvae were set up. One was lost due to contamination in the 11th passage and another in the 7th passage. Of the remaining, one is in the 19th passage and the other is in 8th passage. These cultures mainly consisted of an epithelial type of cells.

Many mitotic figures, some showing diploidy or polyploidy, were seen in cultures of both species. The details are being published in "Current Science", Bangalore.

Probable New Arbovirus (?) for India

Early in 1967, an outbreak of febrile illness in Aurangabad city (Maharashtra State) was investigated. Two agents that produced cytopathic effect (CPE) in BHK-21 cells were isolated from sera of two patients aged 7 and 20 years. The convalescent serum of the latter patient neutralized the virus in tissue culture. The acute serum did not. These agents produced CPE in BHK-21, VERO and primary chick embryo cell cultures but no illness was observed in 0 to 4 day old or adult mice. In addition no pocks were observed on chorio-allantoic membrane. Both these agents were chloroform sensitive. In preliminary tests, these agents seem to be unrelated to the Group A and B arboviruses found in India. Further studies are in progress.

Isolation of Dengue Type 3 Virus in India

Several strains of dengue types 1, 2 and 4 were hitherto isolated in South India by the Virus Research Centre. In October 1965 a strain of dengue 3 virus was isolated from a group of "febrile patients" at the General Hospital, Madras. From a pool of Aedes aegypti mosquitoes caught in houses where the patients were residing, another strain of dengue 3 virus was isolated. This is the first evidence of dengue 3 in India.

Infection of Monkeys (Macaca radiata) with Chikungunya Virus

Macaca radiata monkeys can be easily infected with chikungunya virus by either intravenous injection or through the bite of infected Aedes aegypti mosquitoes. They circulate chikungunya virus up to the 4th or 5th day post-infection, and the intensity of viraemia and its duration vary with the dose of inoculation and possibly the age of monkey. No obvious signs of illness have been recorded in the infected monkeys. The neutralizing antibodies start appearing in the circulation as early as 4th post-inoculation day and by the 7th to 10th day PI fairly high titres of neutralizing antibodies can be demonstrated in the monkey serum. Further studies on the appearance and the pattern of CF and HI antibodies and circulating interferon have yet to be done.

Studies on the rate of infection in Aedes aegypti and Aedes albopictus mosquitoes when fed on viraemic monkeys have been almost completed. The results need final analysis and interpretation. However, Aedes albopictus seems to be more susceptible to infection than Aedes aegypti as evidenced by their capacity to get infected in larger numbers at lower levels of viraemia is high.

Observations on Certain Virological and Immunological Characteristics of Dengue Viruses

The aim was to investigate the biological and immunological characteristics of dengue viruses which could form a basis to understand the similarities, dissimilarities and inter-relationships among the dengue virus strains.

The assay methods of dengue viruses by the tube culture polio-challenge method was standardized. It was found that the titres by the polio-challenge method were virtually identical with the titres obtained in mice and by the plaque method.

Experimental evidence suggested that the mechanism of resistance against

polio virus in dengue infected cell cultures could be other than through the mediation of interferon.

The virus strains could be distinguished by a few of the biological markers. The mk, t and ag markers were found for dengue viruses. Correlation was found between the mouse virulence of a dengue strain and its 't' marker.

Table I summarizes the biological markers of the different dengue virus strains.

A parabolic relationship between the survival time index of groups of mice and the log dose of virus inoculated in them has been described.

The ability to produce haemagglutinin by different dengue virus strains was investigated. Some strains were producers of high titre haemagglutinin while some produced low titre haemagglutinin. All the dengue virus strains could be typed by HI and CF tests.

The role of fresh serum factor in the neutralization of dengue viruses was investigated. It was found that the fresh serum factor did not have any demonstrable effect on the neutralization of dengue viruses. Some samples of fresh sera from rabbits could inactivate the dengue viruses nonspecifically.

The dengue viruses could be typed by neutralization tests. In certain cases intratypic differences could also be found.

Intratypic differences among the dengue type 4 strains were investigated by comparing the area functions of the regression lines fitted to the points obtained with log NI as ordinate and log serum dilutions as abscissa. Two strains (611319 and 642069) differed markedly in area functions from the other two (62231 and 624000).

Agar gel diffusion tests carried out with dengue virus antigens showed that the dengue type 2 antigen reacted with all four types while the other three types reacted specifically. The large antigen was infectious, contained the haemagglutinating fraction and had complement fixation properties. The small antigen was non-infectious, non-haemagglutinating and had no complement fixing properties.

The small antigens of the dengue strains belonging to types 2 and 3 gave two lines of precipitation. Only one line was obtainable with the small antigen with type 4 viruses.

TABLE I

SUMMARY OF THE BIOLOGICAL MARKERS OF THE DENGUE VIRUS STRAINS

Virus Strain		"mk" marker	"ag" marker	"t" marker	Mouse Virulence
Hawaiian	(Dn.1)	mk -	ag -	t -	++
623996	(Dn.1)	mk +	ag -	t -	± -
631292	(Dn.1)	mk +	ag -	t -	± -
P23086	(Dn.1)	ND	ND	ND	
606147	(Dn.1)	mk -	ag -	t -	+ ±
TR1751	(Dn.2)	mk +	ag +	t +	++
New Guinea B	(Dn.2)	mk +	ag -	t +	ND
P8640	(Dn.2)	mk +	ag -	t -	++
P23085	(Dn.2)	mk +	ag -	t +	++
633763	(Dn.2)	mk +	ag -	t +	++
64421	(Dn.2)	mk +	ag +	t +	++
633798	(Dn.3)	mk +	ND	t -	ND
611319	(Dn.4)	mk +	ag -	t -	+ ±
62231	(Dn.4)	mk +	ag -	t -	± -
624000	(Dn.4)	mk +	ag +	t -	± -
642069	(Dn.4)	mk +	ag -	t +	+ ±

A Probable Case of a West Nile Infection

During the course of the study of fevers in children at Vellore, out of 268 cases, eighteen Group B responses were detected. Seventeen of these cases belonged to Vellore town and the serologic evidence pointed to infection with dengue viruses. In the eighteenth case, a rural girl with encephalitis, serologic evidences indicate infection with West Nile or a closely related virus. The only other known human case of a West Nile infection in India was a probable laboratory infection in 1955-56.

REPORT FROM THE MIDDLE AMERICA RESEARCH UNIT BALBOA HEIGHTS, PANAMA CANAL ZONE

Geographic Antigenic Variation of VEE Viruses

Results of kinetic HI analysis of many different VEE viruses indicated presence of several variants which appeared to be related to geographic origin of strains rather than host source or year of isolation (Exchange #14). Recent isolates from Trinidad and Venezuela (Guasapati) submitted by Drs. Spence and Bergold have proven identical to previous Mucambo and VEE variants from these countries. Two distinct variants were originally recognized from Almirante and the Canal Zone area of Panama. In August the first strain from the Darien jungle of the Republic was made available through the courtesy of Dr. Sunthorne Srihongse of Gorgas Memorial Laboratory (GML). This virus has been identified as a strain of Canal Zone type, suggesting that geographic continuity extends from the Colombian border to at least the Canal Zone. Further studies are planned to localize the point at which the Almirante-Canal Zone change occurs.

Viruses from Almirante and the Vera Cruz region of Mexico were identical in kinetic tests. If the basic hypothesis is correct, VEE agents isolated between these two distant places should be similar. Using serological data obtained in the course of a major survey for arbovirus antibodies among humans in Central America, two localities in Eastern Nicaragua were selected and a field team from this laboratory GML and the Nicaraguan Ministry of Health began investigations early in September. Sentinel hamsters are the main hope for detecting virus, but collections of wild animals, mosquitos, and blood specimens from humans and livestock are being made. The goal eventually is to map the geographic variants of VEE virus and attempt to find parallel variation in potential arthopod vectors

and/or vertebrate reservoirs that might provide clues to the origin and evolution of the observed virus patterns.

Drs. William Scherer, Victor Ordonez, Robert Dickerman and colleagues have pursued basically similar studies this past summer in Honduras, Guatemala and Belize.

PATHOGENESIS OF JUNIN VIRUS IN THE HAMSTER

Direct immunofluorescent conjugates were prepared for the four members of the Tacaribe group viruses using globul in fractions of hyperimmune mouse ascitic fluid. Group specific fluorescence was observed in infected VERO monolayer cultures although quantitative differences exist between homologous and heterologous systems within the group. Complete inhibition of the reaction was demonstrated when homologous reagents were used.

In cultures stained with Giemsa, basophilic granules were seen in the cytoplasm of cells in areas showing cytopathic effect. Correspondingly, fluorescence was observed in these same locations. In cultures with more advanced cytopathic effect, the granules were larger and the fluorescence was more intense. In no case were either granules or fluorescence observed in the nuclei of cells. These experiments suggested that specific immunofluorescence might be useful in tracing the course of experimental infection in animals.

The course of experimental infection with Junin virus was traced in suckling hamsters using the direct immunofluorescent technique and by virus plaque assay of tissues in VERO cells. On examination of the sections of various organs stained by this method and later with Hematoxilin-Eosin, it was possible to evaluate the antigen in the cells quantitatively and qualitatively, and to relate it to the general structure of the organs.

Specific fluorescence was first observed in sections of lung, liver, and thymus two days post-inoculation. In all instances, fluorescence started around the vessels of the organs. Parenchymal involvement was variable, usually minimal. For example, hepatic fluorescence was first observed in cells lining the vessels, particularly centro-lobular veins. Intensity of staining increased with time and eventually involved the biliary ducts. Between day 7-9 small scattered foci of intracytoplasmic fluorescence were observed in hepatocytes. At the time of death (12 days) however, no parenchymal antigen was detectable, although the vascular and biliary

changes were persistent. The fluorescent antigen in the lung started diffusely in the endothelium of the small and medium vessels, mainly in the adventitia. Later, the fluorescence was so intense in the septal cells of the alveoli that the architecture of this structure was clearly demonstrated. Fluorescence in the thymus assumed a weak and diffuse character involving groups of cells.

In the kidney, after day 3, the fluorescence was diffuse and rather faint in the tubules and in the adventitia of the small vessels. But after seven days the fluorescence was extremely intense in the cytoplasm of the glomerular cells. After three days, fluorescence appeared in the fibromuscular cells of the spleen, and on day seven the brightness of the capsule clearly defined the shape of the organ. The lymph nodes were equally precocious and exhibited a weak and diffuse fluorescence, inclusively inside small groups of lymphocytes. The pancreas was negative in the acini and islet cells, but positive in the connective interacinar tissue.

Fluorescence appeared on day 4 in the sarcoplasm, sometimes in peripheral cytoplasm of individual muscle cells of the myocardium. The overall picture was that of focal involvement of heart muscle.

Fluorescence was seen in the brain beginning day 5 and it was distributed diffusely in the cortex of the organ and the Ammon horn. Dense clumps of fluorescence were found without specific topography. On day 7 there was diffuse weak fluorescence at the granular layer of the cerebellum, but the brightness in the cytoplasm of Purkinje's cells was intense, probably resulting in the agglutination of small granules to form a "tongue" shape in one of the poles of the cells. Progressive increase in intensity of staining was observed until the animals died.

In general, the antigen showed an avidity for the mesenchymal cells such as in the adventitia of the vessels, septal cells of the alveoli, the splenic capsule, interacinic tissue of the pancreas, and the connective tissue cells of the biliary ducts. On the other hand, antigen shown in the parenchymal cells of certain organs such as those of the brain, heart, and kidney, suggests the hypothesis that those organs, especially the brain, are the main targets of parenchymal virus multiplication.

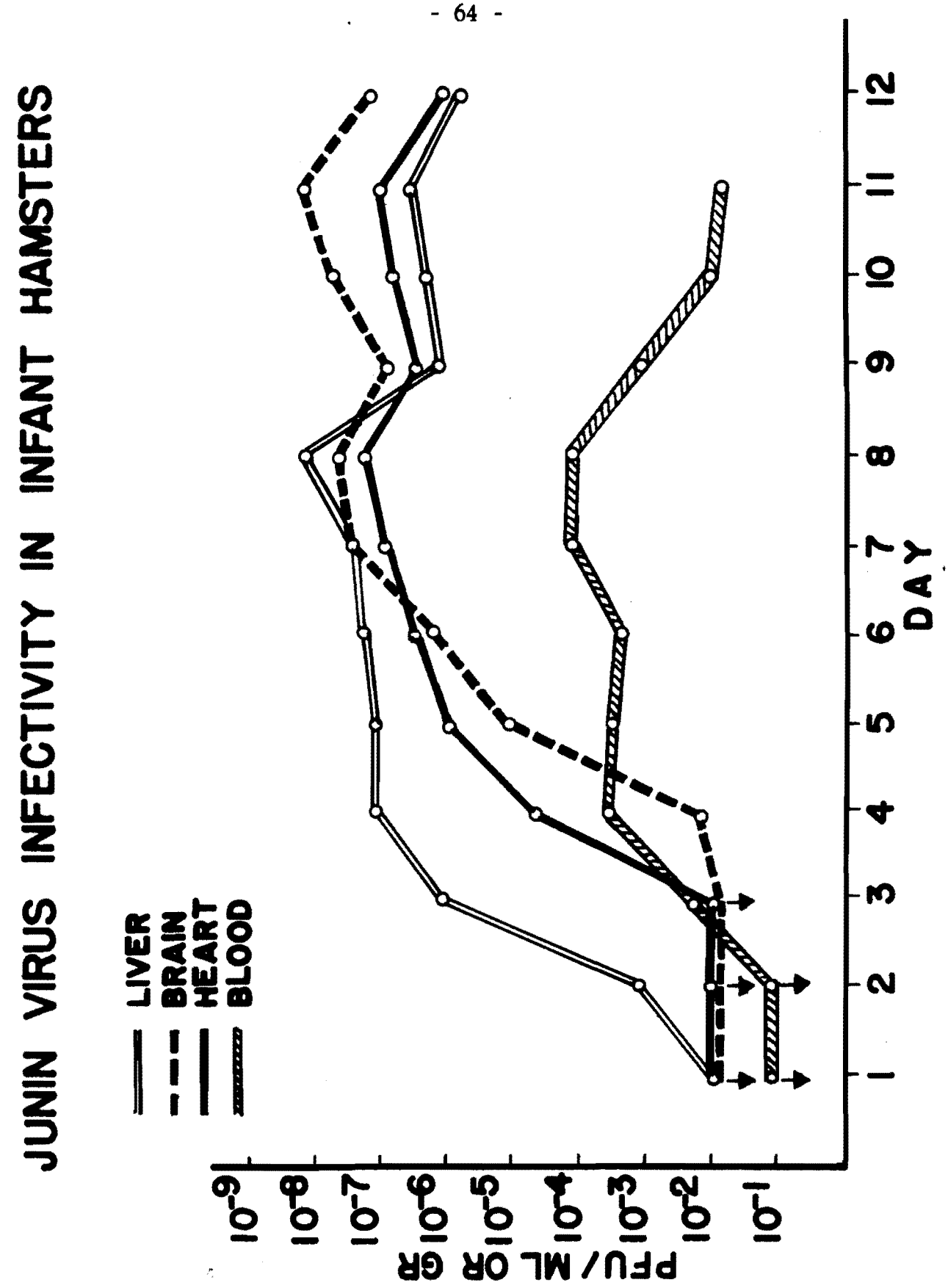
Examination of blood from these animals revealed no clear abnormalities in white cell counts, differentials, platelets or hematocrit.

Gross pathology was absent throughout.

Quantitative virus determination from daily pools of three animals showed

remarkable correlation with fluorescent results. Viremia was of low titer. The pattern for liver shown in the figure is representative for spleen, thymus, kidney and lung. Note the early appearance of virus, the peak at day 8 and the lower sustained titers thereafter. In contrast, virus was not detected in the heart or brain until day 4 and the peaks were sustained until death.

In summary, the data suggest that the virus multiplies widely in "connective" tissues, probably without significant acute or permanent damage. Some or all of these sites may provide the host cells crucial to maintenance of silent, chronic infection. In newborn hamsters, moreover, there is secondary invasion of parenchymal cells of heart and central nervous system, and it is likely that these are the damages responsible for the pathogenicity of the virus. Studies are in progress to determine the precise patterns of infection in adult hamsters which do not succumb to infection.


JUNIN VIRUS INFECTIVITY IN INFANT HAMSTERS
LIVER
BRAIN
HEART
BLOOD
PFU/ ML OR GR
DAY

REPORT FROM THE ARBOVIRUS LABORATORY, INSTITUTO NACIONALDE SALUD, BOGOTA, COLOMBIA

In March, 1966, the "Colonia Penal de Ararcuara", was visited for epidemiologic study of a series of suspect cases of infectious hepatitis. This penal colony is located in southeastern Colombia, along the Caqueta river and approximately at 72° 20′ west longitude and 0° 30′ south latitude. The area is covered by tropical rain forest and small clearings along the river are farmed. The estimated population of 5.000 persons consists of administrative personnel, convicts and Indians. Employees and convicts live in barracks and the Indians in small settlements. The convicts clear jungle, cultivate rice and yucca, and fish to a lesser degree. The Indians are engaged primarily in collecting natural rubber.

396 human blood specimens were studied exclusively from the stand point of arbo-virus particularly to determine to what extent yellow fever virus was the etiological agent. 134 sera belong to Indians who never have left the area. 262 sera belong to people "non-natives" in the area. All those who were bled, stated that they have never been vaccinated against yellow fever.

From one apparently healthy convict Ilheus virus was isolated, this being the only virus isolated from the sample.

HI and NT tests were performed and the serologic results were evaluated according to: type of test and titer, condition of "Indian" or "non-native"; age, sex, occupation, time of residence, and locality where the bleeding took place. However, only totals concerning type of test and condition of "Indians" or "non-natives" are presented in Table I.

Serologic Results for VEE

Only few "non-natives" had antibodies. One "Indian" reacted at the 1:40 dilution in the HI test, but unfortunately there was no specimen left to be tested in the NT test. From these data it is reasonable to assume that VEE virus is very uncommon if not absent in the area.

Serologic Results for Mayaro

"Indians" yielded 44% positivity in the HI test and "non-natives" 6.5%. Among "Indians" the percentage of positive reactors ranged from 50% to 78% above 15 years of age, and no consistent increase was noted according to age groups; so it is very difficult to estimate the periods of activity of the virus. Because of the cross reactivity between Mayaro and Una it

is not possible to ascertain to which of them these results are due.

Serologic Results for Yellow Fever

The average immunity rate in the NT test was 47.7%. "Indians" reached 67.8% in contrast to "non-natives" who showed 37.4%.

The average immunity rate in the HI test was 27.7%. "Indians reached 32.8% and "non-natives" 19.0%.

The proportion of positive reactors, both in the NT and the HI tests, consistently increased with age (5 years grouping) for "Indians", the increase being poorly defined for "non-natives", among whom were not persons less than 15 years of age.

Sera 1:80 dilution proved to be the most frequent titer in the HI test for "Indians", and 1:20 for "non-natives". The few positive reactors in each age group did not allow for establishment of any relationship between age and magnitude of the HI test titer.

Serologic Results for other agents in Group B

Ilheus and St. Louis antigens were also included in this study.

The positive reactor rates in the HI test were almost the same against yellow fever, Ilheus and St. Louis antigens, for "Indians". On the other hand there is a significant difference between the rates against yellow fever and the two other Group B antigens, for "non-natives", which definitely influences the totals or average rates: yellow fever 27.7%, Ilheus 19.9% and St. Louis 18.7%.

Table 2 summarizes the results according to single or multiple positive reaction against the Group B antigens used, and according to type of test and condition of "Indians" or "non-natives".

Almost every person who did not react to yellow fever virus in the NT test (rows 1 to 8) was also negative in the HI test against the Group B antigens. Those few cases with multiple reactions in the HI test (rows 2 to 8) are interpreted as cross reactions produced by infections with Group B agents other than yellow fever, possibly Ilheus.

Considerable number of positive reactors against yellow fever virus in the NT test (rows 9 to 16) were negative for all Group B antigens in the HI test (row 9). To a lesser degree some sera were positive for yellow fever in

in the NT test and for all antigens in the HI test (row 10). Finally there are some multiple reactors in the HI test also positives in the NT test against yellow fever (rows 11 to 16) which are interpreted as cross reaction produced by infections with some Group B agents , particularly yellow fever if the NT test is to be held as the reference test. Apparently there is no relationship between sex, occupation or last locality in the area where the person lived, and the serologic results. The explanation could lie in the fact that all people live in close contact with the jungle and often migrate within the area.

TABLE I

Percentages of positivity in serologic tests performed with human sera from Araracuara* , 1966. -

DONORS	NT **		HI ***				
	VEE	FA	VEE	Mo	FA	I	S.L.
"Indians"	0	67.8	0.7	44	32.8	35.8	28.4
"Non natives"	4.6	37.4	4.5	6.5	19	11.8	13.7
Totals	- - 3.1	47.7	3.2	19.4	27.7	19.9	18.7

* 134 "Indians" sera and 262 "Non natives" sera examined.

** NT test. Mice 25-45 days of age inoculated i.c. with undiluted serum plus 50 to 200 MLD_{50}.

*** Sera positive at the 1:20 dilution or higher, against 8U. of antigen.-

TABLE 2

JOINT SEROLOGIC RESULTS IN THE NT AND HI TESTS WITH HUMAN SERA

Araracuara, 1966.+

NT	HI			TOTAL	
FA	FA	ILH.	ST.L.	Indians	White
N	N	N	N	32	145
N	P	P	P	0	3
N	P	N	N	0	3
N	P	P	N	1	0
N	P	N	P	1	1
N	N	P	P	1	2
N	N	N	P	1	7
N	N	P	N	7	3
				43	164
P	N	N	N	33	51
P	P	P	P	19	14
P	P	N	N	8	21
P	P	P	N	7	2
P	P	N	P	9	6
P	N	P	P	2	3
P	N	N	P	5	0
P	N	P	N	8	1
				91	98

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

Raposo River Field Station

During the first part of 1966 the work carried on at the Rio Raposo was basically the same as in preceding years. While we recognize that there is still very much to be learned about the systematics, ecology, bionomics and behaviour of the haematophagous arthropods in this area, the work of the past several years has already served to give a general picture of the Pacific Coast arthropod fauna, the associated viruses present, and to indicate certain virus-arthropod relationships which deserve closer study

and epidemiological assessment. It was felt that with the limited resources available, it would now be desirable to concentrate attention on specific problems, the appreciation of which had gradually emerged from the broad approach carried on up to this time. Of the several interesting problems which have become apparent, one which is particularly intriguing centers around the most common and very abundant anthropophilic anopheline of the area, Anopheles neivai. From this one species of mosquito, and from no other of the many tens of thousands of mosquitoes of several score of species processed, we have obtained a series of isolations of Guaroa virus, Anopheles A virus, Anopheles B virus, and yet a fourth virus, unidentifiable with any agent previously known from Colombia, which is for the present being designated as Ar 2825. Anopheles neivai is in addition thought to be the principal malaria vector of the high rainfall areas of the Pacific Lowlands. The species is readily identified in the field and thus it is possible to conduct field studies on it without becoming involved in time consuming taxonomic complications. The merit in concentrating attention on this vector of at least four different viruses, and malaria as well, would appear self-evident, particularly since there is virtually no published information on the ecology, bionomics and behavior of the species. With this thought in mind, the general mosquito collecting, the processing of which has consumed such a large share of the mouse colony resources, was gradually decreased, and the collecting of Anopheles neivai intensified. This was accomplished by concentrating on collecting in the evening when Anopheles neivai may be gotten in large numbers on human bait. To obtain a more accurate concept of the frequency of virus infected mosquitoes the pool size of Anopheles neivai was reduced to a standard 50 mosquitoes per pool. As the evening human-bait collections of Anopheles neivai were producing, with the same field effort, considerable numbers of anthropophilic Phlebotomus, it was decided to process these for viruses as well, particularly since we already have several Phlebotomus virus isolations and thus have reason to believe that work with them will be virologically fruitful. Unlike Anopheles neivai, the Phlebotomus are not identifiable alive in the field, and material for virus isolation processing is assumed to consist of a mixture of species. Definitive species identification at this stage of our knowledge calls for dissection, slide mounting and microscopic examination. Ten per cent of the human-biting Phlebotomus gotten are therefore being withheld for taxonomic study, and we may presume that we will presently have some concept of the species composition of the man-biting Phlebotomus population from which viruses are obtained.

Buenaventura

A major change in the Pacific Lowlands field program during the year has been the transfer of the field laboratory from the Rio Raposo to the

laboratory building of the Universidad del Valle at Buenaventura. This building was not in existence several years ago at the time that Raposo was selected as an operating base. The Buenaventura building provides facilities which have not been available at Raposo, such as a regular source of electricity which will permit the operation of low temperature equipment, telephone communication with the Cali laboratory, and access by road to Cali as well as to forested areas suitable for field work and believed to be comparable to those at Raposo. One such area, which appears particularly promising, is accessible through a recently developed African oil palm plantation, Palmeras del Pacifico. At the end of September the meteorological equipment was transferred from Rio Raposo to Palmeras del Pacifico and mosquito collections begun there as well as at several other areas accessible by road. During the last three months of the year, collections were continued at the Rio Raposo on one day of each week to permit continuing although limited surveillance for viruses active there while the Buenaventura-based operation was being developed. It is planned to continue the restricted surveillance at the Rio Raposo until it is established that the Buenaventura-based operation is productive of a comparable arthropod and arbovirus fauna.

The translocation of the field laboratory to Buenaventura also circumvents the logistic problems which have been experienced due to the need to depend on outboard motor marine transportation. Apart from the accessibility of arthropod collecting sites by land from Buenaventura, the facility there enables us to plan surveillance for arbovirus infections in a very much larger human population than was available at Raposo. Additionally, the exploitation of forest for wood pulp by a Cali-based paper products factory is resulting in the extension of logging roads into primary forest, and a government sponsored program for the settling of families from the highlands on newly cleared land. This population presents an opportunity to study the effect of the exposure of nonimmunes to the lowland arboviruses we have established to be present.

Virus Studies

In the adjoining Table appears the information pertaining to the viral isolates from material received from the Pacific lowlands. It will be noted that an important change was introduced in the processing of the arthropods from this area, viz., the standardization of the pool size of Anopheles neivai in order to get a more accurate idea of the dynamics of the arboviruses isolated from this mosquito which has become a species of special interest.

(Pablo Barreto, Vernon H. Lee, Carlos Sanmartin and Harold Trapido)

Raposo River Field Station, 1966
Virus Isolations from Haematophagous Arthropods

Month of collection	Arthropod identification	No. of specimen in the pool	Virus identity
April	Anopheles neivai	230	? (=Ar 2825)
April	Anopheles neivai	232	? (=Ar 2825)
April	Anopheles neivai	233	? (Ar= 2825)
April	Anopheles neivai	220	Anopheles A
June	Anopheles neivai	374	Anopheles A
June	Wyeomyia spp.	272	Wyeomyia complex
July	Wyeomyia spp.	285	Wyeomyia complex
July	Wyeomyia scotinomus	274	Wyeomyia complex
July	Anopheles neivai	41	Anopheles A
July	Anopheles neivai	50	Anopheles A
July	Anopheles neivai	50	Anopheles B
August	Anopheles neivai	50	Guaroa
August	Anopheles neivai	57	? (=Ar 2825)
September	Anopheles neivai	50	Anopheles A
September	Anopheles neivai	50	Guaroa
September	Anopheles neivai	50	Anopheles B
October	Anopheles neivai	50	Guaroa
October	Anopheles neivai	50	Guaroa
October	Anopheles neivai	50	Guaroa
October	Anopheles neivai	50	Guaroa
October	Anopheles neivai	50	Guaroa
October	Anopheles neivai	50	Guaroa
October	Anopheles apicimacula	147	Bunyamwera group

REPORT FROM THE ARBOVIRUS LABORATORY, PASTEUR INSTITUTE, FRENCH GUYANA

The Pasteur Institute opened a section in Arbovirology in January, 1967. The present year is devoted to the establishment of techniques, of personnel, and of a laboratory mouse breeding colony.

Within the framework of the first trials accomplished, it has been possible in our entomology laboratory to capture, with human bait, animal bait and light traps, 54, 346 mosquitoes of which a portion has served to furnish 116 pools inoculated into littermates of mice 24-48 hours old.

The captured mosquitoes represent 28 known species, and others not identified belonging to the genera Culex, Limatus and Wyeomyia. In the accompanying table are included results obtained during the third trimester of 1967.

Our mouse breeding colony makes it possible to obtain on the average 11 litters daily.

By the end of September we had 23 virus isolations. These fall into four groups (A-C-Bunyamwera-Guama). Three isolates appear to be related to Embu virus described in Sao Paulo. Group A is represented by five viruses of the VEE complex.

Group C consists of four viruses related to Murutucu.

The Bunyamwera group is represented by two viruses related to the Chache-Valley complex.

The Guama group is represented by three viruses related to Catu virus.

Three viruses, not grouped, resemble Embu virus. Six viruses are not yet identified. Their grouping is in progress.

Isolations, as related to species of mosquitoes, are included in the Table. As of November 22, 1966, rodents and marsupials are being trapped.

Identification of small mammals will be done by the Natural History Museum in Paris.

To date, the captures and recaptures are as follows:

	captures	recaptures
Marsupiaux	227	49
Rongeurs	55	4
Total	282	53

The aims of this Arbovirology laboratory are, first, to prepare an inventory of the existing viruses, and then to work on existing epidemiologic problems, in close cooperation with other interested laboratories in Latin America.

The first five viruses identification has been made by Belem Virus Laboratory. (Dr. Woodall).

(Dr. Charles Serie)

SPECIES OF MOSQUITOES FROM WHICH VIRUS WAS ISOLATED

Species	Numbers				Virus Isolates			
	Pools Realized	Total Mosq.	Pools having permitted isolation	# of Mosq. from these pools	#	Nature	% of Pools	Median Average
Culex portesi	26	4.652	16	2.983	16	gpe A: 4 gpe C: 4 g. guama : 3 Embu: 1 N.I.: 4	61,5	289
Wyeomyia sp.	32	6.919	3	549	3	Bun: 2 Embu: 1	9,3	2.366
Cansonia renesuelensi	25	8.243	2	778	2	gpe A: 1 N.I.: 1	8	4.121
Haemagogus spegazzinii	5	396	1	122	1	Embu: 1	20	396
Culex sp.	4	550	1	317	1	non-ident	25	550
Total	92	20.760	23	4.749	23			

TABLE

Species	Bait									
	Man			Animals			Light			
	July	Aug.	Sept.	July	Aug.	Sept.	July	Aug.	Sept.	Total
Anopheles										
aquasalis	97	59	154		2	4		21	6	343
brasiliensis			7							7
eiseni	1		11							12
mediopunctatus	5	4	1			1			1	12
nimba			2							2
peryassui			5							5
Aedes										
aegypti			2							2
argyrothorax	14	2								16
fulvithorax	5		1							6
scapularis			1							1
serratus	10		12							22
terrens			7							7
Culex										
portesi	17	6	252	139	146	374		221	992	2.147
sp.	2	19	80		20	81		238	741	1.181
Haemagogus										
spegazzinii	121	19	5							145
Limatus										
asulleptus	4	9								13
durhami	30	6	10		1					47
flavisatosus	57	41	22							120
sp.		185	93					1		279
Mansonia										
albicosta	788	1817	4194	14	64	395		102	53	7.427
lynchii		1	27							28
titillans	26	10	382		3	148			43	612
venezuelensis	3173	1746	3879	312	309	2021		239	1796	13.475
Psorophora										
ferox	3									3
Sabethes										
cyaneus	6									6
Toxorhynchites										
haemorrhoidalis	1									1
Trichoprosopon										
compressum	17	18	44		1					80
digitatum	10	14	8							32
longipes	86	149	213		2					450
Wyeomyia										
melanocephala	447	84	203		6					740
sp.	124	343	1127		27					1.621
TOTAL	5044	4532	10742	465	581	3024	0	822	3632	28.842

REPORT FROM THE TRINIDAD REGIONAL VIRUS LABORATORY

Bush Bush: One strain of Catu virus was isolated from Culex portesi caught in December 1966. Despite a slight recovery of the rodent population no further isolations were made through August 1967. The recovery of the rodent population was most marked for Heteromys anomalus, a species which unfortunately is unsuitable as host for Group C and Guama group viruses. Only VEE virus will circulate to high titers in this species. We continue our maintenance program in Bush Bush Forest.

Turure Forest: A field laboratory was constructed in this forest in the first quarter of 1967. EEE virus which was active from October 1966 until February 1967 was recovered again in June and July 1967. In the intervening period no arboviruses were recovered from Turure Forest materials. Besides EEE virus, Caraparu and Restan and a number of Guama group agents were recovered since June 1967. Strangely enough all 1967 isolations came from sentinel mice despite intensive efforts to obtain isolations from mosquitoes. This situation in 1967 is similar to the one in the early days of the Bush Bush studies prior to the discovery of the importance of Culex portesi. During 1966 Culex portesi yielded several strains of Group C and Guama group agents.

Cumaca studies: Rodents were trapped in this area just north of Turure Forest once weekly. In May a virus was recovered from the organ pool of a Marmosa mitis. This agent is a new group B agent for Trinidad and appears to be related to but different from Rio Bravo virus. Further studies are in progress at YARU.

Cedros: This area in south western Trinidad is visited once a month for 4 - day mosquito catching and rodent trapping operations. Two virus strains, both from Culex "caudelli" (possibly ybarmis) were recovered from this area. These strains, identical to each other, are of a new virus type for Trinidad.

N.I.H. Contract: In February a new contract with N.I.H. for the production of reference reagents was begun.

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

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 I.

Of particular interest are the 59 isolations of Amaparí 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. Amaparí isolations 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 Amaparí 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 were 10% of 61 Neacomys urine samples. In this characteristic, Amaparí 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 transmissions in the experimental area.

(Staff of the Belem Virus Laboratory)

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

New Jersey type vesicular stomatitis (NJVS) outbreaks have not been clinically diagnosed in the South Atlantic states since 1964. Four hundred eighty-four bovine serums from seven Georgia study herds were tested for NJVS to detect undiagnosed or subclinical infection since the large outbreak in 1963. Eleven of 117 cattle, 1 to 3 years of age, were positive to NJVSV by neutralization tests. The following table shows the increase in positive titers with age. Although ages of some of the younger animals need verification to be certain they did not experience the 1963 outbreak, it appears that there has been subclinical vesicular stomatitis in four of the seven study herds.

Age	Positive	Total Tested	Percent Positive
2-3 yrs.	11	117	9
4 yrs.	10	43	23
5 yrs.	46	100	46
6 yrs.	69	94	73
7 yrs. & up	86	130	66
Totals	222	484	45 (average)

Of 79 deer serums obtained from the Southeastern states, one Georgia serum had a weak (1:18) neutralization titer to NJVSV; others were negative. All 79 serums were negative to the Indiana type.

A large outbreak of New Jersey VS involved Texas, New Mexico, and Colorado during the summer of 1966. Indiana type VSV activity was detected in two counties each of New Mexico and Colorado.

Virus isolation efforts were made from a variety of insects, principally diptera collected in Colorado, Texas, and New Mexico. Of 165 pools processed in 1-day old suckling mice, two were positive and all others were negative. Female mosquitoes consituted 92 pools totaling 2,398 insects. New Jersey type VSV was isolated from a pool of 100 Hippelates pusio, eye gnats, caught in a Tinkham trap on an infected premise near Canon City, Colorado. Reisolation was not successful as some mice were missing following a pattern of deaths similar to that observed with the original isolation.

The other isolation was from a pool of 90 Culex tarsalis collected at Aztec, New Mexico. VS has been eliminated, but to this date the sample has not been studied further. This pool was toxic on the first two inoculations. Isolation was made from the pool following dilution with an equal part of diluent and light ultracentrifugation; the sample was depleted without reisolation. This agent kills embryonated eggs and has a death pattern resembling that of the group A arboviruses.

Plans include the use of sentinel mice. More Hippelates gnats will be collected in an effort to determine if they are involved in VS epidemiology. No VS outbreaks were diagnosed in the United States through June of the year 1967.

(E. W. Jenney)

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

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

This report reviews field and laboratory studies on arboviruses during the period May 1, 1966 through April 30, 1967.

The ninth year of evaluation was completed on the immediate and residual effectiveness of intensive control of larval Culex tarsalis as a means to suppress transmission of western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses in an endemic area. For the third year the population of adult C. tarsalis was at a relatively high level in the absence of intensive larval control. WEE viral infection rates in C. tarsalis and immunologic conversion rates in sentinel chickens were among the highest for the 9 year period. There was a low level of Turlock viral activity. For the third consecutive year, there was no virologic or serologic evidence that SLE virus was active.

General epidemiologic observations in Kern County revealed a high level of WEE viral activity in July and August 1966. However, there were no proven human cases of WEE or SLE for the ninth consecutive year. Coincidental to the midsummer increase in C. tarsalis populations and isolations of WEE virus from C. tarsalis, there was a small epizootic of encephalomyelitis in horses. Of 7 suspected horse cases, 5 were confirmed as WEE. Serologic tests of sera from 389 domestic mammals revealed that hemagglutination-inhibiting HAI) antibodies to WEE and SLE viruses occurred frequently in equine sera but rarely in bovine, ovine, and canine sera. Antibodies to Powassan, Modoc, Rio Bravo, California, Bunyamwera group, Buttonwillow, and Turlock viruses rarely were detected in sera from domestic animals.

A human infection with Modoc virus was diagnosed by HAI tests. The patient was a 10 year old boy who was hospitalized on April 18, 1966 with a clinical diagnosis of aseptic meningitis. Nine to 14 days before the onset of illness, the boy had caught and played with wild mice while on vacation with his family at a cabin in the foothills of the Sierra Nevada in Tulare County. No HAI antibody to Modoc virus was detected in sera from the other five members of the family or from 21 rodents collected near the cabin in November 1966.

Studies were continued at 15 localities to correlate mosquito populations, mosquito infection rates, and immunologic conversion rates in sentinel chickens. Locations included suburban, rural community, rural agricultural, desert, and foothill environments. Immunologic conversions in chickens were more sensitive than viral isolations from mosquitoes to detect activity of WEE or Turlock virus at these locations, but this was due in part to the small numbers of mosquitoes that could be collected and tested from some locations. Viral isolations and immunologic conversion rates for WEE and Turlock viruses reached the highest levels in rural agricultural environments. The first isolations of both viruses were made from C. tarsalis just before or at the time the vector population reached its maximum. Immunologic conversions in chickens to WEE virus were detected in the same month or the month after the first viral isolations were obtained; whereas, immunologic conversions to Turlock virus usually were not detected until 2 to 3 months after Turlock virus was first recovered from C. tarsalis. A vector population threshold level below 5 female C. tarsalis per trap night was correlated with little or no evidence of WEE viral transmission.

An intensive study on the ecologic relationships between wild mammalian and ectoparasitic populations and the levels of arboviral activity was continued for the fourth year. Mammals were trapped, banded, bled, and released on a 40 acre grid located in a saltbush desert adjoining agricultural lands. Marked fluctuations have occurred in rodent populations over the 4 year period, and the number of animals captured for most species has steadily decreased. The relative abundance of Ammospermophilus nelsoni and Dipodomys nitratoides in the total population has increased from 60 to 91 percent of total captures, whereas Dipodomys heermanni has decreased from 26 to 2 percent.

Supplemental trapping of wild mammals at Poso Creek and Tracy's Ranch was continued for the second year. As at Lerdo Grid, A. nelsoni and D. nitratoides were the most abundant species at Tracy's Ranch, whereas D. heermanni and Peromyscus maniculatus were the most abundant species at Poso Creek. Parallel studies on bird populations were also continued at these 2 areas. A total of 1,154 resident birds, 154 summer visitant birds, 326 winter visitant birds, and 146 migrant birds were netted, banded, bled, and released.

Collections of ticks from wild mammals were continued, whereas collections of fleas were terminated in August 1966. As in previous years, the infestation rates of ticks on mammalian hosts were highest during the cooler winter and spring months. The predominant Ixodine ticks were Dermacentor parumapertus, which was found most frequently on kangaroo

rats, rabbits, and antelope squirrels; and Haemaphysalis leporis-palustris, which was found mostly on Sylvilagus audubonii.

Ornithodoros parkeri rarely was found on animals but could be collected readily by CO_2 traps from squirrel burrows. Sixty-two hamsters were fed upon by field-caught O. parkeri. None of the hamsters developed HAI antibodies to WEE, SLE, Powassan, Modoc, Rio Bravo, California, Bunyamwera group, Buttonwillow, or Turlock viruses.

Small colonies of O. parkeri, D. parumapertus, and Argas persicus have been established and are available for viral transmission studies.

No virus was isolated from 654 blood samples of wild mammals collected on Lerdo Grid or from 275 fleas collected from A. nelsoni and Citellus beecheyi.

HAI antibodies to WEE, SLE, Powassan, Modoc, Rio Bravo, California, Bunyamwera group, Buttonwillow, and Turlock viruses rarely were detected in plasmas from mammals collected on Lerdo Grid. A high prevalence of HAI antibody to California virus was found in D. heermanni collected at Poso Creek. At Tracy's Ranch, antibodies to WEE, Bunyamwera group, and Buttonwillow viruses frequently were found in plasmas from lagomorphs, whereas antibodies to SLE and California viruses frequently were detected in plasmas from rodents. The highest frequency of HAI antibodies to WEE, SLE, and California viruses in rodents occurred in the winter-spring period. Group B antibodies that were detected in mammalian plasmas most frequently reacted monotypically to SLE virus; whereas, in the past 3 years, monotypic reactions to Powassan virus prevailed.

There was serologic evidence that a number of wild birds had been infected with WEE and Turlock viruses during the summer of 1966 at Tracy's Ranch but not at Poso Creek. A large proportion of plasmas from birds collected in both areas from February to June 1966 inhibited hemagglutination with SLE and California antigens. There were insufficient volumes of plasmas to allow confirmatory neutralization tests.

An ecologic study of the terrestrial habitats of wild vertebrates at Lerdo Grid, Tracy's Ranch, and Poso Creek study areas was initiated in 1966. The purpose of this study was to gain a more complete ecologic perspective between arboviral activity and the population dynamics, reproductive biology, and vegetative habitats of wild mammals. A flood at Poso Creek and a late winter drought throughout Kern County had a pronounced effect on vegetative development. General observations were made on the species, distribution, and growth characteristics of saltbushes, filaree, and grasses.

Aerial photographs were taken of each study area to assist in recording vegetative types.

Studies were continued on the influence of humidity and light intensity on the flight and biting cycles of Culicoides variipennis, C. tarsalis, and Culex erythrothorax. Onset of flight activity of C. variipennis correlated with reduced evaporation rates and not with reduced light intensity. In contrast, C. tarsalis and C. erythrothorax flight onsets were correlated with reduced light intensity and not with reduced evaporation rates.

Tests of 42,198 C. variipennis collected in 1966 yield 40 viral isolations, of which 10 were related by complement-fixation (CF) tests to Buttonwillow virus and 3 to Bunyamwera group viruses. No virus was isolated from 4,657 females of 8 other Culicoides species, 1,486 simuliids, 206 horn flies, 10 stable flies, and 549 Leptoconops.

Studies on the blood-feeding habits of mosquitoes were continued in California, Utah, Texas, Hawaiian Islands, Illinois, and Kentucky. In addition, specimens collected in Virginia and New York were tested.

As in previous years, a high proportion of C. tarsalis collected at Poso Creek had fed on mammals, particularly cattle, horses, and rabbits. This pattern is different from that found in other areas of Kern County where C. tarsalis feed mostly on birds. Anopheles franciscanus, Anopheles freeborni, and Culiseta inornata were strictly mammalian feeders. Anopheline species fed mostly on rabbits and domestic mammals, and C. inornata fed only on domestic mammals.

Culex tarsalis were collected at a marsh, a game farm, and an agricultural site in the region around Salt Lake City, Utah. Most feedings were on mammals at the marsh site, while most feedings were on birds at the agricultural and game farm sites. These differences in feeding patterns between the areas could not be related to host availability.

The host feeding and behavioral patterns of C. erythrothorax collected in Kern County and the Salt Lake City area were markedly different. In Kern County, C. erythrothorax could be collected by light traps but rarely in shelter collections and 99 percent of blood meals were from mammals. In the Salt Lake area, C. erythrothorax were collected predominantly in animal shelters and 97 percent had fed on birds.

In Hale County, Texas, 40.9 percent of C. tarsalis collected in 1964 had fed on mammals, 16.9 percent in 1965, and 58.2 percent in 1966. The significance of this year-to-year variation in proportion of mammalian

blood meals could not be determined because of yearly changes in collection sites. Aedes vexans fed mostly on mammals and Culex quinquefasciatus fed on birds.

In the Hawaiian Islands, 4 species of mosquitoes were studied. Culex quinquefasciatus fed either on domestic mammals or birds, depending on host availability. Aedes aegypti and Aedes albopictus fed mostly on domestic mammals and A. vexans fed only on domestic mammals.

In southwestern Illinois and western Kentucky, 81.2 percent of Culex pipiens fed on birds and there was no evidence of a seasonal shift in feeding pattern. Culex restuans fed mostly on passerine birds and Culex erraticus on columbiforme birds. Thirty of 31 quadrimaculatus were predominantly mammalian feeders.

Culiseta melanura from Virginia and C. melanura and C. pipiens from Long Island, New York fed mostly on birds.

High titered precipitin antisera to a variety of mammalian sera were prepared for identification of blood meals from Culicoides and other small biting flies. Most C. variipennis collected in Kern County had fed either on cattle or rabbits and Simulium bivittatum had fed mostly on horses and rabbits. Two Leptoconops torrens had fed on cattle and 1 on a horse.

Supplemental serologic surveys by HAI tests, and occasionally by neutralization tests, were done on human, domestic mammal, and wild mammal sera collected in areas other than Kern County. None of 216 patients with undiagnosed central nervous system diseases or febrile illnesses in California in 1965 showed a diagnostic rise in HAI antibodies to WEE, SLE, Powassan, Modoc, Rio Bravo, California, Bunyamwera group, Buttonwillow, or Turlock viruses. Of 132 paired human sera collected from similar patients in California in 1966, 2 patients developed a diagnostic rise in HAI antibody to WEE virus and 6 patients had diagnostic conversions to SLE virus. HAI antibody to WEE, SLE, Powassan, Modoc, or California virus at a constant titer was observed in paired serum samples from an occasional patient. Neutralizing antibody to Turlock virus was detected at a stable level in paired sera from 2 patients.

Of 62 paired sera collected from sick horses in California in 1965, 8 had a diagnostic rise in HAI antibody to WEE virus, and paired sera from 30 horses had stable, high titers of WEE viral antibody. Both sera from 1 horse inhibited hemagglutination of a Bunyamwera group antigen. Neutralizing antibody to Turlock virus was demonstrated in paired sera from 17 horses, but no diagnostic rise in titer was observed.

Neutralizing and HAI antibodies to WEE virus were found in 2 of 189 bovine sera collected in Hawaii in 1964, and 1 of the 2 animals was positive by HAI test when it was rebled in 1965. An occasional serum collected in Hawaii from sheep or goats was positive in HAI tests with WEE, California, Bunyamwera group, or Turlock antigens.

Tests on sera from 403 domestic and wild mammals collected in Mendocino County, California revealed that a high proportion of woodrats and jackrabbits had HAI antibodies to California virus, Buttonwillow virus, or both.

Considerable effort is being expended in laboratory studies to develop sensitive serologic and virologic tests for the 12 arboviruses known to occur in Kern County. Tests on the susceptibility of a variety of continuous cell cultures to low passaged arboviruses revealed that plaque assay in Vero cells was as sensitive as intracerebral inoculation of suckling mice for detection of SLE, Modoc, Rio Bravo, Buttonwillow, and Bunyamwera group viruses.

Two viruses isolated from blood of lagomorphs and 18 viruses isolated from C. variipennis were further characterized by CF tests as members of the Bunyamwera serogroup.

An unidentified virus was isolated from the blood of a C. beecheyi. The virus is sodium deoxycholate (SDC) sensitive.

Two viruses were recovered from the lungs and kidneys of a C. beecheyi that was trapped near a cabin in the foothills of the Sierra Nevada where a child probably became infected with Modoc virus. Both viruses were SDC sensitive. Neutralization and HAI tests revealed a possible antigenic relationship between these viruses and group B arboviruses.

Experimental pathogenesis studies were continued with SLE, Powassan, Modoc, and Rio Bravo viruses in 5 species of wild mammals and 5 species of wild birds. Except for an occasional Mourning Dove, none of the birds became infected with Powassan, Modoc, or Rio Bravo viruses. All species of mammals except P. maniculatus were highly susceptible to the 4 group B viruses. Peromyscus maniculatus was resistant to infection with SLE and Rio Bravo viruses. Homologous HAI antibodies reached maximum titers 2 to 4 weeks after infection and were still detectable after intervals of 25 to 52 weeks. Heterologous HAI antibody responses usually were lower in magnitude and shorter in duration than were homologous responses. Modoc viral antibody inhibited hemagglutinins of SLE, Powassan, and Rio Bravo viruses, but Modoc viral hemagglutinin frequently failed to react with heterologous group B antibodies.

Experimental studies were completed with Turlock virus in arthropods, wild birds, and wild mammals. Neutralizing antibody to Turlock virus persisted for 3 or more months in sera from wild birds and mammals. A nonspecific heat labile substance that neutralizied Turlock virus was demonstrated in sera from several species of wild mammals. Culex tarsalis that had fed on viremic chicks transmitted Turlock virus to susceptible chicks after 7, 14, and 21 days of extrinsic incubation.

Two sheep inoculated subcutaneously with 10,000 suckling mouse LD_{50} of Buttonwillow virus failed to develop HAI antibody by 21 days after inoculation.

Culex tarsalis did not become infected after ingestion of WEE virus-immune chicken blood mixtures, thus demonstrating that WEE virus was not dissociated from its antibody during digestion in C. tarsalis.

Basic immunologic studies were continued in an effort to prepare more specific and higher titered precipitin antisera to serum antigens of birds and to develop or evaluate other immunologic techniques that might be more sensitive than the precipitin test for differentiating blood antigens from closely related avian and mammalian species.

Chickens made tolerant at hatching to goose gamma globulin (GGG) produced low levels of antibody against GGG as they "escaped" from the tolerant state 9 to 11 weeks later. These low levels of antibody were detected by the passive hemagglutination test, employing goose erythrocytes that were treated with bis-diazotized benzidine before being coated with GGG. This is the first demonstration of "spontaneous escape" of chickens from immunologic tolerance.

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

(William C. Reeves)

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

Due to interruption of laboratory work following the relocation of the Principal Investigator from Toronto to Vancouver in July 1967, results of serological tests for prevalence of arbovirus antibodies in sera from 748 rodents captured in the mountains and valleys of eastern British Columbia (49 to 50° N, 116 to 120° W) between 8 May and 30 August 1967 have accumulated more slowly than in previous years. Full facilities for arbovirus investigations are now established for the first time in the University of British Columbia, and results of field investigations should now accumulate rapidly.

Powassan neutralizing antibodies were detected in 18 of 313 forest rodents collected near Cranbrook (50° N, 116° W) during May and July, including 8 of 222 Columbian ground squirrels (Spermophilus columbianus), 18 of which were infected with Dermacentor andersoni ticks; 1 of 10 golden mantled ground squirrels (Spermophilus lateralis), 2 of which were tick-infested; 4 of 39 chipmunks (Eutamias amoenus), one of which carried ticks; 5 of 34 red squirrels (Tamiasciuyus hudsonicus), 2 of which yielded ticks; but in none of 4 snowshoe hares (Lepus americanus) or in one other rodent. Sera from animals collected in other areas are currently under investigation (6 September 1967). In conjunction with the Division of Laboratories of the British Columbia Department of Health, sera from 931 human residents of the area were examined for arbovirus antibodies by HI. These included 15 positive reactors to WEE antigen, 8 to Powassan and 11 to California. These results strongly suggest the presence of endemic foci for Powassan, California encephalitis and western equine encephalomyelitis viruses in eastern British Columbia.

(Donald M. McLean)

REPORT FROM THE DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASES SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION SAN ANTONIO, TEXAS

A surveillance program has been in progress since April, 1966 to determine what arboviruses may be present in the baboon colony at SFRE and to identify the vectors. Culex tarsalis and Culex pipiens quinquefasciatus have been captured in significant numbers in the vicinity of the outdoor cages by means of light traps and by sweeping with a hand net in vegetation and other resting places. The population of C. tarsalis showed a pronounced peak density in April-May and a lesser one in October, 1966. Not all specimens have been tested, but ten pools from light traps with a total of 633 mosquitoes yielded two isolations of WEE virus, one collected April 26, 1966 and the other June 1, 1966, and two isolations as yet unidentified. Eight pools of C. p. quinquefasciatus totaling 487 specimens taken during a May-June population peak were negative except for one isolation of SLE virus from a pool taken by sweeping vegetation on June 16, 1966.

WE virus was isolated in 1967 from two infant baboons with CNS symptoms, one from blood and the other from liver. Brain samples were negative for virus by mouse inoculation and no significant lesions were found. The first baboon infection was detected in February when Culiseta inornata was present in appreciable numbers. Collections of this mosquito have not yet been tested for virus. The viremia in the baboon was in high titer and isolation was repeated without difficulty. The second isolation was made in June from the liver of an animal sacrificed during illness; virus was in low titer and reisolation attempts were unsuccessful.

Antibody surveys in the baboon colony are incomplete but scattered samples over a period of several years give evidence of CF antibodies for both WE and SLE viruses.

One experimental infection with WE virus in an infant baboon, initiated subcutaneously and intravenously, yielded a viremia of about four days duration with a peak on the third day post-injection. A rise in temperature was detected but there were no overt symptoms. Serum taken twenty-three days post-injection neutralized about two log units of virus. Pre-infection serum was negative.

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

St. Louis Encephalitis Studies

Since our last report in the Information Exchange our major observation on SLE epidemiology is the continued absence of the virus in this Florida subtropical area. Over 2,000 suspected viral infections in humans have been screened for arbovirus antibodies since January 1, 1963 and none have shown evidence of recent infection with SLE virus. Over 800,000 mosquitoes have been tested, including 500,000 Culex nigripalpus, and all negative for SLE. A wide variety of vertebrates, both avian and non-avian, have been screened in addition to sentinel chickens, pigeons, rabbits and horses; among these there have been no confirmed recent infections with SLE. We have concluded that SLE virus is not enzootic in this area as originally anticipated. We have considered the possibility of periodic re-introduction; however, examinations of over 1,000 migratory birds arriving in the spring from Central and South American have so far yielded no arbovirus isolations.

Our studies of Group B serologic patterns in human residents of the Tampa Bay area has led to an interesting hypothesis; namely, "the long term residents of the area, of whom significant proportion have evidence of immunity to dengue-2 virus, were partially protected against the occurrence of clinical SLE when exposed during the 1959, 1961, and 1962 epidemics." The evidence to date indicates that 14 percent of the white population with long residence histories and 43 percent of the Negroes have dengue-2 immunity. In these dengue immune groups, the SLE clinical disease rates were extremely low compared to the dengue non-immune population. Cross challenge experiments in laboratory mice, using dengue-2 virus as the immunizing agent and SLE virus as the challenge, have also demonstrated significant amounts of cross protection.

A group of SLE cases from the 1959, 1961, and 1962 epidemics have now been followed three to five years for persistence of CF and HI antibodies. Of the 57 tested after a two to three year interval, 38.5 percent had CF titers of 1:8 or greater. Of the 74 tested for HI antibody, 62.6 percent had titers of 1:20 or greater after the same interval. After a four to five year interval, seven of nine tested had CF titers of 1:8 or greater and six of seven had HI titers of 1:20 or greater.

A series of planned experimental SLE infections in wild birds have now been completed. The response of Blue Jays was studied in 1966. Ten of 16

experimentally inoculated birds developed viremia and all 16 had an SLE-HI response. The Blue Jay was added to the list of wild birds which may serve as potential epidemic reservoirs according to both field and laboratory evidence. Other birds on this list for the Tampa Bay area include the Mourning Dove, House Sparrow, Mockingbird and young chicken.

California Encephalitis Studies

Although California encephalitis arboviruses are the most abundant viruses in Florida mosquitoes, the detection of human disease remains a relatively rare event. Of 1,095 suspect CNS infections studied serologically with CE antigens in the last four years, only four confirmed acute human infections have been detected, all in children. Three of these were most likely infected while visiting the summer resort areas of western North Carolina. All four were detected by their HI response to BFS-283 antigen and confirmed by serum neutralizing antibody studies. However, CF tests done in the University of Pittsburgh, using the entire battery of California Group antigens available there, demonstrated that the three children infected in North Carolina had the highest CF response to the LaCross antigen. The single, indigenous, Florida patient has so far shown no CF response to either of the two local Florida strains of CE viruses. The prevalence of HI antibody to BFS-283 antigen in general population surveys has ranged from two to four percent. However, there is ample reason to suspect that the BFS-283 antigen is relatively insensitive in detecting infections with the local trivittatus or Keystone strains of CE virus.

Early serologic surveys with BFS-283 HA antigen suggested that horses, rabbits, squirrels and raccoons have a higher prevalence of CE antibody compared to other mammals in the area. Since rabbits have been repeatedly shown to be likely vertebrate reservoirs for CE viruses in other areas of the United States and the world, a series of experimental infections in domestic rabbits were performed. Three strains of CE viruses were used: local trivittatus, Keystone and BFS-283. Serologic tests with BFS-283 HA antigen, three weeks post-infection, failed to detect antibody except after homologous virus infection. Further studies at the University of Pittsburgh demonstrated the rather narrow specificity of HA antigens prepared from different strains of CE Group arboviruses in detecting HI immune response in these rabbits. The neutralizing antibody response in contrast was considerably broader and heterologous CE Group antibodies were detectable. However, the homologous antigen in the SN test consistently produced the highest neutralization indices. Approximately one-half of the rabbits experimentally infected with either of the three strains of CE virus developed detectable viremia at 48 hours. In one experiment, the viremia profile was determined following infection with Keystone virus. The maximum titer occurred at 48 hours (2.4 $\log_{10}$ adult mouse LD_{50}); the viremic period extended from 24 to 120 hours. Nineteen domestic rabbits

and two wild cotton tail rabbits were exposed to mosquito biting in a swamp with known CE virus during 1966. In three of the domestic rabbits CE virus was detected at the fourth and eighth day after exposure and both wild cottontail rabbits developed viremia. Of the five sentinel rabbits infected in the swamp, only one had a detectable HI titer (1:10) in sera collected three weeks after last exposure and tested with BFS-283 antigen.

California encephalitis virus was again readily obtained from Aedes species of mosquitoes by project entomologist during 1966. Of the total 85 isolations, 63 were from Aedes atlanticus and 10 from Aedes infirmatus. Most of the recoveries were in August when the proportion of positive Aedes atlanticus mosquito pools peaked at 35.3 percent. For Aedes infirmatus the comparable rate in August was 9.3 percent. Precipitin tests were performed on engorged Aedes atlanticus and Aedes infirmatus mosquitoes by the Vero Beach laboratory (Entomological Research Center). Sixty percent of these two species fed on mammals and 30 percent on birds. In both species approximately five percent had fed on multiple hosts. Two preliminary laboratory transmission experiments have been completed using domestic rabbits and Aedes infirmatus mosquitoes. In one, the rabbit was infected with BFS-283 virus and in the second, with Keystone. In neither experiment were we successful in infecting the mosquitoes feeding on the rabbit.

Observations on Other Arboviruses in the Area

The virology laboratory made 136 recoveries of arboviral agents during 1966. Of these, Eastern encephalitis accounted for 13; Western encephalitis, one; California encephalitis, 85; Bunyamwera Group, 17; unidentified cotton rat agents, six; and Hart Park-like agents, 14. Of particular interest was the first reported recovery of California Group arbovirus from cotton rats (from two specimens) and the isolation of Tensaw virus from a marsh rabbit. A viral agent, so far unidentified, has been repeatedly isolated from cotton rats in the area. No attempts were made to isolate virus from ticks or other ectoparasites this year. However, continuing studies of the tick virus termed "Sawgrass" failed to reveal any evidence of neutralizing antibodies for this virus in either man or mammals from the areas where the 1964 tick isolates were made.

(James O. Bond, Emily H. Gates, Wm. L. Jennings, D. J. Taylor, and A. L. Lewis)

REPORT FROM DEPARTMENT OF THE ARMY, FORT DETRICK, FREDERICK, MARYLAND

Immunological Studies on Arboviruses

In studies on arbovirus immunity, Dr. W.P. Allen and Mr. Orville M. Brand have been investigating methods for preparing noninfectious HA and CF antigens. Their success in these endeavors promises to simplify and render less hazardous the preparation of antigens from highly infectious viruses. For several of the viruses we recommend a modification of procedures described by French and McKinney (J. Immunol., 1964). Suckling mouse brains should be harvested at the peak of illness and then triturated in borate saline (pH 9.0) and finally preparing 16 per cent to 15 per cent suspensions in borate saline containing 0.07-0.10 M Tris buffer. To these suspensions is added β- propiolactone (BPL) to a final concentration of 0.3 per cent. The brain preparations were held at 4° C for 24 hours during which time the suspension became noninfectious. After centrifugation at 10,000 rpm for one hour, the supernate was decanted and saved and the pellet was resuspended in fresh borate saline, held at 4° C for an additional 24 hours, and recentifuged. Supernates from both saline extractions contained the virus hemagglutinins. For some viruses (Semliki Forest, Una, Mayaro, and yellow fever) the first saline extraction had the highest titer of antigen; for other viruses (VEE and O'nyong-nyong) the second saline extract had the highest titer or titer equivalent to the first.

The use of BPL has a two-fold advantage: (1) it renders the virus noninfectious, and (2) it facilitates the expression of virus hemagglutinin without the hazardous steps of extraction with lipid solvents. HA titers have been found to be equivalent to those obtained by sucrose-acetone extraction.

To date, this method has been successful for preparing hemagglutinins of VEE, Mayaro, Semliki Forest, Middelburg, Una, O'nyong-nyong, and yellow fever viruses. All but Mayaro have been stable upon storage in the wet frozen state. Lyophilization has frequently caused a 2- to 4- fold loss of titer for some antigens. It was found that the stability of Mayaro antigen was improved when sucrose was added prior to freezing.

With the pantropic, Van Wyck strain of Rift Valley fever virus, the hemagglutinin was not expressed by BPL alone, but needed further extraction with protamine sulfate. Some decay of antigenicity has occurred upon storage of this antigen in the wet frozen state. Further refinements are needed for making a noninfectious hemagglutinin or CF antigen for this

strain of RVF virus.

The hemagglutinins, prepared by treatment with BPL, have been acceptable antigens for the CF test also, except when protamine was used.

Because of acid residues formed by the degradation of BPL, it was necessary to add buffer (Tris hydroxymethyl amino methane) which prevented a shift in pH to below 7.5. It was observed that 0.3 per cent BPL with no Tris buffer caused a shift in pH from 8.8 to 5.0 within 24 hours when held at 4° C and the pH remained at this low level during a four-day test period. When as little as 0.02 M Tris was added to this system, the pH did not shift below 7.0. If the concentration of Tris was increased to 0.05 M, 0.07 M, or 0.10 M, the pH was maintained above 7.7, 7.9, and 8.2, respectively.

The minimum concentration of BPL necessary to render a viral suspension noninfectious was found to be dependent upon the virus strain, virus concentration, and concentration of protein in the suspension. A 0.3 per cent concentration of BPL was sufficient to inactivate 10^9 mouse LD_{50} of all arbovirus strains tested when the virus was contained in a 20 per cent suspension of infected suckling mouse brains. In the presence of 0.07 M Tris buffer, the above treatment was not detrimental to the hemagglutinins.

The need to establish procedures for decontaminating antiserum was realized by these laboratories when we wished to distribute antisera that were derived from potentially infectious sources. A simple procedure was needed that would assure the disinfection of serum samples yet leave antibody reactivity essentially unaltered. Our laboratories are approaching this problem from the standpoint of disinfecting sera that have been deliberately contaminated with VEE or RVF viruses.

Preliminary experiments were designed to test various concentrations of BPL in serum for the inactivation of viral infectivity and for the effects on HI, CF, and neutralizing antibodies. Concentrations of 0.1, 0.3, 0.5, 0.7, and 1.0 per cent BPL in whole serum completely inactivated 10^4 mouse intracerebral LD_{50} of VEE and RVF viruses. The serum-virus-BPL mixtures were incubated for one hour at 37° C and up to four days at 4° C. Infectious virus was not detectable at or beyond 48 hours. The pH of the sera treated with 0.1 per cent BPL dropped from 8.2 to 6.0 within the first 24 hours; for treatment with 1.0 per cent BPL, the pH shifted to 4.5. HI antibody titers of anti-VEE equine sera were unaffected by any of the above treatments. CF titers were reduced 2- to 4- fold with treatments of 1.0 per cent BPL, but were essentially unaffected by concentrations of 0.5 per cent or less. Tests for effects of BPL on neutralizing antibodies have not been completed.

Efforts to control the pH shift in serum treated with BPL revealed that 1.0 M Tris was necessary to maintain a pH above 7.5 when the serum was treated with 0.5 per cent BPL. This was more than ten times the concentration of Tris necessary to control the pH shift in a 20 per cent mouse brain suspension; however, even the 1.0 M Tris did not alter HI or CF antibody titers against VEE virus.

(Arthur N. Gorelick)

REPORT FROM THE DEPARTMENT OF BIOLOGY, THE PENNSYLVANIA STATE UNIVERSITY, UNIVERSITY PARK, PENNSYLVANIA

Beginning in 1963 birds were captured in mist nets in a 32-acre woodlot near State College, Pennsylvania. A routine procedure was established for opening the nets, banding and bleeding the birds, and closing the nets for particular periods of time. The data have been entered on IBM cards and several computer programs written for analysis. To date, approximately 7750 birds have been handled of which about 2650 were examined for antibodies for one or more viruses by the HAI test. These tests were conducted primarily by Mr. Irvin Savidge who worked for the summer of 1966 in the laboratory of Dr. Willian Hann in NIAID in Bethesda. Mr. Savidge then did the tests on the sera for the latter part of 1966 and for the birds from 1967 at Penn State. Duplicate sera run by Dr. Hann and Mr. Savidge gave almost identical results. While many analyses need yet to be done, certain items of interest are apparent. For the present, the definition of positive is a serum of 1:20 or higher. First, we can say that the number of positives for EE and for WE were very low and very irregular and for the moment will be ignored.

For SLE an analysis of immature birds (born locally in the current year) for selected species in 1964 showed that there were 7 positives out of 54 sera and in 1965, 15 positives out of 121. In 1966 there were no positives out of 134, in 1967 no positives have yet been obtained. A rather impressive case was a catbird, three months old, that had a 1:320 serum. In addition, an analysis of the sera of resident species for 1964 and 1965 showed a prevalence of around 10 percent positive for SLE.

Naturally, large numbers of the migratory species were positive, totaling around 10 percent.

No evidence of a differential prevalence of SLE antibodies in males and females was obtained.

In another analysis the conversions within a year and between years were analyzed for 1964-1966 inclusive. Within the year four birds went from positive to negative and 7 went from negative to positive. Between years 6 birds went from positive to negative and one bird went from negative to positive. While these numbers are small, the numerical results suggest that some birds are becoming positive during the year.

REPORT FROM THE MASSACHUSETTS DEPARTMENT OF PUBLIC HEALTH VIRUS LABORATORY, BOSTON, MASSACHUSETTS

California Complex Activity in Massachusetts

As a result of the emergence of California complex viruses as important agents of central nervous system disease in Indiana ('64), Wisconsin ('60-'64) and Ohio, we set up a plaque neutralization test to screen human paired sera from central nervous sytem disease and mammalian sera collected by our Lakeville Encephalitis Field Station. Mr. Andrew Main of the Field Station also set out some indicator rabbits in 1966. From these serial bloods were available.

With the assistance of a graduate student Mr. Joseph Gentile of Northeastern University, approximately 100 selected cases of encephalitis occurring from 1964 to early 1967 have been tested for neutralizing antibody against strain BFS 283, (courtesy of Gladys Sather).

One case, Dr. McK., from Otis Air Force Base was diagnosed by neutralizing antibody, later confirmed by complement fixing antibody. The patient had meningitis, urinary retention, lateral rectus paresis and the E.E.G. showed diffuse and non-specific slowing. Investigation revealed that the patient had come from Ohio approximately 10 days before onset of disease, therefore this was classified as an imported case.

An 11-year-old boy who had left Massachusetts only once in his life, (to Maine)was classified as infected with California virus at an undetermined time (+ neutralizing - complement fixing antibody).

Five out of 9 indicator rabbits converted to California antibody during the

1966 season. Pre-exposure bloods on June 3 were negative. Two had converted on the first post-exposure bleeding, July 7, and at the next bleeding, August 1, all 5 which converted had already done so.

On the strength of this evidence of local activity of a California related virus we have prepared complement fixing antigen in chick embryos (allantoic fluid) in order to screen routinely for this agent.

Most of the 1967 human sera have now been tested with no evidence of recent infection. The 1967 Arthropods collection being inoculated into infant mice, may produce an isolate.

Also added to the antigen for screening human sera is Powassan (allantoic fluid) on the evidence of activity of that virus in Massachusetts. (Information Exchange, Number fifteen, page 63).

(Joan B. Daniels)


USPHS, NCDC, EIP
Ft. Collins, Colorado