ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

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Table of Contents

Table of Contents (continued)

Table of Contents (continued)

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SECOND ANNUAL GENERAL MEETING OF AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

The second annual general meeting of the American Committee on Arthropod-Borne Viruses was held at the Biltmore Hotel in Atlanta, Georgia, October 31, 1962. Ninety members were present. Chairman William C. Reeves briefly summarized the need and evolution of the organization and the present scope of its activities.

Subcommittee on Information Exchange:

Dr. Richard M. Taylor, Chairman of the Subcommittee on Information Exchange, reported on the printing of the second issue of the Catalogue of Arthropod-Borne Viruses, one of the two main functions of the Subcommittee, the other being preparation and distribution of the Arthropod-Borne Virus Information Exchange. The catalogue includes descriptions of unpublished viruses as well as those already documented in the literature. It is of necessity, because of the prohibitive cost of printing, very limited in distribution. One hundred copies of this catalogue have been assembled. Only one is sent to an institution where arthropod-borne virus research is conducted. At the present time, 84 copies have been issued--33 in the continental United States and 51 overseas. To date the catalogue contains 119 virus registrations. There are a good many still lacking, notably registrations of tickborne viruses. These will be added as amendments as they come in (an amendment each quarter) so that the catalogue is kept as current as possible for maximum utility.

The subcommittee also sends out on 3 x 5 cards information on arthropod-borne viruses supplied by three abstract journals--Biological Abstracts, Bulletin of Hygiene, and the Tropical Diseases Bulletin. Currently 1500 of these have been distributed, going back to 1958 in Biological Abstracts and to 1959 for the other two journals. These cards have been coded and labelled flag cards prepared to enable them to be readily referable to the catalogue. This undertaking is still in the experimental stage and comments will be solicited from investigators within the next year to better evaluate the usefulness of this effort. The catalogue will also be reviewed by a group of experts with the goal of establishing a criterion for registration of new viruses. Dr. Taylor concluded by expressing the appreciation of the subcommittee to the National Institutes of Health for financing the catalogue for the current period of three years. Investigators were urged to submit virus registrations promptly and to offer any comments to the subcommittee for improvement of these services.

Dr. Work announced that the sixth issue of the Information Exchange is the largest issue so far--a total of 147 pages--which is, in itself, evidence of the success of this undertaking. It is truly international since even more contributions are received from abroad than from the United States. He explained that only those recipients who continue to participate can remain on the list for distribution. A careful record is kept of every communication sent and received from the investigators on the distribution list and, as explained in the introduction to the sixth issue, individuals are dropped after failure to acknowledge two consecutive contribution requests.

The question was asked about new people just going into the arbovirus field receiving the Infoexchange. Dr. Work explained that because of the cost involved in putting out the Infoexchange, the subcommittee is very careful that issues sent out go to a group of people, so that a single investigator who begins work on arboviruses is not necessarily eligible to receive the Infoexchange. Within the next year, the subcommittee plans to canvass all recipients to find out how many and what category of people have access to it and read it. The way to become a participant is to write a letter to anyone on the subcommittee. This will be forwarded to Dr. Taylor with suggestions as to whether the applicant should be accepted. In some cases, administrators directly responsible for the arthropod-borne virus investigators receive the Infoexchange. Anyone may borrow a copy by writing to the subcommittee. However, as emphasized earlier, because of the informal nature of the exchange, it is not filed in libraries. Dr. Taylor re-emphasized that, while it is not a secret document, reference cannot be made to it in official publications.

The question was asked, "What about reproduction of items from the Information Exchange for internal distribution?" Dr. Work explained that the rules governing use of material in the Information Exchange are carefully spelled out in the sixth issue of the Infoexchange. It is the best policy to obtain the permission of the person submitting the article before reproduction of any kind. Because this is privileged information, the subcommittee cannot give permission; this must be granted by the original source. Most investigators contribute only on this basis and will discontinue participation if this restriction is not observed.

The question was asked, "How much time is allowed for submission of articles for inclusion in the Infoexchange?" When the contribution requests are sent out, a certain deadline is given for receipt of the contribution, varying from one month in the regular academic year to at least two months in the summer.

The question was asked whether certain groups could donate money for the purpose of obtaining more copies of the Infoexchange. Under the present setup, this type of support is not possible.

The system for listing reports in the Infoexchange was also discussed. Dr. Work invited suggestions for other ways of listing the reports. Due to the diversity of subject matter, it is difficult to come up with a better system than the present one of grouping broadly by diseases and geographical areas.

Subcommittee on the Relation of Birds to Arthropod-Borne Viruses:

Dr. Donald D. Stamm, Chairman, introduced the other members of this newly-formed subcommittee of the ACAV, Drs. Hickey, Provost, Newman, and Davis, and gave a brief history of the formation of the subcommittee. The subcommittee was formed as the result of a meeting held at CDC in February 1962. The first act was to organize the proceedings of the meeting for distribution to the members of the ACAV and to 350 ornithologists of the American Ornithologists' Union. Notices were also sent to 15 journals advising of the formation of the subcommittee and offering copies of the proceedings to interested parties. Out of this, 195 requests were received for the proceedings, which certainly indicates interest in these activities.

The subcommittee has further plans to publish a review type article in journals which would be directed to ornithologists to further obtain their active participation in these activities.

The subcommittee has plans for finding out from certain laboratories interested in birds what kind of information they would like to have from ornithologists. The need has also been expressed for some standardized method of field collections. Dr. Davis is presently working on this problem and should be able to issue a mimeographed manual within the next year or so. There is also a need to assemble and evaluate criteria for determining the age of birds. Dr. Hickey is exploring this problem. The problem was also discussed of where ornithologists can submit specimens for laboratory examination. Presently there is no standardized procedure other than arrangements with individual virologists who are already overloaded. The following resolution was subsequently unanimously endorsed by the ACAV: "Whereas the involvement of birds in the ecology of arboviruses is of a character which cannot well be studied or elucidated on a short-term basis, and whereas epidemic situations often result in a demand for short-term research to the detriment of long-term studies, therefore, be it resolved that the American Committee on Arthropod-Borne

Viruses encourage the establishment and maintenance of long-term multidisciplinary, intensive, year-round studies on the role of birds in the ecology of arthropod-borne viruses."

Subcommittee on Serologic Reagents:

Dr. Jordi Casals, Chairman, stated that the function of the subcommittee on serological reagents is to advise the American Committee on Arthropod-Borne Viruses on matters pertaining to development, standardization, and use of diagnostic reference and standard reagents.

As one of the most urgent needs in the field of arboviruses is evidently that of immune sera for typing purposes, it was decided early in the existence of this group that ways and means should be provided for the preparation of such sera to be made available to interested and responsible investigators. At one of the sessions of the subcommittee which met as special consultants to the NIH, held in February of this year, concrete plans were also put forth for the production of a number of such immune reagents. At that meeting, it was decided that in view of the quantities involved, up to 5000 ml of each immune reagent, the possibilities of using immune ascitic fluid prepared in mice was to be considered. Consequently, plans were made to run a number of pilot experiments by members of the subcommittee for preparation of immune ascitic fluids for 11 selected viruses.

In a session of the subcommittee held October 29, 1962, in Atlanta, results of these pilot experiments were presented, discussed, and as a result of these discussions, recommendations were made to the ACAV for further action.

1) The viruses for which immune ascitic fluid were to be prepared on these pilot experiments were as follows: Anopheles A, Anopheles B, Bwamba, CEV, Colorado tick fever, Guaroa, Neopolitan sandfly fever, Sicilian sandfly fever, Oropouche, Tacaribe, and Turlock.

2) For reasons beyond the control of the persons who had undertaken the task, no results or protocols could be submitted for Anopheles B, Neopolitan sandfly fever, and Tacaribe viruses.

3) Results with Anopheles A, Colorado tick fever, and Guaroa viruses were not, in the opinion of the respective individuals who undertook the pilot tests with these reagents, sufficiently good to justify at this time concrete instructions to be shown for production in quantity.

4) Results with Bwamba, CEV, Turlock, Sicilian sandfly fever, and Oropouche viruses were either good or very good; the investigators who had taken up these agents felt that valuable information had been obtained with some of the schedules of vaccination that they had developed so that their protocols could be used as a guide or general directive for the preparation of immune ascitic fluid in quantity. Protocols for these preparations were submitted to the Chairman of the ACAV and through him to the Chairman of the Panel on Arthropod-Borne Viruses of the NIAID.

5) On further discussion, it seemed advisable that in view of anticipated problems involved in the stimulation and extraction of ascitic fluid, the use of mouse immune sera should not be neglected. Consequently, it was decided that, along with efforts to prepare immune ascitic fluid, preparation of immune sera should be continued and, if necessary, the preparation of immune sera in mice be used as a means for procurement of diagnostic reagents, primarily for CF tests.

6) In the process of running these pilot experiments, quantities of AF, with good CF titers, from 200 to 500 cc approximately, were obtained with some of the viruses mentioned. Dr. Telford Work of CDC, one of the subcommittee members, offered to take over these fluids for packaging, lyophilization, and storage. These reagents will be available to interested investigators under conditions to be determined and distributed in 1963.

7) Since the Panel for Arthropod-Borne Viruses has been created and activated by the NIAID, whose function, among others, will be to promote the development of serological reagents, the question arose for discussion whether the subcommittee should be disbanded or deactivated. It soon became apparent that there were many other functions that fell within the province of the subcommittee and as the members of the subcommittee expressed interest and willingness to continue to serve, the committee will continue in existence.

NIH Panel on Serologic Reagents:

Dr. Colvin Gibson stated that the activities of the subcommittee on Serologic Reagents have progressed to the point where obtaining reagents for some of the arboviruses appears feasible in the future. One of the best ways of protecting the NIH investment in this field is in the development of this broad program for the provision of reference reagents which will serve as yardsticks by which identification of

viruses can be made. This was started earlier with the entero- and respiroviruses. Dr. Gibson stated that reference reagents were in production on almost 100 viruses. A central repository will be available for storage of these reagents. The Serological Reagents Program of NIAID has felt fortunate to be able to draw on the Subcommittee for Serologic Reagents of the ACAV to aid in applying NIH resources to put these ideas into effect. An advisory group, the Panel for Arthropod-Borne Viruses, has been established, drawing very largely and heavily on the membership of the present ACAV meeting in Atlanta. Dr. Gibson then introduced Dr. Alfred M. Webb, Executive Secretary of the NIH Panel, who discussed the problems of producing reference antigens in large volumes. It is anticipated that these will be distributed from Washington in the same manner as those for enteroviruses and adenoviruses. He said that the certification and final testing will probably have to be done not in one laboratory in Washington, but by a number of different laboratories, each contributing its result to the overall picture.

Report of the Nominating Committee (Dr. W. McD. Hammon, Chairman)

1) The present Executive Committee, composed of six persons, has served for nearly two years. It is recommended that this year rotation be started beginning with one person and that subsequently one new member be appointed to replace a retiring one each year. In this manner, the term for each new appointee will be for six years. The order of rotation of the present members will be determined by drawing lots. In case of resignation of one or more members, additional new members will be selected.

2) A nominating committee of three members, including a chairman, will be appointed each year by the Chairman of the Executive Committee. Persons serving currently on the Executive Committee should not be included on the Membership Committee. However, such persons may serve subsequently one year or more after they have ceased to serve on the Executive Committee .

3) No member of the Executive Committee will be re-elected to the Executive Committee until after an interval of one year.

4) The chairman of the Executive Committee will be elected annually by the membership of that committee.

5) Decisions on the above recommendations and subsequent ones of a similar nature will be by majority vote of the general voting

membership of the American Committee on Arthropod-Borne Viruses. This general voting membership will be defined as all the professional members of each laboratory group contributing to and receiving the Information Exchange and present to vote at the annual general meeting, now held in conjunction with the annual meeting of the American Society of Tropical Medicine and Hygiene.

The above recommendations were moved, seconded, and unanimously passed. Dr. Edward Buescher was unanimously elected to fill the seat on the Executive Council of the ACAV vacated by the rotational retirement of Dr. Telford H. Work. Dr. Alexis Shelokov will replace Dr. Work as Secretary of the Executive Council.

Dr. Hammon also reported on the Virus Nomenclature Subcommittee Meeting in Montreal, Canada, in August 1962, where it was recommended that arboviruses be recognized as a major group and the term "arbovirus" be used to designate this group. A complete report of this meeting was carried in the sixth issue of the Information Exchange.

Dr. Robert Shope read the tentative agenda for the International Congress of Tropical Medicine which is to be held in Rio de Janeiro, September 1-11, 1963.

Dr. Arturo Saenz of the World Health Organization discussed the WHO program in the arbovirus field. The basic element of this program is the designation of regional reference laboratories around the world. This was recommended by the Study Group on Arthropod-Borne Viruses convened in September 1960 by the WHO in Geneva. It was felt that with the great number of viruses discovered recently, it was impossible for field laboratories to identify and classify them completely. Because of the recognition of this need, the WHO has already designated seven of the Regional Reference Laboratories for Arboviruses. The functions of these laboratories will consist of identification of viruses, maintenance of prototype strains, production of control sera, and other activities like training, collection, and distribution of information, and assistance in epidemics.

The WHO has also initiated studies of disease problems of world health importance, but lack of funds prohibits the number of surveys which can actually be carried out. They also assist in studies of epidemics where they occur, for example the recent yellow fever epidemic in Ethiopia. The WHO realized the international importance of this and began by holding a meeting of yellow fever experts in Geneva in October 1961. This group of experts reviewed the information collected in

Ethiopia by the Pasteur Institute in Addis Ababa. They also reviewed plans drawn up by the Pasteur Institute for future studies of yellow fever and agreed that the WHO should support these plans.

The experts also agreed to assist these studies in their own laboratories. They have been receiving samples of sera collected in Ethiopia for workup and the group will hold another meeting next year to discuss results of these studies and this information will be made generally available.

Dr. Vilches of the Pan American Health Organization discussed the efforts of PAHO in arbovirus research. He mentioned that Dr. Reeves and Dr. Scherer were sent to Latin America by the PAHO last year to survey existing laboratories and relate this to future research possibilities in the arbovirus field.

Dr. Reeves announced that the next annual general meeting of the ACAV will be held in Chicago in conjunction with the meetings of the American Society of Tropical Medicine and Hygiene, November 6-9, 1963.

REPORT FROM DR. R. M. TAYLOR
CHAIRMAN, SUBCOMMITTEE ON INFORMATION EXCHANGE

Arthropod-Borne Virus Catalogue

The issue of nine cards for newly registered viruses during the final quarter of last year brought the number of viruses now registered in the catalogue as of January 1, 1963, to 128. Since the first of the year, registrations of one virus from Australia and three from Malaya have been received. Registration cards of these viruses will be distributed in April. However, registration of several known viruses of the RSSE complex and from India are still lacking.

Fifty-six abstracts from Biological Abstracts, 48 from Bulletin of Hygiene and Tropical Disease Bulletin, and 17 Current Information slips, as well as 113 cards from Biological Abstracts for years 1958 through 1961 which had not been previously distributed, coded for reference to the Catalogue, were issued during the fourth quarter of last year. This brings the total of abstracts issued to date to 1,717, and of Current Information slips to 47.

A preliminary analysis, utilizing the punch card system, of the data on the registration cards of the 128 registered viruses has been made for presentation at the March meeting of the American Committee on Arthropod-Borne Viruses (ACAV). This analysis has been useful in testing the application of the punch card system, in sorting and correlating data, and in indicating certain desirable changes in the coding. A tabulated analysis of information obtainable from the registration cards will be distributed as a supplement to a future issue of the Information Exchange.

EDITORIAL NOTES

Accidental Exposure and Infection with Arboviruses

The accelerating interest and activity in the investigation of arboviruses and their related disease problems has brought a consequent increase in laboratory accidents and exposures to a number of these agents. Many laboratories became acutely aware of this in 1962 and it is not surprising that a laboratory such as Dr. Hanson's at the University of Wisconsin should become one of a number of focal points for inquiries, instruction, and resource of immune substances for dealing with such accidental exposures. Prompted by this, Dr. Hanson composed a letter which was distributed to a number of other individuals and laboratories concerned with this problem. The full text of this letter follows:

> "On December 26, seven long distance telephone calls between Kansas City, Madison, Atlanta, and Washington were required to locate a supply of eastern encephalitis hyper-immune serum. A technician in a Kansas City laboratory, who had not been vaccinated in six years, was accidentally exposed to the virus at 3 o'clock in the afternoon. The serum was located about 7:00 p.m. at the Walter Reed Medical Center. The physician was first referred by us to CDC and when he failed to get help there, Dr. A. Evans of the Wisconsin Laboratory of Hygiene called Col. Buescher who had some antiserum.
>
> "This episode illustrates the lack of any protocol or agreement for the handling of laboratory accidents with encephalitic viruses.

"We know of three other accidents within the past year and a half. In the first, seven technicians in a Georgia laboratory were exposed to an aerosol of eastern encephalitis when a tube of virus broke in a centrifuge. We obtained plasma from our vaccinated staff with the help of the Wisconsin Laboratory of Hygiene and the University Blood Bank, sent it by air through the courtesy of the U.S. Air Force, and it was administered within 48 hours of the accident.

"In the second, an individual on our Veterinary Science staff accidentally inoculated himself with Powassan virus. Dr. McLean of Toronto, whom we immediately called, did not have hyper-immune serum. He suggested that we obtain, if possible, serum from an individual who had had Russian Spring Summer encephalitis. A Polish veterinarian was located on the campus who had lived and worked in the Carpathian Mountains where RSSE is prevalent and who had left there within the year. His serum was administered within 6 hours of the accident.

"The third accident involved an individual on the staff of the Wisconsin State Laboratory of Hygiene who found on a roller drum a broken tube containing Japanese B encephalitis virus. Believing it to be a para-influenza virus, he did not adequately protect himself from an aerosol of the virus. At the suggestion of Col. Buescher, he was given a plasma transfusion from an individual known to be immune. The recipient suffered a mild attack of serum sickness.

"The Wisconsin Laboratory of Hygiene and the Department of Veterinary Science collaborated in the handling of each of these accidents. In all instances, the exposed individuals suffered no adverse effects from the virus. Whether they would have become ill if they had not received treatment is unknown.

"Eight of the individuals in these 3 accidents worked in laboratories in which encephalitis virus was being handled but did not work with the virus and they had not been vaccinated. The other two individuals worked with encephalitis virus but either had not been vaccinated recently or with the appropriate virus.

"It appears to us that two problems need further amplification. One, is the definition of the procedure that should be followed to avoid an incapacitating laboratory accident and the second is determination of the availability of vaccine and immune serum.

"We have vaccinated our staff with the Eastern and Western encephalitis vaccine made available by Col. Buescher of the Army Walter Reed Laboratory. A booster shot is given each year. All personnel are instructed concerning the danger of exposure and the areas in which virus can be handled are restricted.

"We understand from Col. Buescher that he recommends cutting a virus infected wound to promote bleeding and then sterilizing the wound with formalin.

"We have followed the procedure of giving plasma or serum as soon as possible after exposure but we have felt that specific antibody may be useful even if given 3 days after exposure. This is based on experience with laboratory animals. Dr. Buescher recommends that the antibody preparation be given within 3 hours in order to prevent the completion of the first growth cycle of the virus. This cycle takes about 9 hours.

"If Dr. Buescher's recommendation is to be followed, gamma globulin should be available in any center where the virus is being handled. If a longer period is permissible, one or two centers of supply in the United States might be adequate. In any case, it is most important that responsible individuals know either where such supplies are located or that the provision of such a supply is their responsibility. The responsible individuals include not only the project leader in research institutions, most of whom are members of the information exchange, but also many of the laboratory officers of concerns that produce veterinary biologics. Several serious accidents are known to have occurred in commercial laboratories.

"In summary, here are the specific questions which we feel should be considered by the Committee for Arthropod-Borne Virus Information Exchange.

"Providing a vaccine is available, who should be vaccinated and how frequently should a booster shot be given?

"What can be done to avoid accidents?

"What procedure should be followed in the event of an accident?

"If vaccines and serums are to be used, how are they to be obtained?

"Should a roster of individuals known to be immune to specific agents be prepared?

"Who is to be responsible for providing information on these questions to the laboratories in which risk of infection exists?

"Should we at Wisconsin prepare specific gamma globulin for our own emergency use?"

Dr. Hanson's letter resulted in discussion of various means by which this problem could be approached at a series of scheduled meetings in Washington in March. These were the Commission on Viral Infections of the Armed Forces Epidemiological Board, the NIH Panel on Arboviruses, and the Executive Council of the American Committee on Arthropod-Borne Viruses. No concrete proposals were formulated by the Commission other than a request for analysis of results of experimental vaccination of laboratory workers with vaccine produced by the Veterinary Public Health and Virus Diseases Division of Walter Reed Army Institute of Research (WRAIR). The deliberation of the NIH Panel on Arboviruses resulted in an acceptance of responsibility by the NIH Research Reference Reagents Branch for provision of prophylactic and immune substances when appropriate protocols for their production are forthcoming. The Executive Council of the ACAV designated Dr. E. L. Buescher of WRAIR as convener of a conference to be attended by a selected group with experience with and current problems of laboratory and field exposure to arboviruses. It is expected that such a group will define the principle aspects of the problem and determine whether a new subcommittee of the ACAV should be established to deal with procedures to be followed in the safe handling and accidental exposure to arbovirus infection.

Seventh International Congresses of Tropical Medicine and Malaria

The Seventh International Congresses of Tropical Medicine and Malaria are to be convened in Rio de Janeiro, Brazil, from September 1 to 11, 1963. The address of the Secretary General, Dr. F. Nery Guimaraes, is: Av. General Justo No. 365-9.0, Cx. Postal 1859, Rio de Janeiro, Brazil.

A series of seminars on arboviruses and their diseases in man and animals has been organized for the congresses. There will be time for some individual presentation of contributions submitted prior to April 30. All inquiries regarding papers and abstracts for the virus disease sessions should be sent to: Dr. J. Rodrigues da Silva, Faculdade Nacional de Medicina, Universidade do Brasil, Caixa Postal 1859, Rio de Janeiro, Brazil.

Special arrangements are being investigated for a group travel itinerary which would allow for scheduled visits to the arbovirus laboratories in Caracas, Port-of-Spain, Belem, Buenos Aires, Bogota, Cali, and Panama. Besides making the most of limited available time for observation of the laboratory and field activities, local arrangements for accompanying wives is also being scheduled. Further information can be obtained from the Information Exchange office or by writing to Room 1611, Fulton National Bank Building, Atlanta, Georgia.

The sessions of the congresses, the special arbovirus meetings being planned for Rio by the Pan American Health Organization, opportunity to visit the laboratories in Latin America where so much productive investigation of arboviruses has been accomplished, and the fascinating scenery and warm hospitality of Latin America warrant embarking on this once-in-a-lifetime opportunity for investigators of arthropod-borne viruses in other parts of the world.

The international activity of the Subcommittee on Information Exchange of the American Committee on Arthropod-Borne Viruses will also be presented and discussed during the forthcoming congresses in Rio.

Encephalitis in the Caribbean area, 1962

The lead group of reports in this issue of the Information Exchange are from investigators and laboratories which dealt with various aspects of the unprecedented activity of mosquito-borne viral encephalitis which occurred in the Caribbean area in 1962. It is too early to have a definitive evaluation of what really happened and this problem will doubtless be the subject of subsequent reports from the many laboratories involved.

Telford H. Work, M.D.
Editor

REPORT FROM DR. ANTONIO M. VILCHES
COMMUNICABLE DISEASES BRANCH
PAN AMERICAN HEALTH ORGANIZATION, WASHINGTON, D.C.

The Pan American Health Organization has initiated plans to hold two separate meetings of interest to arbovirus investigators at the International Congresses of Tropical Medicine and Malaria in Rio de Janeiro, Brazil, September 1-11, 1963. The subjects will be: 1) Arbovirus Disease Epidemics in the Western Hemisphere: How to Cope on a Regional and International Basis with Such Emergency Problems; and 2) Arbovirus Disease Problems Involved in the Movement of Populations in Central and South America. An agenda designating specific persons to discuss recent problems covered by the subjects is being prepared. The meetings will be scheduled for a half-day session, although they will be held on separate days to allow for additional discussion should this be indicated. Dr. Mauricio M. da Silva is responsible for plans and preparation of these meetings.

Dr. Telford Work, as a consultant of PAHO, made a trip to Argentina to appraise the present status of the studies on the hemorrhagic fever outbreaks (Mal de Rastrojos) which have occurred in the last few years in the north of Buenos Aires province, and to recommend the course that these studies should follow in the future. His report containing an exhaustive analysis of the problem has been received in this office for submission to the Government of Argentina.

Dr. Chester A. Gleiser visited Jamaica as a consultant of PAHO during the first two weeks of December 1962 to review and evaluate measures against the outbreak of eastern equine encephalitis then occurring in the southeast tip of the island; his report and recommendations were submitted to the Government of Jamaica.

In order to cooperate with the governments and scientific institutions interested in the hemorrhagic fever problem in the Western Hemisphere, this organization is presently establishing a coordinating service for collaborative studies on this subject.

REPORT FROM DR. A. C. SAENZ
WORLD HEALTH ORGANIZATION, GENEVA, SWITZERLAND

Seminar on Japanese B Encephalitis and Other Arthropod-Borne Infections

This seminar took place in Tokyo from 5-14 November 1962. It was organized under the sponsorship of the Western Pacific Regional Office of WHO, with the support of the Japanese authorities.

Twenty-one participants from 15 countries in the Western Pacific and Southeast Asia regions attended the seminar. In addition, 21 members of the staff of different research institutes and laboratories interested in these problems were also present. Dr. M. Kitaoka of NIH Tokyo, Dr. N.I. Grashchenkov, Moscow, Prof. J.A.R. Miles of Otago University, New Zealand, and Prof. W.F. Scherer, Cornell University, New York, acted as consultants and discussion leaders. Dr. D.R. Huggins of the Western Pacific Regional Office, Prof. C.Y. Chow, also of this office, and Dr. A.C. Saenz, HQ, represented WHO.

The information brought to the seminar by the participants and the discussions held during it have made it possible to put together in the final report an up-to-date picture of the distribution of arboviruses and of the public health importance of the diseases caused by them in that region. This report will be distributed to the recipients of the Arthropod-Borne Virus Information Exchange as soon as it is available in printed form.

REPORT FROM DR. OTTIS R. CAUSEY
BELEM VIRUS LABORATORY, BELEM, BRAZIL

The Utinga wild animal recapture program, although begun only in June, has given results which indicate that wild rodents and marsupials in their natural habitat can serve as effective sentinels. Their habit of returning to the traps affords excellent opportunities to isolate the viruses contracted by them under the most natural circumstances, and to obtain sera at suitable intervals to test for the presence and persistence of antibodies.

From an area 500 by 500 meters square, 187 rodents and 86 marsupials were collected a total of 1467 times. The majority of recaptures were spaced from 3 to 7 days apart. Less than 20 per cent of the marked animals failed to return, and these included animals killed by accident in the laboratory. Approximately one-third returned 6-15 times and there were 10 individuals which were captured 17 to 34 times. The rodents were captured usually in the same trap but would occasionally be found at a neighboring station 150-200 meters distant. Twenty-five viruses were isolated from the 1467 captures (1.7%) of the 273 animals in this study. There were in addition 17 HI antibody conversions to viruses that were not isolated, as well as at least one additional CF conversion, making a total of 43 (15.8%) infections so far recorded for the 273 animals during 27 weeks of observation. However, serological tests have been made for only 20 of the 47 virus types known from the Amazon region with sera collected in January and later.

REPORT FROM DR. A. L. BRICENO ROSSI
INSTITUTO NACIONAL DE HIGIENE, CARACAS, VENEZUELA

During the months of November and December, 1962, there was an epidemic outbreak involving a total of 6,737 persons in the rural area in the northern part of the State of Zulia, including the towns of Paraguaipoa, Sinamaica, El Mojan, Isla Toas, Sta. Cruz de Mara, Sabaneta de Palma, Guerrero, and Puerto de Altagracia. Such persons consulted the local public health centers with complaints of fever with severe cephalagia, bone pains, and, in some cases, signs of acute encephalitis. The clinical picture was a sudden one and it was seen at once that an epidemic of encephalitis had occurred. The Demography and Epidemiology Department of the Ministry of Health and Social Assistance went immediately into action, and we travelled to the center of the outbreak in order to collect the samples that were to be studied

in investigating the entire epidemic wave. From the outset, both the Venezuelan Institute for Scientific Investigations (IVIC) and the Instituto Nacional de Higiene accomplished the isolation of viruses from blood specimens of the first cases, identified as VEE.

The Virus Section of Instituto Nacional de Higiene received a total of 247 blood specimens and throat swabs for study.

The isolation method employed--intracerebral inoculation in suckling mice--gave consistently good results 24 to 48 hours from the time of incubation. Less than one-day-old chicks, inoculated subcutaneously, were also found quite sensitive but at times the skin of these animals was too fragile, often releasing the infecting material from the inoculation wound. For this reason, these animals, although as sensitive to VEE as the suckling mice, were discarded from this study. In 120 inoculated chickens, the rate of infection was equal to the one found in suckling mice; that is, therefore, a suitable technique as wherever there is no breed or colony of white mice, laboratories can use the easily available less-than-one-day-old chick. Care must be taken, though, on the one hand to protect the person doing the inoculation and on the other, securely to isolate the chicks from mosquitoes.

Neutralization tests were performed in 3-week-old white mice. Viruses were isolated from 79 per cent of the blood specimens received at the Virus Section, Instituto Nacional de Higiene. Such specimens had been collected within the first 3 days after the onset of the disease in each case. The highest rate of cases was found among children less than 14 years old but adults in the 49-59 year old age group were equally affected.

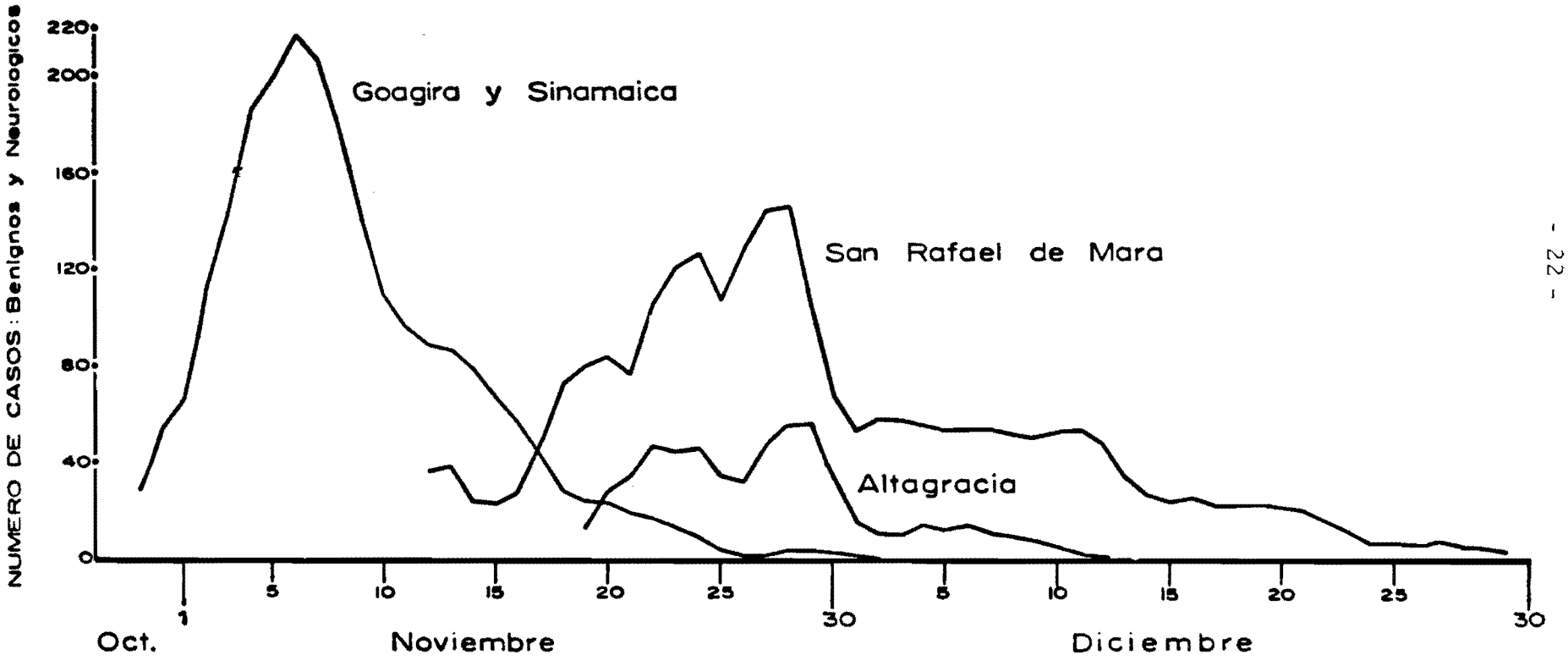
The enclosed graph and table on this epidemic shows its epidemiological power and mortality rate. Three types were differentiated by the clinic, viz. (1) cases with high, 40°C temperature, vomiting, severe permanent cephalagia, conjunctival hyperemia leaving a particularly hyperemic line on the palpebral opening, and ataxia-like signs; (2) nervous type with convulsions--this was frequent among children less than 10 years old; and (3) the grippal type of the disease.

Viruses, now under study, were twice isolated from 33 throat swabs. One of our laboratory technicians was infected while inoculating mice during this study. Virus isolated from her blood was identified as VEE.

Epidemia de Encefalitis VEE en el Norte del Estado Zulia (1962)

Municipios (towns)	Duracion de la epidemia (Date) Fechas	(Days) No Dias	Casos (Patients) Benignos	Neurologicas	Defunciones (deaths)
Goagira (Capital Paraguaipoa)	29/10 a 2/12	35	1607	115	10
Sinamaica (Capital Sinamaica)	30/10 a 29/11	31	998	95	18
Luis de Vicente (Capital Carrasquero)	21/11 a 14/12	19	80	7	2
San Rafael (Capital El Mojan)	11/11 a 30/12	50	2437	99	5
Padilla (Capital El Toro	19/11 a 4/12	16	361	4	2
Monagas (Capital San Carlos)	25/11 a 2/12	8	90	1	2
Ricaurte (Capital Sta. Cruz)	24/11 a 15/12	22	224	19	2
Altagracia (Capital	18/11 a 13/12	26	543	57	1
Total			6340	397	42

EVOLUCION DE LA EPIDEMIA DE ENCEFALITIS VEE
EN LOS MUNICIPIOS GOAGIRA, SINAMAICA, SAN RAFAEL DE MARA
Y ALTAGRACIA DEL ESTADO ZULIA
(PROMEDIO MOVIL: 3 TERMINOS)



REPORT FROM DR. G.H. BERGOLD, HEAD, VIRUS DEPARTMENT INSTITUTO VENEZOLANO DE INVESTIGACIONES CIENTIFICAS CARACAS, VENEZUELA

Dr. Robert F. Sellers from Wellcome Research Laboratories in England has succeeded Dr. Manfred Mussgay as Head of the Laboratory for Domestic Animal Viruses of this Department.

A line of baby hamster kidney cells (BHK 21) was kindly supplied by Dr. Stoker from Glasgow. The susceptibility of this cell line for growth and plaque formation by different arboviruses, groups A and B, was investigated by Dr. Sellers. It was found that Venezuelan equine encephalitis (VEE), Mayaro, St. Louis encephalitis (SLE), Ilheus, and French Neurotropic (FN) and 17D strains of yellow fever gave cytopathic effect. These 6 virus strains also produced plaques using an overlay containing 2.5% calf serum, 0.35% lactalbumin hydrolysate, 0.07% yeast extract in Earle's saline. It is interesting that VEE, Ilheus, and SLE produced irregularly shaped plaques, whereas on the 2nd, 3rd, or 4th day, Mayaro and both strains of yellow fever gave rise to somewhat triangular or cirriform plaques similar to those described by Ellem and Colter for the S-variant of Mengo virus. This is interesting because it may indicate the mechanism for the growth and release of virus particles. These particular cells grow in characteristic sheaves and susceptibility of cells within the sheaves may be greater than that of cells of the neighboring sheaf.

Experiments (G.H. Bergold) using the BHK 21 cell line for plaque assay of various yellow fever strains gave at first good results; however, it seems that lately, after several passages, the cell line lasts much less under agar overlay than when first received from Stoker. It appears that the BHK 21 cell line is not suitable for plaque assay of viruses which require 4 or more days to form plaques. Preliminary experiments with the methyl-cellulose overlay recently suggested by Schulze and Schlesinger does not appear to increase survival of these cells. The suitability of KB cells as suggested by the same authors is presently being checked. However, some good plaque assays were occasionally obtained with chicken fibroblasts using Noble agar in the Coleman overlay.

A rather severe epidemic occurred in the State of Zulia in the fall of 1962 with more than 60 children's deaths. The first blood and brain samples of heavily diseased and deceased children and donkeys were collected on November 9 in the areas of Sinamaica and Paraguaipoa, NE of Maracaibo. Dr. Sellers investigated these samples and on November 11 was certain that it was an encephalitis type virus judging from the

quickness of the cytopathic effect in BHK cells. Protection tests carried out simultaneously with WEE, EEE, Mayaro, St. Louis, and VEE, again in BHK cells, indicated with certainty on November 12 that the viral agent was VEE.

At about the same time, with the kind cooperation of the Direccion de Malariologia y Saneamiento Ambiental and the Hospital Univeristario de Maracaibo, over 2,000 mosquitoes were collected, 22 birds shot, and more blood and brain samples of human cases received. Dr. Sellers was able to isolate VEE virus from most of the blood and brain samples of human cases and from donkey's brain, as well as from Aedes taeniorhynchus, Anopheles acquasalis, Aedes serratus, and Psorophora confinis. One of the birds had a viral agent, but which was not VEE.

A small expedition into the infected area in the middle of January, 1963, by Dr. Sellers and O. Suarez and A. Morales, all from our department, collected about 900 mosquitoes, 13 iguanas, one snake, some lizards, bats, marsupials, rodents, and birds. They have been stored in dry ice. This material is presently under investigation, and its results and some other aspects of the outbreak will be reported later.

Stimulated by discussions with Dr. O.R. Causey from the Rockefeller Foundation Laboratory and Dr. M. Bruno Lobo from the Evandro Chagas Institute at the occasion of a visit of Dr. G.H. Bergold and O. Suarez to Belem, it was decided to make an expedition to the Casiquiare area in the Territorio Amazonas to investigate the presence of arboviruses in that region. This region was chosen because the Casiquiare is the famous water connection between the Amazonas and the Orinoco water basins, and any migration of arboviruses would most likely take place along that channel. Furthermore, this area is well known and feared for the presence of great quantities of mosquitoes.

The expedition was organized with the cooperation of the Direccion de Malariologia y Saneamiento Ambiental, and carried out between October 22 and December 2, 1962. Apart from the technical staff, the members of the expedition were: Drs. G.H. Bergold, R.F. Sellers, O. Suarez, and A. Morales, from the Virus Department; Dr. L. Warren, Parasitologist from the Physiopathology Department; and Dr. V. Vareschi, Botanist from the Universidad Central de Venezuela.

Major problems arose in the need of having continuous dry ice supply in that remote area; therefore, the staff was divided into 3 groups each one preceding with dry ice on definite scheduled dates. The Venezuelan Air Force was able to provide an additional shipment for us. About 3,000

gallons of gasoline had to be distributed for refueling at different river locations. The expedition started by boat from Puerto Ayacucho up the Orinoco until Tama-Tama and then down the Casiquiare until Cano Monomi, where camp was set up for about a month. The cargo boats took about 8 days (with outboard motors) and the speed-boats about 3 days to reach the camp, about 700 kms. (400 miles) from Puerto Ayacucho. More than 12,000 kms. (7,500 miles) were covered by boat, counting all the water travel necessary to carry out the expedition.

From the camp, one side trip was carried out up the Orinoco some 400 kms. (250 miles) to the area of the Guaika Indians (Mavaca, Platanal, and Tucusito) in order to take blood from these indians who belong to one of the most primitive tribes living on earth. In the past, there had occurred several mysterious epidemics among these indians. About 50 blood samples were obtained from the Guaikas and about a dozen from the Bare indians in the Casiquiare area.

Four special virus and antibody free Cebus apella apella were kindly given to us by Dr. Causey and were exposed for about a month on 4 different locations, 10 mts. high, along a 1/2 km. trail cut from the black water forest of the Monomi to the white water forest of the Casiquiare. About 50 newborn litters of white mice were exposed to mosquitoes for 24-48 hours along the trail, using various gadgets. None died, but several showed some suspicious symptoms. About 1100 mosquitoes were captured on high stands during the day and the evening using light traps and ventilators as proposed by Dr. Causey, and preserved in dry ice. The classification of these mosquitoes by O. Suarez gave the following results:

Aedes fulvus fulvus	4	Limatus fluvisetatus	13
Aedes serratus	48	Limatus sp.	79
Aedes terrens	14	Mansonia humeralis	234
Aedes sp.	2	Mansonia sp.	3
Anopheles mediopunctatus	1	Psorophora ferox	60
Anopheles neivai	24	Sabethes albiprivus	3
Anopheles nimbus, thomasi	305	Sabethes cyaneus	3
Culex (Carrollia) sp.	3	Simulidae sp.	190
Culex sp.	77	Trichoprosopon digitatum digitatum	18
Culicoide	3		
Haemagogus sp.	36		
Haemagogus uriartei	36		
Limatus durhami	17		

Nine bats (Phyllostomidae spp.) and 7 rodents were captured in traps set up along the trail and the sera preserved in dry ice. Eight monkeys (2 Saimiris sciureus sciureus (Linneaus), 2 Callicebus torquatus torquatus (Hoffmansegg), and 4 Cebus apella apella (Linneaus)) of a very large variety were shot and 6 serum samples could be obtained. Presently, we are busy testing the samples, but so far it appears that this area is very healthy and very suitable for old-age retirement. All serum samples will probably be checked for presence of antibodies in cooperation with the Rockefeller Foundation Laboratory in Belem.

REPORT FROM DR. HERNANDO GROOT
INSTITUTO CARLOS FINLAY, BOGOTA, COLOMBIA

Outbreak of Infections Caused by Venezuelan Equine Encephalomyelitis Virus:

A serious epidemic of a febrile disease was observed in Guajira, Colombia, between mid-October 1962 and the first week of December 1962. Not less than 3,000 human cases were recorded among the rural inhabitants of the coastal plains. The clinical picture may be summarized as follows: sudden onset with malaise, chills, and fever; nausea, headache and muscle and bone pains. The fever lasted usually from two to three days and was accompanied by severe headache and, commonly, by stupor. Although no precise statistical figures are available apparently more cases were observed in children and young adults than in the older population. Both sexes were equally affected. about 30 deaths were attributed to the disease.

From 18 out of 19 people bled during the febrile stage, VEE virus was isolated from the blood stream. From the nineteenth case, no virus was isolated.

During the same period of time, an epizootic in horses and donkeys, accompanied by high mortality, was observed. Both the epidemic and the epizootic apparently started two to three weeks after the beginning of the rainy season.

REPORT FROM DR. HENRY K. BEYE
MIDDLE AMERICA RESEARCH UNIT
BALBOA HEIGHTS, CANAL ZONE

Encephalitis Virus Activity Along the Pacific Coast of Panama in 1962:

During 1962 surveillance was maintained for arbovirus activity in the Canal Zone and along the Pacific side of the Republic of Panama. Figure 1 shows the three areas where field investigations were conducted.

The Canal Zone and Panama City are located within Area 1. Steep hillsides covered by tropical secondary growth forest are characteristic of this area. The Panama Canal bisects it. Total rainfall at Gamboa, C. Z. during 1962 was 73 inches, while at Portobello on the Atlantic Coast, it was 167 inches. The terrain of areas 2 and 3 is flatter and generally drier. The 20-foot Pacific tides flood many thousands of acres of tidelands and salt marshes along its seaward edge. Total rainfall at the junction point between areas 2 and 3 was 68 inches during 1962. Area 1 is much less suited and used for agriculture than are areas 2 and 3 which are engaged in dairy farming, beef raising and cane growing activities. There is little similarity between these areas and the dense tropical rain forest of the Atlantic coast from which they are separated by a ridge of mountains 3,000 to 10,000 feet high.

The VEE virus was isolated in December 1961 from a resident of a town which adjoins Panama City. This resulted in continuing surveillance until September 1962 when many cases of horse encephalitis due to EEE occurred in and around the village of El Rincon in area 3. This village was canvassed for human illness and ten febrile persons were found. The virus of VEE was isolated from the serum of five of the ten.

Table 1 shows the number of isolations of VEE, EEE, and SLE and the number of serological conversions which have been demonstrated within the 3 areas during the year of 1962. Table 2 indicates the months, between April 1961 and February 1963, during which isolations of VEE, EEE, and SLE viruses have been made in Panama.

EEE virus was the only agent recovered from encephalitic horses. VEE virus was isolated only from humans in the two localities which

were investigated following the reports of horse illness. Clinically, the human VEE illnesses were acute, undifferentiated fevers without distinct encephalitic manifestations. The unique pattern of simultaneous VEE and EEE virus activity is under continuing investigation. Serologic study of both human and equine populations of the El Rincon area is in progress.

Since March 1962, a number of staff changes have occurred. The assignment of Dr. Karl M. Johnson as Head of the Virus Diseases Section brings to MARU special competence in the respiratory viral agents, as well as in the enteroviruses and in general viral epidemiology; that of Dr. Merle L. Kuns adds to capability in ecology and virology; and that of Maj. Bryce C. Walton in parasitology. We are also pleased to report that Dr. Arnold S. Monto with special training in microbiology, Mr. Robert T. Rawles, a well trained serologist, and Miss Elizabeth Earley with competence in newer tissue culture system are now with MARU. The arrival of Col. Chester Gleiser as part of the U.S. Army Mission to Panama has given MARU support in animal pathology.

The return of Dr. James M. Brennan and Dr. Conrad E. Yunker to their home base at the Rocky Mountain Laboratory after two years with MARU has been a loss, as well as the departure of Dr. Evert A. Bruckner for work in pathology with Dr. Gustav Dammin, and that of Miss Dorothy Longfellow for the Malaya Army Unit in Kuala Lumpur.

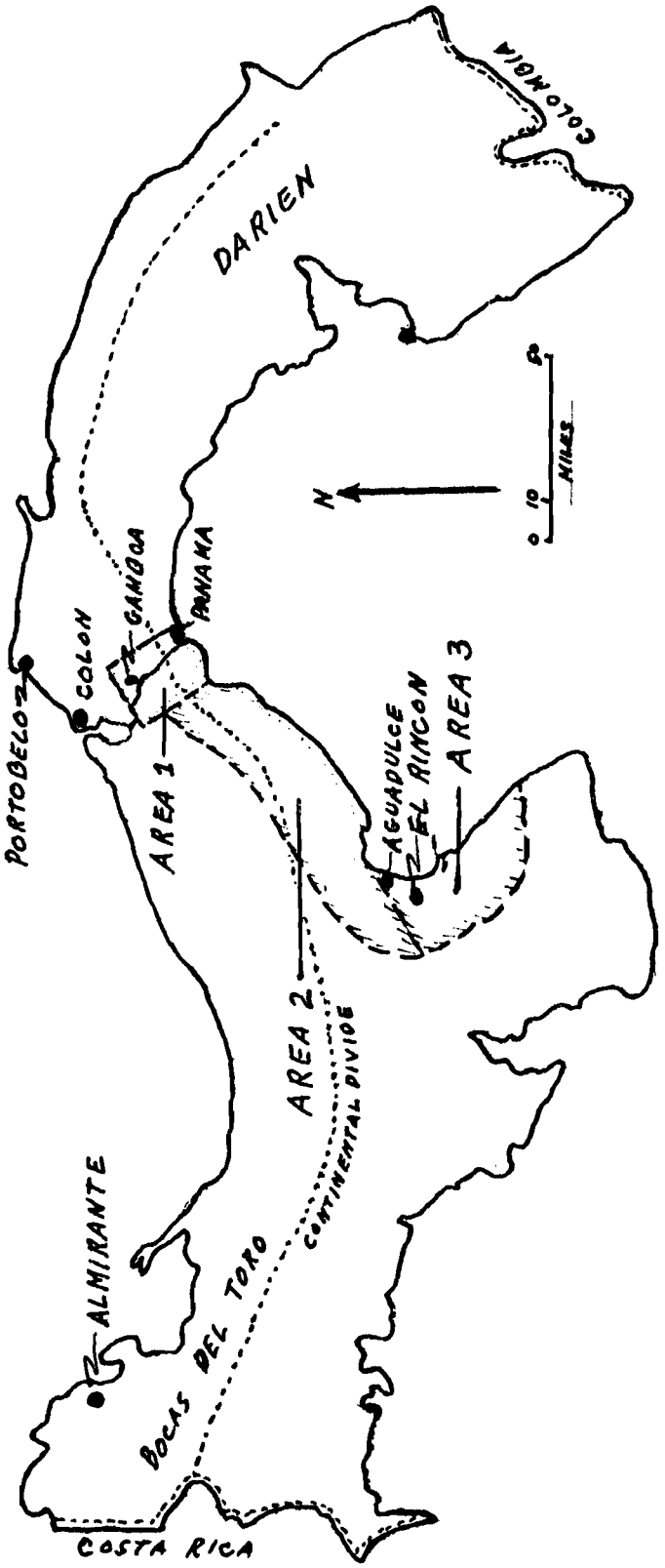

COLOMBIA
DARIEN
PANAMA
PORTOBELO
COLON
GAMBOA
PANAMA
AREA 1
AREA 2
AREA 3
AGUADULCE
EL RINCON
CONTINENTAL DIVIDE
ALMIRANTE
BOCAS DEL TORO
COSTA RICA
N
0 10 20
MILES

FIG. 1

Table 1

Numbers of VEE, EEE and SLE virus isolations and demonstrated serological conversions in 3 areas of Panama (shown in Fig. 1) during 1962.

	AREA 1			AREA 2			AREA 3		
ISOLATIONS	VEE	EEE	SLE	VEE	EEE	SLE	VEE	EEE	SLE
Human serum	2	-	-	-	-	-	5	-	-
Horse brain	-	2	-	-	1	-	-	-	-
Sentinel mouse litters	8	-	1	-	-	-	-	-	-
Wild spiney rat[a]	1	-	-	-	-	-	-	-	-
Wild ground dove[b]	-	-	1	-	-	-	-	-	-
SEROLOGICAL CONVERSIONS									
Horse	-	-	-	-	-	-	7[c]	2[d]	-
Cattle	-	-	-	-	-	-	e	-	-

a Proechimys semispinosis

b Columbigallina talpacoti

c Serologically negative on 1 Feb; neutralizing antibodies found in sera taken on 14 Nov*.

d One of the two demonstrated clinical encephalitis.

e One of 176 from a herd of 500 bled on 23 July had neutralizing antibodies. Fifty one percent of 102 of same herd bled on 14 Nov had neutralizing antibodies*.

* Tests done at Department of Veterinary Science, University of Wisconsin.

Table 2

The months and years during which VEE, EEE and SLE viruses have been isolated within the 3 areas shown in Fig. 1 since April 1961.

	VEE	EEE	SLE
Jan.	+('63)		
Feb.	+('63)		
Mar.			
Apr.	*+('61)		
May	*		
June	*		
July	*	+('62)	
Aug.	*	+('62)	+('62)
Sept.	*+('62)		
Oct.	*	+('62)	
Nov.			
Dec.	+('61, '62)		

*Isolations made in Almirante area in 1961. (See joint Gorgas Memorial Laboratory - MARU Report in March 1962 issue of Information Exchange).

REPORT FROM DR. MARGARET GRAYSON, DR. PAULINE PERALTA, AND DOROTHY LONGFELLOW, GORGAS MEMORIAL LABORATORY, PANAMA, AND MIDDLE AMERICA RESEARCH UNIT, CANAL ZONE

This report presents the results of serological surveys relating to our studies on the ecology of Venezuelan equine encephalitis virus in Almirante, B. T. As previously noted (Information Exchange, March, 1962), VEE was highly active in Almirante from April, 1961, to November, 1961, during which time a total of 49 isolations of VEE virus was effected from humans, mosquitoes, wild rodents, and sentinel animals. The fact that no VEE virus isolates were obtained from field materials collected between September, 1959, and April, 1961, suggested that the virus was not active in the area during that time.

In an attempt to assess Group A arbovirus activity prior to and during the 1961 VEE outbreak, screening tests for complement-fixing (CF) and hemagglutination-inhibiting (HI) antibodies to Venezuelan equine encephalitis, eastern equine encephalitis, and Una viruses were performed on 543 serum specimens collected from residents of Almirante and on 71 sera of domestic animals in the area. Sixty-four paired specimens collected from 32 residents of Almirante in October, 1960, and August, 1961, respectively, were also tested for CF and HI antibodies to VEE, EEE, and Una viruses.

Antibody patterns in 172 and 371 sera obtained in October, 1960, and August, 1961, respectively, from 543 residents of Almirante are shown in Table I.

Of the 1960 samples, 31.9% exhibited antibodies only to VEE virus, 12.8% to VEE and EEE, and 4.1% to VEE, EEE, and Una viruses. Of the 1961 samples, 31.8% reacted solely with VEE virus, 4.9% with VEE and EEE, 0.3% with VEE and Una, and 2.9% with all three Group A agents. All of the VEE CF positive sera were also positive by HI test. However, two of the EEE and three of the Una CF positives were HI negative. No EEE and/or Una positive, VEE negative sera were observed. These data suggest that most, if not all, of the infections detected were due to either VEE or an unknown, closely related Group A virus.

VEE antibodies were widespread through populations sampled, occurred nearly uniformly in both sexes, and showed progressive age-specific increments. Three of 11 children less than five years of age were

positive in 1960, suggesting that the virus had been active in the region at some time between 1955 and 1960. These results indicate the distinct probability that VEE virus had been active in Almirante prior to its first recovery in Panama early in 1961. No serological conversions were observed in 32 paired sera collected in 1960 and 1961 but VEE antibodies were detected in seven serum pairs. Infrequent low-titered antibodies to EEE and Una viruses were noted in the VEE positive sera. VEE antibody titers in the seven positive individuals are depicted in Table II.

The nearly uniform stability of both CF and HI antibodies during a period of nine months is of some interest. Based on observations of investigators at the Rockefeller Foundation Virus Laboratories (Annual Report 1956), the CF results suggest that several of these infections may have occurred during 1959 or 1960. Further careful study of the temporal patterns of such antibodies following documented natural infection is needed in order to determine whether serologic data alone can be used to establish the temporal occurrence of VEE infections in humans.

Sera from 69 equines and two dogs were also collected in August, 1961. Antibody patterns in equines were similar to those observed in humans. Thus, 69.6% of the equines had antibodies to VEE virus, with significantly smaller numbers exhibiting EEE and Una antibodies. There were no EEE and/or Una positive VEE negative sera among equines, further suggesting that VEE rather than EEE or Una was the infecting virus. CF and HI antibodies to VEE virus were demonstrated in the sera of both dogs. Since dogs are common animals closely associated with human habitation in Panama, the use of dogs as serologic sentinels in epidemiologic investigations of VEE virus seems worthy of consideration.

TABLE I

Distribution of Group A Antibodies in 543 Residents of Almirante, B. T.

	1960 (172 Sera)		1961 (371 sera)	
	% positive by		% positive by	
VIRUS	HI	CF	HI	CF
VEE	48.8	36.6	39.9	29.9
EEE	16.9	1.7	7.3	2.7
UNA	2.9	2.3	2.9	0.8

TABLE II

VEE Antibody Titers* in Seven Residents of Almirante, B.T.

			CF		HI	
Subject #	1960 Age	Sex	1960	1961	1960	1961
1	6	M	32	16	320	160
2	12	M	16	32	80	20
3	9	M	8	8	40	80
4	74	F	32	32	160	160
5	12	M	8	<4	20	20
6	10	F	32	32	320	640
7	13	M	4	4	40	40

* Expressed as the reciprocal of the highest serum dilution exhibiting CF or HI activity.

REPORT FROM DRS. ENID DE RODANICHE AND PEDRO GALINDO GORGAS MEMORIAL LABORATORY AND UNIVERSITY OF PANAMA SCHOOL OF MEDICINE, PANAMA, R. DE P.

The isolation of Ilheus encephalitis virus from mosquitoes collected in eastern Panama and from birds captured near Almirante in Western Panama in March 1960[2] was previously reported. The latter isolations were obtained from the Little Blue Heron, Florida caerulea and the Keel-billed Toucan, Ramphastos sulfuratus.

The present report includes further work on the ecology of Ilheus virus in the Almirante area carried out between March 1960 and April 1961.

A total of 370 vertebrate sera were inoculated intracerebrally into 2-day-old mice for virus isolation attempts. Of these, 330 were avian sera, 23 reptilian, 4 from lower mammals, and 13 from humans. Five viruses were isolated, 3 from birds and 2 from humans. Only a single isolate, obtained from the blood of the Scarlet-rumped Tanager, Ramphocelus passerinii, proved to be Ilheus virus. This bird was captured near Almirante on January 24, 1961.

Large numbers of mosquitoes were also inoculated into mice for virus isolation attempts. Ilheus virus was isolated five times from the following pools of mosquitoes. One pool of 101 Psorophora lutzii captured between July 17 and July 21, 1960. One pool of 117 Psorophora ferox captured between October 11 and October 29, 1960, two pools containing 204 mosquitoes each, of Culex nigripalpus captured between November 8 and November 12, 1960, and one pool of 154 Aedes angustivittatus collected between November 15 and November 19, 1960.

From these isolations, we can conclude that Ilheus encephalitis virus was active in the Almirante area from March 1960 to at least February 1961.

A number of neutralization tests with sera of birds, lower mammals and humans were conducted to determine the immunity response of the vertebrate population of Almirante to Ilheus virus. Twenty-three species of birds, belonging to 14 different families, were tested and 19 gave a clearly positive reaction, showing that infections with Ilheus virus are widely distributed in the avian fauna of Almirante. The rate of infection was 14.8%, with 35 positives out of 236 sera tested. The rates of infection in the various species of

birds found positive follows: Great Tinamou (Tinamus major) 1/3; Little Blue Heron (Florida caerulea) 1/6; Snowy Egret (Leucophoyx thula) 1/3; Laughing Falcon (Herpetotheres cachinans) 1/3; Middle American Jacana (Jacana spinosa) 1/1; Blue-ground Dove (Claravis pretiosa) 2/3; Short-billed Pigeon (Columba nigrirostris) 3/21; Mealy Parrot (Amazona farinosa) 1/8; Rufous Motmot (Baryphthengus martii) 1/3; Swainson's Toucan (Ramphastos swainsonii) 2/9; Collared Aracari (Pteroglossus torquatus) 1/6; Black-cheeked Woodpecker (Centurus pucherani) 6/17; Black-crowned Tityra (Erator inquisitor) 2/8; Purple-throated Fruit-crow (Querula purpurata) 2/3; Unidentified Flycatchers (Family Tyrannidae) 2/11; Yellow-tailed Oriole (Icterus mesomelas) 2/11; Scarlet-rumped Tanager (Ramphocelus passerinii) 3/7; Blue-gray Tanager (Thraupis virens) 2/2; Saltator sp. 1/8.

A total of 255 lower mammals including two species of marsupials, 5 species of bats, one species of carnivore, one species of squirrel, and six species of rats and mice, were tested. Only three specimens (1.2%) gave a positive reaction. Two of these were Alfaro's Water Rat (Nectomys alfari) and one was a Cotton Rat (Sigmodon hispidus).

Neutralization tests were also conducted on 643 human bloods collected in December 1960 from residents of Almirante. Ninety-six specimens (14.9%) neutralized 100-200 LD/50. Twenty of these positive sera retested against 1000 and 2000 LD/50 of Ilheus virus were found to neutralize this concentration of virus. The percentage of positive males (18.2%) was significantly higher than that of females (9.8%). Males also became positive at an earlier age, the youngest being 5 years old whereas the youngest female found positive was 20. In the age group 0-15 years, there were 9 positives among 127 males tested, while all 71 females tested were negative. These results seem to indicate an infection contracted in the field rather than a domestic one. This is corroborated by the mosquito isolations which were obtained from "field" rather than from "domestic" or "peridomestic" species.

Present findings re-emphasize the importance of birds and of Psorophora and Aedes mosquitoes in the ecology of Ilheus virus and brings out as a likely vector the bird-feeding species Culex nigripalpus.

References

1. Rodaniche, Enid de, and Galindo, P., 1961. Isolation of the virus of Ilheus encephalitis from mosquitoes captured in Panama. Am. J. Trop. Med. &Hyg. 10:393-4.

2. Galindo, P., and Rodaniche, Enid de, 1961. Birds as hosts of Ilheus encephalitis virus in Panama. Am. J. Trop. Med. &Hyg. 10:395-6.

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

Field activities in Trinidad were restricted to Bush Bush Forest in the Nariva swamp in 1962.

We succeeded in isolating more viruses in the 1962 dry season than in previous years. All of these dry season isolates were from mosquitoes; all well known agents (VEE, Caraparu, Guama group agents) were represented among them.

The sentinel mouse program continued with exposure of the mice in mosquito traps. Valuable information was collected by this method with regard to transmission capacity of different mosquito species under field conditions.

A yet unnamed Culex (Culex sp. #9) appears to hold a key position in the virus cycles of VEE, Guama and Bimiti viruses and is possibly also important for the maintenance of Caraparu and Catu viruses. Detailed studies on this mosquito are in progress.

Laboratory studies using wild rodent species from the laboratory colony infected with Caraparu virus and with the VSV-like agent TRVL 40233 (proposed name Cocal) were completed and results prepared for publication.

Longevity studies on field rodents were started in 1962 and are still in progress. Mosquito age-dating studies were also initiated during 1962.

Four virus strains of the same agent (prototype TRVL 42336) were isolated from Ornithodoros capensis ticks collected on Soldado Rock off the southwest point of Trinidad. These agents appear in CF tests to be identical with or related to an agent isolated by personnel of the Rocky Mountain Laboratory from the same species of ticks collected on Dry Tortugas Island in the Florida Keys.

Virus isolations in 1962 from Bush Bush material are presented by source in Table I. At least one of the yet unidentified isolates seems to be new in Trinidad and possibly to science.

Virus isolations of agents slow in killing baby mice were increased considerably by passing inocula from which fast killing agents had been isolated through immune serum to the fast agents. Eleven viruses which had been masked by fast agents came to light in this way.

Field expeditions were made to British Guiana (October) and to Jamaica (December) for the investigation of outbreaks of equine encephalitis. In British Guiana TRVL staff worked with staff of the Ministries of Agriculture and Health and in Jamaica with staff of the Ministries of Agriculture and Health and the Department of Microbiology, University of the West Indies. During the B.G. outbreak, EEE virus was isolated at this laboratory from two horses only. A total of 5,020 B.G. mosquitoes (mainly Aedes taeniorhynchus) and 255 Culicoides were tested for virus but found negative. Blood specimens from equines, wild birds and humans are presently being studied. The Jamaican outbreak presumably will be discussed by the Jamaican investigators. Suffice it to say here that there were numerous equine and a few human cases. A total of 15,750 mosquitoes as well as a few other haematophagus arthropods were collected for virus isolation attempts during a two week period in mid-December.

TABLE I

Identification and source of arbovirus isolations from Bush Bush material in 1962

Immunological group	Virus	#isolations	Source			
			Sent. mice*	Rodent	Mos-quitos	Marsupi-als
A	VEE	39	7	4	27	1
	UNA	1			1	
B	SLE	1			1	
	ILHEUS	7			7	
C	CARAPARU	20	2	1	17	
BUNYAMWERA	WYEOMYIA	1			1	
GUAMA	GUAMA	28	6	4	18	
	BIMITI	18	2	2	14	
	CATU	9		2	7	
UNGROUPED	TRVL 9223	2			2	
	TRVL 40233	2		1	1	
UNIDENTIFIED		6	1	3	2	
TOTAL		134	18	17	98	1

* One or more isolations of the same virus from a sentinel mouse family regarded as a single isolation.

REPORT FROM DR. LOUIS S. GRANT UNIVERSITY COLLEGE OF THE WEST INDIES KINGSTON, JAMAICA

During our investigation on equine encephalomyelitis in November of last year, several pools of mosquitoes were processed for possible virus isolation. A pool of eighty-two (82) Anopheles grabhami collected at Dalvey St. Thomas (Jamaica) on the 27th November was inoculated on the same day intracerebrally (0.02 ml per mouse) and intraperitoneally (0.03 ml per mouse) into one group of two-day old suckling mice. As a result, a virus was isolated. Subsequent passages showed that the virus was pathogenic for baby mice by either route. Our colleagues at the Trinidad Regional Virus Laboratory identified this agent as a strain of Cache Valley virus. This is the first evidence of this virus occurring in Jamaica.

During the past two months we have collected 381 bird sera in search of EEE, WEE, and VEE antibodies. Several pools of organs (brain, spleen, liver, kidney, and heart) from 202 birds were inoculated into mice. The results thus far have shown no isolate. Serological tests on the sera have not yet been done.

REPORT FROM DRS. CARLOS CAMPILLO-SAINZ AND JULIO DE MUCHA-MACIAS, INSTITUTO NACIONAL DE VIROLOGIA, MEXICO 7, D.F.

Accidental Exposure to EEV in the Laboratory:

In August 1962, when an ampoule of lyophilized VEE virus (strain BEAN 8007) was opened, two accidental infections occurred at the Arbor Virus Section of the I.N.V.S.S.A.

One person who handled the virus (case no. 1) had previously received vaccination with the Berge attenuated strain of VEE virus at CDC on the 22nd of August, 1961. Tissue culture attenuated VEE vaccine (TC-80 prepared in tissue culture from the Trinidad strain of VEE) was administered to him. The dose given was 0.5 ml subcutaneously, after which he presented no outward clinical signs. Serological response to vaccination was titrated in a 2nd blood sample obtained on September 19, 1961. The first sample showed no HI antibodies to VEE, whereas the post-vaccinal serum had a titer of 1:40 of HI antibodies and a neutralization index of 2.84 log.

The other person (case no. 2) had not been previously vaccinated. She presented a short incubation period (13 hs). Clinical picture was: headache, sore throat, photophobia, and malaise. Fever appeared suddenly, rapidly reaching a level of 40°C. The febrile period lasted 3 days, and general symptoms 4. During convalescence, this person was noticeably weak. Clinical picture of case No. 1 was essentially the same as that of case No. 2, with the exception of having a longer convalescent period (72 hs), which could be attributed to resistance conferred through vaccination.

In both cases, two blood samples were taken: in the acute phase, as well as five weeks later. There were no isolation attempts.

Serological test results were:

	First Sample		Second Sample	
	HI*	NI**	HI	NI
Case No. 1	1:10	0.7	1:5120	2.8
Case No. 2	<1:10	ND***	1:640	3.5

*Inhibition of hemagglutination
**Neutralization index (in log values)
***Not done.

Note: In both serological tests (antigen and virus), strain BEAN 8007 was employed.

According to results obtained, the etiology of the infections that appeared in the laboratory was definitely established. It should be stressed that case no. 1, notwithstanding its previous vaccination with a positive serological response, developed an illness very much like that of case no. 2.

Encephalitis Outbreak in Campeche, Mexico:

In July 1962, an outbreak of encephalitis occurred in the state of Campeche, Mexico. Twelve cases appeared in Campeche City and another in Champoton, Camp., 60 kms. south of the former. Every case recorded was in human beings.

Two cases occurred in the 7 months to 5 years age-group, 5 in the 11-16 group, 3 in the 22-32 group, another in a person 47 years old and a last case in one 55 years of age. The clinical stories of these patients point to an attack of varying intensity to the nervous system, and agree with signs and symptoms produced by viral encephalitis. There were 5 deaths (47, 29, 14, 11 years, and 7 months). Three cases show at present neurological sequelae, and five have recovered. The only case that we were able to study was that of Champoton, Camp. This was a 16 year old boy, who at the time of exploration presented neurological sequelae which, together with the clinical background, allowed us to diagnose an attack of encephalitis.

Blood samples from 97 residents of Campeche were taken, among whom the originally reported ill could not be found. Mosquitoes collected were all Culex quinquefasciatus. Entomological collections in February 1962 in Campeche City disclosed the aforementioned as predominant species as well as: Aedes scapularis, Aedes taeniorhynchus, Aedes serratus, and Psorophora ferox.

The HI test was employed with blood samples collected, using EEE, WEE, SLE, VEE, and Ilheus antigens. Thirty per cent of sera studied had low titer HI antibodies to group A (EEE, VEE) arboviruses. The rest were negative to the above mentioned encephalitis virus.

Serum belonging to the case found in Champoton, showed a titer of 1:160 of IH antibodies to VEE and a neutralization index of 2.64 log. to the same virus.

Isolation attempts in suckling mice from collected mosquito samples were negative.

According to results obtained, at least one of the cases (Champoton) in this outbreak could be due to VEE. There were no infections in equines.

REPORT FROM DR. JOSE SOSA-MARTINEZ, CHIEF INFECTIOUS DISEASE RESEARCH LABORATORY HOSPITAL INFANTIL DE MEXICO, MEXICO 7, D.F.

This laboratory is conducting investigations on viral infections of the central nervous system in children. However, as a fundamental part of these investigations, studies are being done at the same time on the distribution of arbovirus infections as well as of other encephalitic viruses inasmuch as this is a reference hospital to which children from different areas of the country are sent.

Though a survey of antibodies in blood samples of adult humans from various sources has been made, the most significant and consistent results have been obtained from sera sent to us from the Yucatan Peninsula. The antibody assay was made by means of the neutralization test in mice.

Positive reactions for the St. Louis virus have been numerous and without considering it necessary for the moment to give percentage figures, we can say that among the residents of the Yucatan Peninsula there is a wide dispersion of St. Louis virus infection. Although not as extensive as in the area mentioned, the same type of infection has been found in most of the states bordering the Gulf of Mexico.

All the sera with neutralizing antibodies for the St. Louis virus have so far given negative results for the WEE and EEE viruses.

These same tests together with many others are being made on patients with neurologic manifestations admitted to various hospitals of the country.

REPORT FROM DR. JAMES O. BOND, DIRECTOR TAMPA BAY REGIONAL ENCEPHALITIS LABORATORY TAMPA, FLORIDA

September 10 - December 15, 1962:

The Tampa Bay Regional Encephalitis Laboratory was established on a temporary basis on September 10 by the Florida State Board of Health and the Communicable Disease Center, U.S. Public Health Service. Space for the laboratory was donated by the Southwest Florida Tuberculosis Hospital. On December 15, the staff of the CDC terminated their temporary duty and the following report is a summary of the activities jointly carried on up to this date. The combined number of field and laboratory personnel working on the project at some time was 36, of whom 15 were from CDC and 21 from the Florida State Board of Health.

Field collections of human diagnostic specimens were carried out by physicians, health department staffs, and hospital personnel in the four county area. The laboratory received 1183 specimens of sera representing 566 individuals. Of these, 158 were confirmed as recent cases of SLE infection, and 72 showed evidence of past infection at an undetermined time. In 168 individuals, paired sera were negative for SLE. The remaining sera from suspects are either in process or awaiting

the collection of additional specimens. The distribution of the confirmed and presumptive cases in the four county area is given in the following table:

Human Cases of SLE - 1962

	Pinellas	Hillsborough	Manatee	Sarasota
Confirmed	113	16	14	15
Presumptive	63	5	4	0
Total	176	21	18	15
Rate/100,000	47.0	5.3	26.0	19.5
Deaths*	13	2	1	1

*Deaths with serological or virological evidence of past infections with SLE virus included in the totals.

In all, 73 post mortem tissue specimens were received from 12 fatal cases. From 3 patients a virus was isolated and identified as SLE. These were a 79WF from Pinellas County who died on 8/16, a 68CF from Hillsborough County who died on 9/13, and a 73WF from Manatee County dying on 9/16. Spinal fluid specimens from 148 individuals were examined for viruses, and no isolations obtained.

Blood specimens were received on 229 household associates of suspected cases in the four county area. Of these, 220 were tested and 7 found positive at a dilution level of 1:40 for HI antibodies, and an additional 11 at the lower titer of 1:20. The distribution of these results by county are as follows:

		Positive at 1:40 HI		Positive at 1:20 HI		Total Positive	
	No. tested	No.	%	No.	%	No.	%
Pinellas (St. Pet.)	114 ×	5	4.4	8	7.0	13	11.4
Sarasota	33	1	3.0	1	3.0	2	6.0
Manatee	33	1	3.0	2	6.0	3	9.1
Hillsborough (Tampa)	40 ×	0	0	0	0	0	0
Total	220	7	3.2	11	5.0	18	8.2

In Manatee and Sarasota Counties, a serological survey of the entire population was carried out in October with the assistance of the Florida State Board of Health, county health departments, and county medical

societies. Using the CDC quota sample technique, 549 households, including 1457 individuals, were selected and visited, and 1174 sera were obtained. Of these, 1008 were suitable for testing; 18 were positive at the 1:40 level and an additional 65 at the 1:20 level. Thus a total of 8.2 per cent of the general population have evidence of past infection with the SLE virus. The actual proportions of these recently infected will not be known until presently planned complement fixation tests are completed.

Extensive collections of avian and mammalian specimens were carried out by biologists, veterinarians, and ornithologists from the Florida State Board of Health, CDC, and the University of South Florida. Of these 1450 specimens were submitted to the laboratory either for serology or virus isolation attempts. A summary of the serological results shows 17 species of birds and 5 of mammals have been infected with the SLE virus in the Tampa Bay area. Many other species were not adequately collected to determine the presence or absence of past infection.

A survey of the serological tests positive at a 1:10 level (HI) is given below :

Species	Number of Birds or Mammals Tested	Positive
Birds:		
Chickens	374	95
Ducks (3 species)	151	53
Doves	111	31
Pigeons	11	3
Red Wing Blackbird	118	5
Rookery Birds	19	10
Laughing Gull	6	2
Little Blue Heron	2	2
Cattle Egret	5	5
Wood Ibis	1	1
Cardinal	33	3
House Sparrow	100	3
Blue Jay	8	2
(Goose, Peafowl, and Parakeet were positive in 1961)		
Mammals:		
Cotton rats	36	2
Squirrels	19	1
Opossums	3	1
Dogs	77	12
Cattle	80	2

No isolation of viruses was obtained from 600 bird bloods collected in the Tampa Bay area. These included samples from ducklings, doves, pigeons, and various small wild birds. One isolation of SLE virus was obtained from a pigeon collected in Apopka, Florida, and sent to the Tampa Laboratory for processing.

Two ornithologists were employed to secure bird counts and ecological data in both the St. Petersburg and the Tampa areas. Several significant differences were found including a 25 per cent denser wild bird population in St. Petersburg than in Tampa, 5 times more mourning doves and cardinals, and twice as many sparrows, flickers, and redbellied woodpeckers. Tampa, in contrast, had 5 times as many pigeons. St. Petersburg was also found to have large exotic bird populations, notably parakeets and domestic ducks. It has more dense, low growing vegetation than Tampa, and much less activity by dogs and housecats, and has a comparative abundance of open, grassy areas, parks and dense shrubbery, with numerous fresh water ponds and lakes. Perhaps the most important and significant activities of the laboratory and field team were directed toward the suspected mosquito vectors of SLE in the area. Entomologists from the Florida State Board of Health and CDC collected 62, 645 adult female mosquitoes in the four county area, using miniature battery-operated light traps and duckling-baited bird traps. The collections were as follows:

ADULT FEMALE MOSQUITOES

	No. processed By TABREL	No. processed By CDC	Total No.	Per Cent
C. nigripalpus	30, 067	16, 061	46, 128	73.6
A. crucians	2, 857	5, 753	8, 610	13.7
20 other species	4, 456	3, 451	7, 907	12.6
	37, 380	25, 265	62, 645	100.0

The TABREL inoculated 1167 pools of these mosquitoes into suckling mice, and the remainder were processed at the CDC. Identifications are based only on CF tests at present and final confirmation must await the results of neutralization tests. Preliminary results through December 15 are as follows:

PRESUMPTIVE VIRUS ISOLATIONS
(December 15, 1962)

Mosquito Species	SLE	Cache Valley-like
Culex nigripalpus	33	2
An. crucians	1	25
Cu. (melanoconium)	1	

The SLE virus isolates came from the four counties as follows: Pinellas 21, Hillsborough 8, Manatee 10, and Sarasota 1. The proportion of positive pools in each county is unknown at present, but collections were most intense in Pinellas.

The last positive collection of mosquitoes was reported on 10/18; various later collections are still in process.

This demonstration that C. nigripalpus is an important vector of SLE is the single most important accomplishment of the temporary laboratory. The significance of the finding that Cache Valley virus is readily obtainable from A. crucians is unknown, although it is presumed that this virus does not cause significant disease in man.

In addition, the laboratory confirmed the occurrence of the infection in 158 humans, and the virus was actually recovered from 3. Household associates of cases were shown to be at somewhat greater risk of infection as compared to the general population. The level of silent infection in the Manatee-Sarasota area was 1.8 per cent, compared to approximately 20 per 100,000 who developed clinical disease. No single species of bird or mammal was proven by virus recovery to be a reservoir of the infection. There were 17 different species of birds and 5 of mammals having demonstrable evidence of past infection. There was a strong epidemiological association between the presence of large numbers of doves and ducks, in the St. Petersburg area, and the large number of human cases.

The epidemic investigation begun by the temporary laboratory and field team will be continued as a permanent 5-year project supported by the National Institutes of Health, the U.S. Public Health Service, the Florida State Board of Health, and the four county health departments and the four county mosquito control districts. The S. W. Florida Tuberculosis Hospital will make available a laboratory building to serve this research project. The purpose of the project will be to continue the careful field

and laboratory studies into the complex life cycle of the SLE virus in this area of Florida, to identify those links which may be effectively destroyed or altered to prevent human infections and epidemics with their attendant serious losses to the health and economy of the area.

REPORT FROM DR. MAURICE PROVOST FLORIDA STATE BOARD OF HEALTH ENTOMOLOGICAL RESEARCH CENTER, VERO BEACH, FLORIDA

At the Entomological Research Center, Vero Beach, Florida, the Florida State Board of Health has been studying the winter biology of Culex nigripalpus and developing a new bait trap for mosquitoes. Except for a brief period following the severe cold of December 12-14, blood-seeking females have been caught in substantial numbers in chicken-baited traps all winter, yet routine examination of oviposition sites indicated a practical cessation of egg-laying activity at the end of November. A year-round study of biting and oviposition correlations has consequently begun, to elucidate this phenomenon and its implications. The primary objective of the new bait trap is to concentrate the mosquitoes in small cages which can be removed from the rest of the trap for quick servicing and deployment to the laboratory in cold boxes. It is planned to maintain a single hen in the center of the trap as bait.

REPORT FROM DRS. WILLIAM L. POND AND JOEL EHRENKRANZ DEPARTMENT OF MEDICINE, UNIVERSITY OF MIAMI SCHOOL OF MEDICINE, JACKSON MEMORIAL HOSPITAL, MIAMI, FLORIDA

In the fall of 1962 we investigated an encephalitis epidemic appearing in Sarasota, Florida, which was occurring as part of a larger epidemic involving the Tampa-St. Petersburg-Sarasota area. Suckling mouse inoculation of specimens from these patients (at the bedside) yielded one definitive isolation of St. Louis encephalitis virus from blood. We are following up these particular patients by obtaining serial serum specimens, with a view to determining the rate of antibody titer change (neutralizing, complement fixing, hemagglutination inhibiting) during convalescence and thereafter.

Investigations are also under way to determine the past experience of all major vertebrate populations in the Miami area with several of the arthropod-borne viruses suspected to be active in this area. These serological determinations will augment those results scheduled to be published soon in the American Journal of Medicine.

We have recently studied two patients with group B arbovirus encephalitis at the Jackson Memorial Hospital. One, a resident of Dade County, Florida, was admitted on November 1, 1962; the other, a resident of Okeechobee County, was admitted on December 11, 1962. There were several interesting features of their illnesses. In the first patient, headache was not present at the outset and occurred only as a minor symptom after her mental confusion cleared. SLE HI antibody titer of 1/5120 was found in this patient and sera from several of her birds (kept in an outside aviary) also revealed SLE HI antibodies. In the second patient, the finding of papilledema suggested brain abscess. The papilledema gradually subsided during the course of her illness. Her SLE HI titer was also 1/5120.

The cases illustrate the advantages of close association with an arbovirus laboratory that accrue to a medical service dealing with arbovirus infections. Diagnosis was correctly established within seven days of admission of both patients, permitting proper management as well as timely clinical and epidemiological investigation.

REPORT FROM THE ARBO-VIRUS UNIT DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF ARIZONA TUCSON, ARIZONA

Isolation and serological studies for the determination of the prevalence of arthropod-borne viruses in the Tucson area continued much along the same lines as indicated in our last report to the Information Exchange (No. 6, September, 1962).

In addition to our serological studies with SLE and WEE antigens, we have been testing sera from various animals by the HAI technique with EEE antigen. The results with EEE using human, bird, mammal, and horse sera have all been negative, although in the case of birds and horses, only a small number of sera samples have been tested (4 and 11 respectively). Some very interesting results have been obtained from chicken sera and are indicated in the following table.

Sentinel Chickens

Antigen Used	No. Sera Tested	No. + HAI*
SLE	183	2
WEE	183	3
EEE	71	18

*Titer of 20 or greater considered +, test run by microtiter technique.

Only one chicken positive for EEE was also positive for WEE. This strongly suggests the presence of an agent which, if not identical to, is at least related to EEE in this area. It is of interest to note that 15 of the 18 positive sera were obtained from the same group of chicks; 15 of 24 bled had antibody titers ranging from 20 to 320. These sera were obtained in September of 1962. Additional samples of sera have been obtained from these same chickens and are in the process of being tested. We plan to test the EEE positive sera, as determined by the HAI technique, by the neutralization method using both mice and tissue culture and thus determine if it is EEE we are dealing with or a serologically related virus.

We have also isolated a virus from a pool of engorged or gravid Culex quinquefasciatus mosquitoes which were caught in the same chicken house containing the EEE positive sentinel chickens described above. The mosquitoes were caught in October, 1962. The isolation was initially made in hamster kidney tissue culture and the agent was subsequently reisolated

from the original mosquito pool in suckling mice. All preparations tested have been bacteriologically sterile. The agent has been found to be pathogenic for both adult and suckling mice by the I. C. and I. P. routes and to be nonpathogenic for the less than 24-hour-old chick. Preliminary neutralization studies indicated that the agent is not WEE but may be SLE or related to SLE. One of the 24 chicken sera tested for HAI antibody in this area had an HAI titer of 80 against the SLE antigen. This serum was EEE and WEE negative.

To the best of our knowledge, this is the first reported isolation of an arthropod-borne virus in the state of Arizona. A preliminary study has been initiated to determine whether or not Colorado tick fever (CTF) virus is present in Arizona. Sera samples from 118 Papago Indians from southern Arizona have been tested by the CF technique for CTF antibody. All were found to be negative. Tests on sera from Indian tribes living in northern Arizona will be undertaken.

REPORT FROM DR. J. V. IRONS AND J. E. GRIMES LABORATORIES OF THE TEXAS STATE DEPARTMENT OF HEALTH AUSTIN, TEXAS

With the help of Arbovirus Laboratory of CDC, one of our 1961 Culex tarsalis isolates has been tentatively identified as Turlock virus by cross-complement-fixation tests. This isolate, EM 556 B, was reported on in the last Exchange. The virus possessed a hemagglutinin for goose erythrocytes and the HA antigen was used for the testing of serum from several sources. No antibodies were found in human or mammal sera. Only chicken and wild birds from the Lubbock area of Texas showed presence of HI antibody.

Another of our 1961 Culex tarsalis isolates, EM 569 B, was shown to be related to some "unknown" viruses isolated by the CDC Arbovirus Laboratory. We have twenty of these isolates which appear to be similar if not identical. Antisera and antigens are being made to do cross testing. Through the courtesy of Miss Elinor Whitney of the New York State Department of Health, it has been shown that two of the above "unknowns" (related to EM 569 B) were related to some virus isolations made by the New York State workers.

A second isolation of California virus has been made in Texas. This one, EM 570, was from unfed Psorophora confinnis mosquitoes collected in the Lubbock area in 1961. This virus did hemagglutinate goose erythrocytes with the first batch of antigen. Two subsequent batches

have failed to agglutinate goose cells but we hope to be able to get the virus to hemagglutinate again. Cross-neutralization tests with California virus (BFS 283) have shown very slight differences and cross-hemagglutination-inhibition tests showed our virus and the California virus to be practically identical. HI tests with EM 570 and California have shown the presence of HI antibody in both the acute and convalescent serum from an unconfirmed case of encephalitis in 1961. The titer was the same in both sera and in neutralization tests against EM 570 both sera protected against 2.2 logs of virus. It is supposed then that these results indicate some previous exposure to California virus. Thirteen other human sera were negative in HI tests. We hope to test convalescent sera from unconfirmed illnesses occurring in other years.

No virus isolations were made from 211 pools of mosquitoes tested during 1962. Four locations of the state were represented in the collections.

Sentinel chicken sera from the San Antonio and Orange County areas have been tested with negative results.

REPORT FROM DR. S.S. KALTER
SOUTHWEST RESEARCH CENTER, SAN ANTONIO 6, TEXAS

In the last issue of the Arthropod-Borne Virus Information Exchange, the results of CF and NT tests on approximately 40 baboon serums were given. Of these, sufficient serum remained on 21 specimens for the Arbovirus Laboratory of CDC to perform HI tests against the following viruses:

EE New Jersey	Bunyamwera RI-1
WE Fleming	Guaroa J-C2
Chikungunya Chic L	West Nile AR 248
Louping Ill DXL IV	Yellow Fever Asibi
Sindbis AR 1055	Jab B G8924
Semliki RI-1	Langat TP-21
Calif H&R	SLE Fla P-15
VE 38873	MVE IIA
Cache Valley-like A9171B	Marituba Be An 15

Dengue II TR 1751

All serums were acetone treated and tested against 4 and 8 units of the above sucrose acetone treated hemagglutination antigens. Only 1 animal was positive with a 1:10 titer to yellow fever virus. The 2 animals previously indicated as positive with EE antigen in the CF test were not found to be positive in this HI test. The reason for this is obscure at this time. Further studies on these and additional baboon serums are in progress.

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

In previous experiments (Information Exchange No. 5) following subcutaneous inoculation of small doses of JBE virus into various species of insectivorous bats, only occasionally was there evidence that infection became established in the CNS of these animals. Although virus was demonstrated in low titer in the brain tissue of a few bats, none showed overt signs of encephalitis and sections of infected brains exhibited no evidence of viral pathology. These results indicated that either the brain tissue of the bat is capable of supporting JBE virus multiplication without suffering injury, or that so few brain cells are infected following subcutaneous inoculation that their destruction by viral multiplication is not injurious enough to produce evidence of encephalitis in the bats nor to be detected by histopathological techniques. To learn more about the mechanisms of JBE virus multiplication in these animals, an effort was made to increase the virus concentration in the CNS by introducing virus directly into the brain. Bats received approximately 150 LD_{50} doses of JBE virus (OCT-541) in 0.03 ml into the left hemisphere of the brain. One-half the brain, one lobe of brown fat, and one kidney from each animal were assayed and the remaining portions of the respective tissues were fixed in formalin for histopathological studies. Since virus was introduced into the left hemisphere of each animal, right and left sides of the brain were taken alternately for virus assay and histologic study. In addition to evidence of marked viral proliferation in the brain, it was found that virus introduced intracerebrally could subsequently be demonstrated in the blood, brown fat, and kidneys.

The big brown bat (Eptesicus f. fuscus) is significantly more susceptible to i.c. inoculation of JBE virus than the little brown Myotis. Virus was widely distributed in the former species, titers in many instances reaching over 5.0 log units. In only one instance did virus reach a titer of 3 log units in the brain of an experimentally infected little brown bat. Both species were equally susceptible to infection with this virus strain following subcutaneous inoculation. Although the virus

inoculum was introduced into the left cerebral hemisphere in all instances, virus was subsequently demonstrated with equal frequency in the right and left hemispheres, indicating that virus spread and multiplied throughout the brain. None of the animals showed clinical evidence of disease, nor were histopathologic changes seen in section of brain or brown fat. These results indicate that JBE virus is capable of replicating in various tissues of the bat without noticeably damaging cells or producing overt signs of disease. Although virus titers in the brain and brown adipose tissue of these animals reached concentrations commensurate with levels demonstrable in the brains of mice dying of infection with this virus, the bats appeared to suffer no ill effects and evidence of viral pathology was not observed in sections of infected brain or brown fat. The innocuousness of this viral infection in these animals indicates a particularly well balanced host-virus relationship in which the cells of the bat are capable of functioning while producing significant quantities of infectious virus particles.

REPORT FROM DRS. EDWIN M. ELLIS AND EDWIN W. JENNEY NATIONAL ANIMAL DISEASE LABORATORY, AMES, IOWA

Vesicular Stomatitis Studies in Georgia:

An entomologist was assigned for the summer of 1962 to study the epidemiology of vesicular stomatitis (VS). Efforts were made to isolate viruses from arthropods trapped in Louisiana and Georgia during outbreaks of vesicular stomatitis.

Observations in Endemic Areas. The Louisiana investigation was made between June 22 and July 10 during unusually heavy populations of Tabanus spp., many mosquitoes and hornflies (Siphona irritans). Daily afternoon thundershowers left many temporary pools excellent for mosquito breeding. This vesicular stomatitis outbreak was very limited, and was in the VS endemic area 35-60 feet above sea level, located among rivers, swamp, and bayous with many trees.

Investigations were transferred to Georgia where the outbreak started in the Chattahooche River basin at an elevation of 650-1200 feet. Nearly every farm had at least one spring or small stream which in many cases were dammed to make ponds. Rainfall was abundant but the runoff was good and the streams contained many small fish which limited the breeding places for mosquitoes. Much of the area was timber tracts (pine) or small pastures. A multitude of species of trees grew along the streams. During the last week in June and first week in July the area experienced the densest population of horseflies in recent years.

Many tabanids were taken by blacklight in Louisiana but very few specimens were attracted in Georgia. It appeared that the heavy horsefly population in west central Georgia had dropped by mid July.

Conclusions. Dr. Brooks did not believe the transmitting agent for vesicular stomatitis to be a mosquito or a black fly because he was unable to find more than a few dozen mosquitoes during his five weeks in Georgia. He located only four black flies. Dr. Brooks interviewed only one person who had seen black flies in large numbers and this person reported no vesicular stomatitis in his animals.

Stable flies were not common in the Georgia piedmont. Their general distribution across the United States and their great abundance in such states as Iowa where VS does not occur is an argument against them as a vector. The hornfly fell in the same category as the stable fly.

Dr. Brooks reported, "In both Louisiana and Georgia the VS epidemic peak and the tabanid population peak roughly coincided from the information I could gather. One must consider at the same time that in Louisiana during the last week in June the tabanids were quite numerous and yet the disease stopped. However, if only one species was the vector, then this would not be an argument against the tabanid because adults of that one species could have died out earlier than the other species." He suggested an investigation of southern tabanids as possible mechanical carriers from animal reservoirs.

We were unable to isolate any virus from 13 pools of insects collected in Louisiana or three submitted by a veterinary diagnostician from Alabama. Insect collections made in Georgia were inadequate for testing. Suspensions of all samples were inoculated into swine kidney tissue cultures, suckling mice and embryonated eggs.

The quantity of samples submitted was too small to be significant and the observations rather limited and of short duration. Additional investigations are planned for the summer of 1963.

Note: Four men developed clinical cases of New Jersey type VS in Alabama during November following treatment of infected cattle on a farm. Three of the cases were confirmed by complement fixation, the fourth by serum neutralization.

Summary of Inoculations for the Detection of Arthropod-Borne Viruses

Group No.	Received 7-2-62	No. in pool	Diluent ml.	Farm	Address	Bovine serology from host farm	Embryonated egg*	Suckling mice**	Tissue*** culture(SK)
1.	Tabanus cymatphorus	6	6.	McMorris	Denham Springs, La.	Pos. serum SN	Negative	Negative	Negative
2.	Chlorotabanus crepuscularis	2	1.	Gomez	Corbin, La.	Pos. tissue CF	Negative	Negative	Negative
3.	Haematobia irritans (Linn)	300	12.	Gomez	Corbin, La.	Pos. tissue CF	Negative	Negative	Negative
4.	Tabanus (species unidentified)	4	4.	McMorris	Denham Springs, La.	Pos. serum SN	Negative	Negative	Negative
5.	Haematobia irritans (Linn)	77	3.08	McMorris	Denham Springs, La.	Pos. serum SN	Negative	Negative	Negative
6.	Chrysops vittala Wied	2	1.	McMorris	Denham Springs, La.	Pos. serum SN	Negative	Negative	---****
7.	Tabanus lineola Fab.	6	3.	McMorris	Denham Springs, La.	Pos. serum SN	Negative	Negative	Negative
Group No.	Received 7-5-62								
1.	Psychodidae	100	2.	Easterley	Denham Springs, La.	Pos. tissue CF	Negative	Negative	Negative
2.	Culex nigripalpus	20	1.	Easterley?	La.		Negative	Negative	Negative
3.	Anopheles barberi	24	1.	Easterley?	La.		Negative	Negative	Negative
Group No.	Received 7-7-62								
1.	Culex salinarius	21	1.	Gomez	Corbin, La.	Pos. tissue CF	Negative	Negative	Negative
2.	Culex restuans	24	1.	Gomez	Corbin, La.	Pos. tissue CF	Negative	Negative	Negative
3.	Culex interrogator	30	1.2	Gomez	Corbin, La.	Pos. tissue CF	Negative	Negative	Negative

* Inoculation of .1 ml. inoculum into chorioallantoic sac of 7-8 day hens eggs.
** Intracerebral inoculation of .01 ml. into 1-7 day suckling mice.
*** Inoculation and 3 blind passages in swine kidney tissue cultures (.1 ml. doses).
**** Insufficient material for inoculation.

REPORT FROM DR. R. P. HANSON
DEPARTMENTS OF VETERINARY SCIENCE AND ENTOMOLOGY
UNIVERSITY OF WISCONSIN, MADISON, WISCONSIN

A colorimetric neutralization test for arboviruses was standardized. The procedure is based upon color change of the pH indicator produced by metabolizing HeLa cells when the test virus is neutralized by specific antibody, or a failure of the indicator to change color when the unneutralized test virus destroys the cells. The test is carried out in serially cavitated polystyrene panels. The quantitative methods for 7 viruses, Eastern, Western, Venezuelan, and St. Louis encephalitis, New Jersey and Indiana vesicular stomatitis and encephalomyocarditis, have been worked out. One individual can perform approximately 1,000 neutralization tests a week.

The epizootiology of vesicular stomatitis in Panama was studied. Both serotypes of the virus are enzootic in the frost-free area. Clinical cases appear principally at the beginning of the dry season in December and January. Risk of infection varies greatly with the phyto-geographic regions. Half of the susceptible individuals (cattle, swine, and horses) become infected within a year of birth in the moist forest of the lower montane region. In the dry forest or savanna, only about 5 per cent of the susceptible individuals become infective during their first year. In relatively susceptible animal populations of this area, epizootics of New Jersey serotype occur in milking herds. The virus in these epizootics is believed to be transmitted on the hands of the milkers but the origin of the primary case is unknown. Clinical disease in cattle was associated only with the New Jersey serotype. Antibodies to the Indiana serotype but not to New Jersey virus were found in arboreal species (kinkajou, capuchin monkey and three-toed sloth) of the rain forest.

Virus of the New Jersey serotype that had been adapted to chick embryos was lyophilized, potency and safety tested, and used to vaccinate 375 heifers reared on the savanna in Panama. The ability of vaccinated animals to resist clinical infection is under study.

Immunodiffusion readily differentiated the blood from the gut of insects that have fed on unrelated groups of animals (snakes, turtles, and birds), but failed to differentiate related species (cattle, sheep, and deer). An adsorption procedure that would eliminate cross reaction was not found.

A total of 2,465 arthropods (of 38 species distributed among 4 orders - Culicidae, Ceratopogonidae, Simuliidae, and Tabanidae) were collected in

the Wisconsin River valley near Mazomanie during the summer of 1962 and identified, triturated, and inoculated into suckling mice. No virus was isolated.

Colonies of Haemaphysalis chordalis and H. leporis palustris were established and the biology of these ticks is being studied. Transmission experiments with selected encephalitides have been initiated.

One hundred and sixty-three small mammals (primarily Peromiscus, Microtis, Blarina, and Sorex) were trapped in the Wisconsin River valley during March 1962. Neutralizing substances for group B arbovirus were found in 17 of 87 animals. Two virus-like agents as yet unidentified were isolated from two of the animals. These agents induced signs of encephalitis and death in suckling mice but were sodium desoxycholate resistant.

Eastern encephalitis virus was isolated from 28 of 39 experimentally infected ground squirrels (Citellus tridecemlineatus) that died from the fifth to the forty-eighth day of hibernation but not from 4 animals that eventually awakened.

Serological surveillance of Wisconsin wild animals and livestock revealed reactors to California encephalitis, 39/520, and encephalomyocarditis, 42/500. The positive sera came from turkeys, cattle, horses, and swine. Some conservation department personnel and some veterinarians had antibodies to these viruses. Eleven reactors (2 horses, 4 swine, and 5 turkeys) were found to St. Louis encephalitis virus and eleven reactors (2 turkeys and 9 horses) to western encephalitis virus. There were no reactors in this group of sera to eastern encephalitis virus. Antibodies to arboviruses were also found in wildlife sera submitted from Minnesota, western encephalitis 11/118, California encephalitis 7/57, St. Louis encephalitis 2/105; from Nebraska, western encephalitis 3/5, California encephalitis 2/5; and from Ontario, eastern encephalitis 7/554, western encephalitis 4/473, St. Louis encephalitis 3/584.

The paper disc method of collecting blood from small wild animals was further evaluated. A good correlation was obtained among antibody titers of blood that were tested as serum, as fresh blood on discs and as dried blood on discs obtained under various simulated field conditions.

A difference in survivorship was found between vaccinated and unvaccinated pheasants released on the Mazomanie public hunting grounds 55 days before their harvest. Five hundred pheasants were released in 1962. Bags of 875 hunters were examined. Seventy-seven of 250

vaccinates and 52 of the 250 unvaccinated birds were recovered. In 1960 and 1961 no difference in survivorship was found.

A study of a snowshoe hare population in north central Alberta was initiated in May 1962 in cooperation with the Department of Wildlife Management of the University of Wisconsin. One virus has been isolated in suckling mice from a suspension of a clot from a snowshoe hare. Identification of this isolate, which resembles Silverwater virus, is currently in progress.

Serological screening for neutralizing antibodies to California Encephalitis Virus, Western Encephalitis Virus, St. Louis Encephalitis Virus, and encephalomyocarditis virus by means of the metabolic inhibition test is nearly complete. All of these or closely related viruses appeared to be active on the study area. California encephalitis was by far the most active. Antibodies to this virus occurred in over 90% of 160 adult hares tested during the June-September period. Some circumstantial evidence implicates California encephalitis in the May die-off of about 40% of the adult hare population. Screening for Powassan virus and Silverwater virus antibodies remains to be done.

Human serology was also carried out on a small scale. Of 66 human sera tested, from Alberta and Northwest and Yukon Territories, 13 carried neutralizing antibodies for CEV.

Participating in these studies were Merle Kuns, now at MARU, Vernon Lee, now in Columbia with Rockefeller Foundation, Thomas Yuill, Lloyd Lauerman, Paul Knipping, Daniel Trainer, Iraj Javidpour, and David Nassif.

REPORT FROM ENCEPHALITIS SECTION, USPHS COMMUNICABLE DISEASE CENTER, GREELEY, COLORADO

Report from Greeley, Colorado, Dr. A.D. Hess, Dr. L.C. LaMotte, and Dr. G.J. Love:

Poor results were obtained from larviciding programs as a means of controlling encephalitis virus transmission in Texas in 1962 and in Washington in 1961. In 1962 a premises spraying program was initiated at selected rural premises in the Columbia River Basin in Washington. DDT (5% solution) was applied to the exterior surfaces of all buildings and to surrounding vegetation in the treated areas prior to the mosquito breeding season, and again at the height of the season when the original

application appeared to be breaking down. Effectiveness of the treatment was measured by light trap and shed trap collections of mosquitoes on the treated premises and on untreated premises. WE virus activity in all areas will be determined by HAI and neutralizing antibody tests on sera from flocks of sentinel chickens. Based on collection data from 1962 and preceding years, C. tarsalis were reduced by more than 40 per cent of what might have been expected in light trap collections at treated premises and by 75 per cent in shed trap collections. Laboratory results are not available at this time.

For the third consecutive year, the first post-hibernation female C. tarsalis was collected on almost the same date. The first female C. tarsalis was found on February 11, 1961, and on February 12 in 1962 and 1963. This occurrence might be due entirely to chance, but wide variations in the severity of the past three winters suggest that some factor other than temperature is involved in the hibernation phenomenon. The winter of 1960-61 was very mild and the winter of 1961-62 was severe. The winter of 1962-63 was mild through December but January was cold.

An interesting serologic phenomenon was recognized in the Greeley laboratory with the sera of sentinel chickens exposed to natural infection during the summer and fall of 1961. As shown in Table 1, there was reasonable agreement between the several strains of western virus used as HAI antigen with the chickens exposed in Texas, Washington State, North Dakota, and Oregon. Neutralization tests performed in mice and/or wet chicks generally confirmed the HAI results, as had been the case in past years. However, the chickens held in Colorado and Utah developed an antibody-like inhibitor to the Rockefeller strain of WE (Olitsky), but usually not to Dr. Casals' WE (McMillan) strain or the WE-117 strain isolated in Colorado in 1960. The titers of this Olitsky inhibitor usually were high (1:160 to 1:1280) when tested simultaneously against 4-8 units of each antigen. The sera had been acetone-extracted to remove non-specific lipid inhibitors, and inactivation for one hour at 56° C had no effect on the inhibitor. However, we were unable to obtain unequivocal neutralization confirmation of any specimen which was HAI positive only to Olitsky. Interval bleedings on flocks in both Texas and Colorado indicated a similar gradual accrual of HAI antibody during July, August, and September. This is the time when we would expect virus activity, based upon past experience with virus isolations.

A review of the 1961 virus isolations from several of these areas indicated that Texas had a mosquito infection rate of 10 per 1,000 (98 positive pools containing 9,797 mosquitoes). Although all of the isolates are not yet identified, the majority appear to be WE. The identified WE isolates are uniformly pathogenic for two-week mice, duck embryo TC,

Table 1

INHIBITION OF SELECTED HAI ANTIGENS AND NEUTRALIZATION BY SENTINEL CHICKEN SERA FROM SIX WESTERN STATES, 1961

Area	HAI					NEUTRALIZATION		
	Number Tested	Percent Positive				Number Tested	Percent Positive	
		WE (Olit)	WE (McM)	WE (117)	SLE (Par.)		WE	EE
Greeley, Colorado	170	75	4		4	49	10	0
Fargo, North Dakota	79	61	31	48	0			
Malheur Valley, Oregon	59	54	44	44	22			
Umatilla County, Oregon	70	11	14	13	14			
Abernathy, Texas	42	90	90	90	24			
Plainview, Texas	18	83	83	83	17	18	88	0
Quincy A, Washington	97	66		60	2			
Quincy B, Washington	78	85		85	0			
Quincy C, Washington	81	63	41	48	4	51	55	0
Quincy D, Washington	110	61	54	55	0			
Bothwell, Utah	39	49	0	0	0			

chick embryo TC, and hamster kidney TC. This is the third consecutive year where Texas has had high mosquito infection rates and correspondingly high chicken antibody rates. In contrast, there were no isolations of western virus from 8, 264 mosquitoes collected in 1961 in Colorado, and tested in mice. Thus we have concluded that Colorado either had little or no western virus in 1961 or the strain of WE which may have caused the Olitsky HAI inhibitor in chickens was avirulent for mice. In previous years, WE isolations have been made regularly in the same Colorado area using two-week mice as the host system; mosquitoes have had WE infection rates varying from 0. 3 per 1, 000 in 1956 to over 6 per 1, 000 in 1955 and 1957.

Recent study of a number of WE strains isolated in Colorado as well as in other areas of the country suggests that at least several WE strains are active in Colorado. The 1961 Texas isolates appear to be similar; they have a uniform titer and pathogenicity for a variety of host systems. However, even in Texas, there is some evidence of different WE strains. We have recently isolated at least two WE types from a single pool of Texas mosquitoes having different plaque characteristics on duck embryo TC.

The most plausible explanations for the observed serologic response of 1961 chickens in Colorado and Utah are, (1) another agent, antigenically related to WE was active in the areas during 1961, (2) a mouse-avirulent strain of western was quite active in the chickens, but was antigenically inadequate and did not cause neutralizing antibody or HAI antibody to other laboratory strains of WE, (3) an age-related physiologic change in chickens (such as hyperlipemia) occurred in Colorado and Utah but not in the other areas at about the time when arboviruses are usually active, and a non-specific HAI inhibitor appeared which reacted against only one strain (Olitsky) of WE virus.

REPORT FROM DR. WILLIAM C. REEVES, PROFESSOR OF EPIDEMIOLOGY, UNIVERSITY OF CALIFORNIA SCHOOL OF PUBLIC HEALTH, BERKELEY, CALIFORNIA, ON COOPERATIVE RESEARCH PROJECT WITH ENCEPHALITIS SECTION, C. D. C. AND CALIFORNIA STATE DEPARTMENT OF HEALTH

WEE and SLE viruses had low levels of activity in Kern County, California, in the summer of 1962. Serologic surveys of farmers' flocks of chickens less than one year old revealed an annual prevalence of 3 per cent of WEE and 2 per cent of SLE antibodies, and for 9 wild bird species of all ages, 8 per cent with WEE and 4 per cent with SLE antibodies. In Culex tarsalis attracted to chickens in bait traps in rural areas, the total

summer infection rates were 0.8/1,000 for WEE virus and 1.0/1,000 for SLE. The transmission rate for the same vector population was 0.3/1,000 for both viruses. The first WEE virus isolation was on July 12 and the first SLE virus isolation on July 31. There were no confirmed human cases and only one suspected presumptive WEE case in the Kern County area; and one suspected but unconfirmed horse case was reported. The above pattern is that of a typical year of low endemicity for both viruses.

Investigations have continued on the wintertime persistence of WEE and SLE viruses in the Kern County area. No additional WEE or SLE virus isolations have been made from vectors or vertebrate hosts collected in the midwinter periods since 1961. The last isolation was one of SLE virus from C. tarsalis collected in February 1961, and the two WEE virus isolations from Citellus nelsoni and Mus musculus in February and March of the same year. A total of 4 isolations of what appears to be a new arbovirus have now been made, 3 from Sylvilagus audubonii and 1 from Lepus californicus. There is a slight indication, one way partial HAI cross, that this agent may be in the California antigenic group.

HAI tests have been completed on over 1,500 sera from rodents, rabbits, and hares. WEE antibodies are extremely rare in these hosts, but 5 to 15 per cent of sera from various species inhibit reactions with SLE, Powassan, or Modoc antigens, indicating that some group B agent is active in the population of small mammals. This still must be clarified by virus isolations and neutralization tests.

A third year of evaluation was completed on the effect of intensive C. tarsalis control in a 50 square mile area on virus transmission levels. WEE and SLE viruses remained active in the controlled and in two comparison areas, indicating a failure to reduce the C. tarsalis population to a critical threshold level where transmission would cease.

Precipitin test studies have now been extended to over 12,000 specimens of blood-engorged C. tarsalis. There is a consistent pattern of preference for passerine and columbiform birds, with the common domestic mammals and birds, wild rabbits and hares being fed on at relatively high rates in those environments where they are abundant. No feedings have been detected on rodents, humans, or cold-blooded vertebrates. Tests on 50 Anopheles franciscanus have revealed a most surprising pattern in that 66 per cent of feedings were on rabbits and hares. This is the first indication of this type of host predilection for an Anopheles in the Northern Hemisphere and of interest with regards to myxomatosis and Cache Valley viruses.

There is an urgent need for pools of serum from any species of wild birds or cold-blooded vertebrates that can be obtained to allow the further development and evaluation of the precipitin test procedure for mosquito blood-meal identifications. Please write for details of methods of handling and shipping, if you can assist.

A pilot study of the survival of adult mosquitoes, marked with fluorescent dusts, through the winter period has yielded interesting results. From October 16 through November 2, 1962, a series of 7,000 female C. tarsalis reared from pupae, were marked and released in the field; and between December 20, 1962, and January 23, 1963, 4 marked females were recovered. Considering the small numbers released and the degree of effort to make recoveries, this is an unexpectedly high number of recoveries after a two months' period.

REPORT FROM CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH DR. EDWIN H. LENNETTE, CHIEF, VIRAL AND RICKETTSIAL DISEASE LABORATORY, AND DR. HARALD N. JOHNSON, DIRECTOR, ROCKEFELLER FOUNDATION ARBOVIRUS STUDY UNIT BERKELEY, CALIFORNIA

There were 9 confirmed and 6 presumptive cases of SL encephalitis in California in 1962. There was one fatality, a rancher from Inyo County, east of the Sierra Nevada Mountains. This was the only case with onset in July. There were 5 cases in August and 9 in September. The latest onset date was September 29th. All but 2 of the cases were from the northern half of the Central Valley. Blood specimens from cases of horse encephalitis are tested against both WE and SL antigens in the CF test. Two of the horses which had WE encephalitis also showed a diagnostic rise in CF antibody for SL virus, indicating a concurrent infection with both viruses. Three other horses had CF antibody for SL virus.

There were 5 cases of encephalitis where a presumptive diagnosis could be made of WE encephalitis. The onset date of one case was on July 21st; the other cases occurred in September. There were 17 confirmed and 19 presumptive cases of WE encephalitis in horses. Most of the cases occurred in the northern half of the Central Valley. On September 13th, a sick ground squirrel, Citellus beecheyi, was killed in Butte County and the brain specimen was sent to the Viral and Rickettsial Disease Laboratory for examination for rabies because a dog was exposed to the sick animal. WE virus was isolated from the brain of this animal. This is the third isolation of WE virus from ground squirrels found sick in nature in California. Tree squirrels, Sciurus griseus, also develop encephalitis

when exposed to WE virus in nature. There have been 11 isolations of WE virus from this tree squirrel in California, 1 in 1953, 2 in 1955, 4 in 1956, and 4 in 1958.

There were 11 confirmed cases of Colorado tick fever in California in 1962. The infected individuals were exposed in the Great Basin Plateau region of California or in Nevada.

In 1962, a special study was made of Microtus montanus, meadow mice, on a ranch near Klamath Falls, Oregon, where there was a moderately high population of these animals. Two different types of viruses were isolated from these animals. One of these viruses, which is pathogenic for both infant and adult mice, was isolated from brain and lung specimens of mice collected in January 1962. The other type of virus, which is pathogenic only for infant mice, was isolated from blood specimens of mice collected during June and July. No relationship has been found between these viruses and those previously isolated in Western United States. Five strains of rickettsia were isolated from blood specimens of Microtus collected in July and August. Colorado tick fever virus was isolated from Dermacentor andersoni ticks collected in Klamath County, Oregon, in June 1962, and from a goldenmantled ground squirrel, Citellus lateralis, and a yellow pine chipmunk, Eutamias amoenus, collected in September, 1962.

A strain of WE virus of low pathogenicity has been developed by cloning on chick embryo cells and selecting clones on the basis of pathogenicity for mice. The B628 clone 15 strain of WE virus has been selected for study as a live virus vaccine.

REPORT FROM THE DEPARTMENT OF VETERINARY SCIENCE UNIVERSITY OF SASKATCHEWAN, SASKATOON, AND THE ENTOMOLOGY RESEARCH INSTITUTE, RESEARCH BRANCH CANADA AGRICULTURE, OTTAWA, CANADA

(R. Connell, J. McLintock, J. Spalatin, A. Burton)

Studies of the ecology of WEE disease in the Province of Saskatchewan have continued in 1962-63 and the results are briefly summarized as follows:

Mosquito Investigations, 1962 - J. McLintock.

The accompanying table summarizes the collections of female mosquitoes from Southern Saskatchewan in 1962. In the table, the majority (90%) of the indeterminate aedines were rubbed specimens of either Aedes dorsalis or A. campestris that could not be distinguished with certainty. Over 3,000 of the A. vexans in column 7 were taken on one farm in the southeast corner of the Province from September 5 to 9; they formed part of a localized outbreak of Aedes that did not occur elsewhere in the southern part of the Province. If we ignore the A. vexans in column 7, it is apparent that Culiseta inornata, Culex tarsalis and Aedes dorsalis were the most abundant species in the southern part of the Province from June 15 to September 17. These dates include the period when outbreaks of WEE usually occur in the Prairie Provinces of Canada. Culex restuans was notable for its scarcity; this species was known previously in Saskatchewan only in the Regina area and the 1962 records have extended its known distribution.

At all the light trap sites, Culex tarsalis reached its population peak for the summer during the week that ended on August 13. For C. inornata and A. dorsalis, population peaks were reached in the period from July 16 to August 27, depending on the locality.

Compared with normal weekly temperatures and precipitation, the period during which the light traps were in operation (columns 1 to 6) was generally cool and wet. Hence, it appears that C. inornata, C. tarsalis, and A. dorsalis are capable of building up large populations when recorded temperatures are below the meteorological normals. This might be of some epidemiological significance with reference to C. inornata, a species known to be tolerant of low temperatures in the laboratory.

Out of the 20,481 female mosquitoes collected, 12,709 divided into 565 pools and representing 14 species, were preserved for examination for virus.

A more detailed account of the above has been prepared for publication.

Virus Isolation - R. Connell, J. Spalatin, A. Burton

Attempts at virus isolation have been completed on circa 500 mosquito pools from the above mentioned mosquito collections. From 6

mosquito pools, we were able to isolate virus which we identified as WEE. Three of these isolations were made from Culex tarsalis, and one each from Aedes dorsalis, A. flavescens, and Culiseta inornata. The isolated strains have been sent to Dr. Casals, Rockefeller Foundation Virus Laboratories, New York, for further study with reference to strain properties. These are the first isolations of WEE virus from mosquitoes collected in the Province of Saskatchewan.

Further attempts to isolate virus from bird and mammal specimens have not been successful to date.

Studies on natural and experimental exposed snakes (Thamnophis species) and frogs (Rana pipiens) to the WEE virus are in progress.

A serological survey involving chicken flocks, other than sentinel flocks, was begun in the fall and early winter of 1961 and a follow-up of this survey was carried out during the same period of 1962. This was considered to be an aid in determining the extent of WEE virus distribution, since 10% samplings were collected from flocks scattered throughout the Province.

Results of serum-neutralization tests done on blood samples collected in 1961 indicated that 4 birds out of 600 were positive and 37 were suspicious reactors. Only one-third of the blood samples taken in 1962 thus far have been examined and it would appear that there is a significant rise in the number of positive reactors. These findings correspond with virus activity in 1962 as indicated by the number of mosquito isolations and the number of reported cases in horses during this season.

Relative abundance of female mosquitoes in Southern Saskatchewan, 1962.

	(1)		(2)		(3)		(4)		(5)		(6)		(7)	
Location	Saskatoon		Outlook		Swift Current		Melfort		Craik		All Light Traps		Miscellaneous* catches	
Period Operated	June 26-Sept. 17		Aug. 1-Sept. 17		July 21-Sept. 17		July 14-Sept. 17		July 19-Sept. 16				June 15-Sept. 9	
Number of Trap Nights	75		48		58		65		61		307			
	Number	per cent	Number	per cent	Number	per cent	Number	per cent	Number	per cent	Number	per cent	Number	per cent
Culiseta inornata	2795	52.2	1337	37.2	410	27.1	744	57.2	69	20.5	5355	44.2	2512	30.0
Culex tarsalis	1321	24.7	891	24.8	382	25.3	41	3.2	101	30.0	2736	22.6	616	7.4
Aedes dorsalis	508	9.5	485	13.5	240	15.9	75	5.8	85	25.2	1393	11.5	684	8.2
Aedes campestris	152	2.9	106	3.0	25	1.7	58	4.5	22	6.5	363	3.0	207	2.5
Aedes vexans	44	<1	23	<1	41	2.7	6	<1	4	1.2	118	1.0	3352	40.0
Aedes nigromaculis	0		22		27	1.8	0		15	4.5	64	<1	288	3.4
Anopheles earlei	16		3		3	<1	19	1.5	0		41		35	<1
Aedes riparius	11		1		0		8	<1	0		20		153	1.8
Culiseta minnesotae	7		7		1		4		0		19		2	<1
Aedes fitchii	0		0		0		0		0		0		9	
Aedes flavescens	2		1		0		5		0		8		69	
Aedes excrucians	0		0		0		0		0		0		8	
Aedes punctor	1		1		0		2		3	<1	7		297	3.6
Aedes cinereus	0		0		0		0		1		1		7	
Aedes canadensis	0		0		0		0		0		0		4	
Aedes spencerii	2		0		1		0		0		3		139	1.7
Culex restuans	0		2		0		1		0		3		0	
Culiseta incidens	0		0		0		3		0		3		0	
Mansonia perturbans	0		0		0		0		0		0		2	
Indeterminate Aedes	490	9.1	715	19.8	383	25.3	261	20.0	37	10.9	1886	15.5		
Indeterminate Culiseta	2		2		0		73		0		77			
Totals	5351		3596		1513		1300		337		12097		8384	

*Miscellaneous catches= Catches by aspirator, traps baited with CO_2, and with light + CO_2.

REPORT FROM MR. ELMER T. FELTZ
CHIEF, VIROLOGY LABORATORY
ARCTIC HEALTH RESEARCH CENTER
ANCHORAGE, ALASKA

Dr. Jacob Brody has arrived at the Arctic Health Research Center. His new assignment has absorbed him into several existing projects as well as turning his energies to several new ones concerning respiratory and entero viruses. However, plans are now beginning to materialize for a study of the Arbovirus Story in Alaska. A small, isolated laboratory is being considered for preparation of appropriate antigens, for the subsequent serological screening of human and animal sera for the arboviruses. An initial approach will depend upon the demonstration of significant antibodies to the arbovirus group. If this preliminary study is encouraging, then our program will develop along the lines of virus isolation from possible reservoir animals, infected birds, mosquito vectors, and human illnesses.

REPORT FROM DR. SUSUMU HOTTA
DEPARTMENT OF MICROBIOLOGY, KOBE MEDICAL COLLEGE,
KOBE, JAPAN

Propagation of Japanese B encephalitis and related viruses in human and animal tissue cultures:

Human and animal tissues of various kinds were cultivated by the plasma-embedded roller tube method. Coverslip cultures were prepared for purpose of morphological examination. Viruses used were Gl strain JBE virus, of the 230th to 240th mouse brain passages, JATH strain JBE virus, of the 2nd or 3rd mouse brain passage, and Negishi strain virus, a member of the Russian Spring-Summer encephalitis group, of the 7th to 9th mouse brain passages. At intervals following inoculation of the viruses of a given concentration, portions of the culture fluids were taken out and measured for active virus contents. The viral growth patterns revealed could be divided into three categories: (1) "Convex" curves, indicating good multiplication of virus; (2) "Flattened" curves with few peaks, suggesting a low grade multiplication or a significantly prolonged persistence of virus; and (3) "Continuously declining" curves, indicating no growth of virus. Almost all the tissues thus far investigated were shown to belong to the category of either (1) or (2). Some of the examples were: Human embryonic brain (cerebrum or cerebellum), adult lung, kidney, thyroid, lymph node, intestine, tonsil, nasal mucosa; rabbit testicle, lung; hamster kidney; puppy cerebellum, lung, kidney, spleen, liver, heart, lymph node; kitten cerebellum, testicle, kidney, and other tissues. It was noted that

human adult spleen tissue cultures did not support the growth of JBE virus, at least under the experimental conditions adopted, although further experiments are required. The puppy nerve cells in virus-inoculated cultures exhibited specific degeneration which was recognized in morphological changes of the nuclei, dendrites, neurites, and neurofibrils (refer to Arbovinfoexchange No. 5, p. 49).

Cytological changes of virus-infected cells in tissue culture, with special reference to hamster kidney cell cultures infected with Japanese B encephalitis, dengue and yellow fever viruses (Hotta, Ohyama, and Takami):

Hamster kidney monolayer cell cultures on a coverslip were infected with JBE, G1 strain; type 1 dengue (DG), Mochizuki strain; and yellow fever (YF), 17D strain viruses. "One-step growth curve" experiments were performed, obtaining the following growth patterns for each virus:

	JBE	DG	YF
Latent phase	0-6*	0-24	0-12
Logarithmic phase	6-54	24-132	12-120
Stationary phase	54-72	132-240	120-192

*Time, in hour, after inoculation of virus.

Cytological alterations of the infected cells were observed by staining and phase-contrast microscopy. Major subjects of observation were the changes that occurred during the period from the end of the latent phase through the logarithmic phase. This period was further divided into four stages: Stage 1: Aggregates of basophilic granules were observed in the cytoplasm, particularly in areas surrounding the nucleus; Stage 2: Intensely basophilic granules were seen in the cytoplasm, and thickening of the nuclear membrane was noted; Stage 3: Vacuoles of various sizes were seen in the cytoplasm, and distortion of the nucleus was revealed; Stage 4: A number of cells had become detached from the surface of glass, and the remaining cells contained densely stained masses in the cytoplasm.

Phase-contrast microscopic pictures were compatible with those seen as above in the Giemsa-stained preparations. By Feulgen stain, Feulgen-positive masses of particular shape and distribution were revealed in the nuclei of the infected cells. The cytoplasmic vacuoles and nuclear Feulgen-positive masses appeared larger and more distinct in the cells infected with YF virus than those seen in the cells infected with JBE or DG virus.

REPORT FROM DR. K. FUKAI
DEPARTMENT OF PREVENTIVE MEDICINE, INSTITUTE FOR MICROBIAL DISEASES, OSAKA UNIVERSITY, OSAKA, JAPAN

An Infectious and Ribonuclease-sensitive Fraction from Mapanese B Encephalitis Virus Infected Mouse Brain (Fukai, Igarashi, and Kitano):

An infectious and ribonuclease-sensitive fraction (infectious RNA) was obtained from adult mouse brains infected with Nakayama strain of Japanese B Encephalitis Virus (JBEV).

The infected brains were homogenized with 9 volumes of 0.14M NaCl in 0.05M tris buffer, pH 8, and centrifuged at 8,500 x g for 15 minutes. RNA was extracted either from this supernatant or after partial purification by differential centrifugation (90,000 x g 90 minutes and 2,000 x g 15 minutes). Cold extraction was carried out according to Gierer and Schramm (1956). After 4 times extraction with 90 per cent phenol containing 0.001M EDTA and several extractions with peroxide-free ether, RNA was precipitated 3 times with 2 volumes of cold ethanol.

The final precipitate which has been dissolved with 0.14M NaCl in 0.02M phosphate buffer (PBS), pH 7.4, exhibits ultraviolet absorption spectrum characteristic for nucleic acids. The ratios of both E_{max}/E_{min} and E_{260}/E_{280} are about 2.0 (1.9 to 2.2).

The infectivity of RNA thus obtained was assayed on primary culture of chick embryo cell monolayer using 1M $MgSO_4$ as RNA diluent and 20 minute adsorption at room temperature (at 20 to 25°C), in contrast to the virus titration in which dilution with 0.75 per cent bovine plasma albumin (BPA) in PBS, pH 8, and 2 hours adsorption at 37°C are used.

Chick cells were prepared by the method of Porterfield (1960) with following modifications; rubber stoppered 2 ounce prescription bottles were used instead of petri dishes and humidified chamber; tris growth medium was replaced by YLH (0.1 per cent yeast extract and 0.5 per cent lactalbumin hydrolysate in Hanks' balanced salt solution) supplemented with 10 per cent calf serum. Tris-Gey overlay medium of Porterfield (1959) was slightly changed by omitting serum and supplementing with 0.4 per cent BPA and 0.6 per cent tryptose phosphate broth. The final concentration of lactalbumin hydrolysate was doubled and that of neutral red was 0.005 per cent.

Plaque-numbers produced are proportional to the relative RNA concentration inoculated in the range less than 25 plaques per bottle.

Infective agents which can be recovered from plaques formed by RNA manifest the same symptom as JBEV in mice, produce plaques on chick cell sheets, and are neutralized by anti-Nakayama guinea pig serum.

The infectivity of RNA assayed by this method is about 0.1 per cent of that of the starting material and is completely destroyed by ribonuclease as shown in Table 1, while JBEV does not lose its infectivity with 0.1 mg RNase per ml. at 37°C for 15 minutes incubation. RNA can be precipitated by 2 volumes of cold ethanol without loss of infectivity, but JBEV becomes largely noninfectious by this treatment. The presence of protamin sulfate (0.5 mg/ml.) arrests the RNA infectivity; however, JBEV suffers no appreciable effect even with 5 mg of protamin per ml.

Table 1

Infectivity of RNA and its Inactivation with Ribonuclease

Experimental	Infectivity (PFU/ml.) of Starting Material	RNA fraction before RNAse treatment	RNA fraction after RNAse treatment
#20 (crude supernatant)	1.7×10^7	1.0×10^4	0
#21 (partially purified)	1.3×10^7	1.5×10^4	0
#22 (partially purified)	6.4×10^6	2.2×10^4	not tested

Ribonuclease treatment was carried out with 0.01 mg of RNase per ml. at 0°C for 15 minutes.

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Heterogeneity of Japanese B Encephalitis Hemagglutinin (Fukai, Igarashi and Kitano):

The heterogeneity in hemagglutinating agent (HA) of Japanese B encephalitis virus (JBEV) was found by density gradient centrifugation technique.

The virus used was Nakayama strain of JBEV grown in adult mouse brains. Infected brains were homogenized with 9 volumes of 0.14 M NaCl in 0.05 M tris buffer pH 8 (TBS) and spun down at 8,500 g for 15 minutes. The supernatant was treated with protamin sulfate (0.5 mg/ml as the final concentration) at 4°C for an hour, and freed from precipitates by centrifugation (8,500 g, 15 minutes). Partially purified virus was prepared from this supernatant by two cycles of differential centrifugation (90,000 g, 90 minutes and 2,000 g, 15 minutes).

Sucrose density gradient centrifugation was performed in RPS-40 rotor of Hitachi 40 P centrifuge at 39,000 rpm for 4 hours, in a 20-49% sucrose gradient in TBS. After the run samples were divided into 10 fractions from the top of centrifuge tubes. Hemagglutinating activity and infectivity were estimated by Clarke and Casals' method (1958) and mouse LD_{50} titration, respectively.

CsCl equilibrium density gradient centrifugation was performed according to Meselson et al. (1957) in SW-39 rotor of Spinco E centrifuge at 35,700 rpm for 40 hours. Samples were collected by droplet fractionation from the bottom of the tubes. Density was determined refractometrically using the formula of Ifft et al. (1961).

Sucrose rate sedimentation of JBEV (both protamin treated supernatant and partially purified virus) resulted in the separation of HA into two components: rapid-sedimenting hemagglutinin (Hr) and slow-sedimenting hemagglutinin (Hs). Isolation and recentrifugation of two peaks showed that each formed a single peak at the position where it had originally been observed. Infectivity also separated into two components which had their peaks approximately in the same fractions of HA peaks. Deviation of Hs peak from the peak of slow-sedimenting infectivity might be explained by the existence of normal inhibitor for HA.

Two peaks of HA are also present in the equilibrium density gradient columns of CsCl. When Hr and Hs isolated by sucrose gradient sedimentation are rebanded in CsCl, each shows a single peak which lies in the position corresponding to the two HA peaks of original sample. The buoyant density of Hr is about 0.08 g/cm^3 larger than that of Hs (Table 1).

Hemagglutinating activity of Hr and Hs is inhibited by anti-Nakayama guinea pig serum to the same extent as the original material (Table 2). The optimum pH for hemagglutination of Hr exists slightly on the acidic side than that of Hs (Table 3).

Separation of Hr and Hs is successful also in virus materials of different strains of JBEV grown in MK2 cells (Dr. K. Fujinaga's personal communication), so it would be rather common phenomenon in JBEV. Difference in buoyant density and optimal pH for HA suggests some differences in chemical compositions of Hr and Hs, especially of their surface structures.

Table 1

Buoyant Density in CsCl Equilibrium Density Gradient Centrifugation

Initial density	Buoyant density (g/cm^3 at 4°C) of	
	Hr	Hs
1.24	1.241	1.317
1.25	1.243	1.325
1.26	1.247	1.318
average	1.244	1.320

Table 2

Inhibition of Hemagglutination

Antigen	HA antigen titer	HI titer against anti-Nakayama guinea pig serum
Hr	16	40
	8	80
	4	160
Hs	16	40
	8	80
	4	160
Original (protamin treated supernatant)	8	80
	4	160
	2	320

Table 3

pH Dependency of Hemagglutination

pH of the reaction media	HA titer (HAU/ml) of	
	Hr	Hs
5.93	32	8
6.12	64	32
6.26	128	64
6.50	32	64
6.65	8	8
6.85	2	2
7.05	0	0

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REPORT FROM DR. IRVING J. GREEN
U.S. NAVAL MEDICAL RESEARCH UNIT NO. 2, TAIPEI, TAIWAN

We have recently isolated 2 viruses from the sera of patients clinically appearing to have a hemorrhagic fever-like illness in Manila, Philippine Islands. These viruses were isolated in HeLa cells. Cytopathic effects were observed and in appearance seemed to resemble dengue virus in our HeLa cell cultures. However, qualitative tissue culture neutralization test suggests that these isolates belong to the Russian tick-borne complex, as they were not neutralized by most of our available group B arbovirus antisera, including anti-dengue types 1-5, nor by Chikungunya, but are neutralized by Louping Ill antiserum.

If these preliminary observations are confirmed, the presence of a hemorrhagic fever virus related to the Russian tick-borne complex may account for some of the mortality attributed to Philippine Hemorrhagic Dengue Fever.

REPORT FROM DR. DILOK YENBUTRA, FACULTY OF MEDICINE, CHULALONGKORN HOSPITAL, BANGKOK, THAILAND

Possible first isolation of JBE virus in Bangkok, Thailand (Dasaneyavaja, Robin, and Yenbutra):

During the study of Thai Hemorrhagic Fever vectors in Bangkok in 1962, 11 agents were isolated from 69 pools of wild caught mosquitoes. Most belong to group A.

One of the isolated agents (Q 180) was proved to be group B. It was isolated from a pool of 250 Culex pipiens quinquefasciatus, collected by light trap at 6 PM to 6 AM during August 1962 in suburban area of Bangkok.

This agent kills adult mice by I.P. route as well as I.C. route within 9 days and 5 days, respectively. In pig kidney stable cell line (P.S. cell) tissue culture, it showed definite cytopathogenic effect on the 7th day. It produced good complement fixing and hemagglutinating antigens on suckling mouse brain preparation using sucrose acetone extraction. The HA titer is 1:40,960 at the optimum pH 6.5 and temperature 37°C, on the 14th mouse passage.

Hemagglutination Inhibition Studies (Clarke & Casals, 1958)

Mouse antisera		ANTIGENS 4 UNITS Q 180	Deng II Tr 1751	Chikungunya	J.B.E. Nakayama
Q 180	1	40*	0	0	40
	2	640	160	0	640
Deng II	1	0	0		
	2	0	10		
Chik	1	0		1280	
	2	0		2560	
J.B.E.	1	80			80
	2	320			320

1 - serum 10 days after the first inoculation.
2 - serum 10 days after the second inoculation, i.e. 24 days after the first inoculation.
40* - highest dilution of serum that inhibits hemagglutination.
0 - no hemagglutination inhibition at the serum dilution of 1:10.

REPORT FROM DR. ALBERT RUDNICK
UNIVERSITY OF CALIFORNIA SCHOOL OF MEDICINE
AND
THE INSTITUTE FOR MEDICAL RESEARCH
KUALA LUMPUR, MALAYA

Classical dengue and probable dengue-related hemorrhagic fever cases have been observed in the Federation of Malaya. The "hemorrhagic fever" cases have all occurred in the city of Penang in the northwest coastal area and are believed to be new to that city, according to local clinicians. Four deaths have occurred in a possible eighteen cases reported since October, 1962. This is the first report of "hemorrhagic fever" in Malaya. Virological, serological, and entomological studies are being conducted.

Preliminary studies of the ecology of the dengue viruses have been initiated. Dengue HI antibodies have been demonstrated in wild jungle monkeys from several areas of Malaya. Some mice surviving inoculation with sera of these monkeys have resisted dengue challenge, suggesting the presence of dengue or dengue-related viruses in the monkey sera. Mosquitoes from canopy and ground-level sites and mammals are being collected in several selected areas of differing ecology and these are being processed in the laboratory.

Three cases in cattle of ephemeral fever, diagnosed clinically, have been observed in Malaya. Paired sera were negative by HI for group B antibodies. Virus isolations are being attempted from the acute phase sera.

REPORT FROM DR. LIM KOK ANN
DEPARTMENT OF BACTERIOLOGY, UNIVERSITY OF SINGAPORE, SINGAPORE 3

Sensitized Erythrocyte Agglutination (SEA) Test:

Serological diagnosis of Group B arbovirus infection in a region where more than one such virus is prevalent is often inconclusive because of the cross-reactions obtained. This has given rise to difficulties in the interpretation of diagnostic tests for either Japanese encephalitis virus or dengue virus type infection (e.g. Singapore Hemorrhagic Fever) in Singapore, by either complement fixation tests or hemagglutination inhibition tests.

Following the work of Hale and Pillai, 1960, it has been discovered that the sera of patients suffering from dengue type virus infection were able to agglutinate one day old chick erythrocytes that had been treated with hemagglutinin of dengue type virus. Agglutinating titres were very high, often exceeding 1/10240,and cross-reactions with erythrocytes treated with HA of JE virus was minimal, usually less than 1/40. This was so even when CF and HAI tests gave positive results to high titres with both JE and dengue type 1 antigens. In this presentation, agglutination refers to agglutination of sensitized erythrocytes by antibody, and hemagglutination to virus activity.

The tests were initially performed with one day old chick erythrocytes, but as the use of goose erythrocytes has become practically universal, the latter has been adopted more recently. Results obtained were similar, but for reasons not clear at the moment, less cross-reaction was obtained with chick erythrocytes.

The procedure generally employed methods used for the performance of hemagglutination inhibition tests as described by Casals. Goose or chick erythrocytes were collected in acid citrate dextrose and washed four times in dextrose glucose veronal (DGV) buffer. Virus hemagglutinatinin was prepared by sucrose acetone extraction of infected infant mouse brain. HA was diluted in pH 9 borate buffer and mixed with erythrocytes suspended in virus adjusting diluent (VAD) for the pH at which HA activity was best. For most viruses tested, this pH was 6.6.

Time was allowed for adsorption of virus. The sensitized erythrocytes were washed twice in DGV and stored cold in this solution. Serum dilutions were made in pH 9 borate buffer plus 0.4% bovine albumin. The stored sensitized erythrocytes were centrifuged and resuspended in VAD to a concentration of 0.5% at pH 7.6 and added to serum dilutions. Positive results were observable within a half to one hour, but were much clearer after storage overnight in the cold.

Some conclusions are summarized as follows:

1. The agglutinability of sensitized erythrocytes determined by serum titres is dependent on the concentrations of HA and erythrocytes in the sensitizing mixture.

2. When excessively high concentrations of HA are used for sensitization, the erythrocytes are auto-agglutinable even at pH 7.6. A sensitizing dose should be selected so that sensitized erythrocytes form a "button" at this test pH, yet giving maximum titres of HA with a reference immune serum.

3. The absorption of HA is rapid and logarithmic, 50% being absorbed within about 2-4 minutes. Practically all the HA that can be absorbed is absorbed within 15 minutes.

4. Non-specific inhibitor in serum causes "slippage" of the agglutinated erythrocytes. It does not interfere with agglutination by antibody which usually has a higher titre than inhibitor, but gives rise to a "prozone". This can be removed by treatment with kaolin.

5. Sensitized erythrocyte agglutination tests were satisfactorily performed with the following Group B viruses: Nakayama strain of Japanese encephalitis virus, two JBE isolates, dengue types 1, 2, 3, 4 viruses, and Langat.

6. Attempts to employ the method with Chikungunya virus were unsuccessful because we could not produce HA of sufficiently high titre. With yellow fever virus, it was discovered that although HA was rapidly absorbed, the erythrocytes were not agglutinable by immune serum, possibly because of "elution" of virus.

7. The sensitized erythrocyte agglutination test could be a useful diagnostic procedure for viruses in which it is applicable.

(a) Sensitized erythrocytes, prepared beforehand, can be stored for days.

(b) Non-specific inhibitors give false negatives at low dilutions of serum, an interpretation different from that in HAI tests in which non-specific inhibitors give false positives.

(c) Although kaolin treatment can remove non-specific inhibitors, it is not essential if significance is placed only on high agglutinating serum titres.

(d) In regard to JE and dengue infections, the tests had a much higher order of specificity allowing a diagnostic significance to be given to tests where CF and HAI are not conclusive.

8. Representative results of SEA tests on sera of patients suspected of JE or dengue type infections are presented in the following table.

Representative serological reactions of suspected cases of arbovirus infection, 1962.

Lab No.	Sex/ Age	Clinical Diagnosis	Day of Serum	Serological Reactions						Diagnosis[4] Confirmed?
				CF[1]		SEA[2]		HAI[3]		
				JEV	D-1	JEV	D-1	JEV	D-1	
437	M.6	enceph.	2*	0	0	0	0	0	0	
			16	0	64	0	640	20	640	No
438	M.6	H.F.	2*	0	0	0	320	0	80	
			26	0	256	160	10240	640	5120	Yes
469	M.2	enceph.	6	0	0	0	0	Not tested		
			24	0	0	40	40			No
457	M.50	enceph.	6	8	8	640	0	10	0	
			33	64	64	1280	20	80	10	Yes
460	F.13	enceph.	2	0	0	0	20	10	10	
			30	0	0	0	40	10	10	No
477	M.19	enceph.	4	8	8	0	0	0	0	
			22	32	32	0	0	0	0	No
479	M.18	H.F.	9	0	0	0	40	0	20	
			26	0	0	0	40	0	20	No
493	M.4	H.F.	4	256	256	0	10240	2560	5120	
			20	256	256	40	10240	5120	5120	Yes
494	M.9	H.F.	4	0	0	0	160	20	160	
			20	64	256	80	10240	2560	5120	Yes
498	M.5	H.F.	7	256	256	80	10240	5120	5120	
			21	256	256	40	10240	2560	5120	Yes

*Virus isolated from this serum specimen has not been identified

1) Serum dilutions from 1/8 to 1/256. Titers expressed as reciprocals.
2) Serum dilutions from 1/20 to 1/10240. 0 = less than lowest dilution.
3) Serum dilutions from 1/10 to 1/5120.
4) In all instances the laboratory confirmation or otherwise of the clinical diagnosis was shown to be consistent with the outcome of the illness.

REPORT FROM DR. H. A. E. VAN TONGEREN
DEPARTMENT OF MEDICAL MICROBIOLOGY
FREE UNIVERSITY, AMSTERDAM, HOLLAND

New Guinea Study:

Since the preceding report, the serological survey of residents of the former Netherlands New Guinea (West-Papua) has been carried on with a number of arboviruses belonging to Casals' Group A.

Neutralizing antibodies against Sindbis or an antigenically related virus were not found in sera of the Dani population living in the Baliem Valley in the Central Highlands. In the southwestern coastal region (Merauke area), however, in 247 out of 401 sera tested, neutralizing antibody against Sindbis virus could be detected.

In 20 per cent of the 332 serum samples collected in the Baliem Valley, neutralizing antibody was found against Semliki Forest virus and less frequently and in lower titer against Chikungunya virus. This observation may be a possible indication that Semliki Forest virus and not Chikungunya virus is the cause of infections in New Guinea.

Although a relatively small number of sera collected in the southern coastal area have as yet been screened for antibody against Semliki Forest virus, preliminary results indicate that 80-90% of the population have been infected with this virus.

REPORT FROM DR. IAN D. MARSHALL
DEPARTMENT OF MICROBIOLOGY
JOHN CURTIN SCHOOL OF MEDICAL RESEARCH
AUSTRALIAN NATIONAL UNIVERSITY, CANBERRA, AUSTRALIA

A further two trips were made to the field study area at Maprik, New Guinea, during 1962. The first, between May 4 and June 1, was at the end of the wet season, and the second, between November 11 and December 10, at the end of the dry. However, these seasons are not as pronounced nor total rainfall as high in the Sepik District as in some other areas of New Guinea. Rainfall during the "wet" November to April (3 years average) is 42 inches, and during the "dry" May to October, 24 inches.

Maprik is about 800 feet above sea level and is situated on the Screw River in the low foothills on the inland side of the Torricelli Ranges. An earth road connects Maprik with Pagwi some forty miles to the south on

the Sepik River. Other roads, which are generally only negotiable by four-wheel drive vehicles even in dry weather, fan out from Maprik into the mountains, and one of these continues through to the coast at Wewak. When negotiable, these roads allow access to a fairly broad range of ecological situations; lowland hill forest with steep ridges and deep valleys to the north, and rolling kunai grass plains extending south to the Sepik River. Broad strips of forest are associated with swamps and streams crossing the plains and converging on the Sepik River, and much of the trapping is carried out in and around these forest areas.

Apart from dogs, there are no domestic animals in the area, although there is a moderate population of free ranging fowls and many feral pigs. The latter contribute an important ceremonial food, but there is no attempt at husbandry, the native gardens being laboriously stake-fenced to protect them from damage.

During the May trip, a collecting and reconnaissance excursion was made to Chambri Lake, some forty miles downstream from Pagwi. This extensive lake is formed by anabranches of the Sepik River, and harbors a wide variety of water birds. More extensive collections were made here during November.

Arthropods:

Mosquitoes were collected by small portable Chamberlain-type light traps powered with NIFE alkaline storage cells, by double cone portable traps baited with native birds, mammals, or domestic fowl, and by aspiration from humans.

The light traps have proved to be the most profitable source of mosquitoes, and catches increase significantly with distance from the foothills (15 per light trap night) and towards the Sepik River (321 per light trap night). Although this trend bears a superficial correlation with the activities of the Malaria Control unit, catches outside the control area tend to confirm that the apparent differences in mosquito density reflect natural ecological situations.

On the plains, the forest and the forest-kunai margins produce the largest catches, and numbers tend to decrease with distance into the kunai grass. However, apart from bats, most of the mammals are found in the kunai grass, so these trapping stations continue to be exploited.

Small numbers of ticks and mites have been taken from birds, mammals, and reptiles.

The 15,000 mosquitoes trapped during November 1961 were sorted into over thirty species by Mr. S. Christian of the Malaria Control School, New Guinea Public Health Department, but the 18,000 trapped during 1962 have been pooled into groups of fifty mosquitoes without any attempt at identification.

Birds:

Collections were made by mist netting and by shooting. Identification was carried out by Mr. Warren Hitchcock, Division of Wildlife Research, Commonwealth Scientific and Industrial Research Organization, and all suitable specimens were bled, banded, and released, or killed for organ sampling and preparation of animal skins. Some of the 141 land and 74 water birds represent first records for this district. Blood was also collected from 38 domestic fowls in an attempt to obtain evidence of recent activities of arboviruses.

Mammals:

Mist nets left in position overnight have yielded 63 bats and have contributed very largely to the collection in an area notoriously lacking in wild mammals. There has been relatively little damage done to the nets, even by large flying-foxes (Pteropus spp.).

Ground mammals were trapped with Sheridan and National live traps, and natives were encouraged to collect and sell specimens. Fifty rodents were trapped with Sheridan traps in villages. The five marsupials collected included a bandicoot which appears to be a previously undescribed species.

Blood and organs were collected from all suitable specimens. The trapping, identification, and processing has been largely carried out by Mr. Kent Keith, Division of Wildlife Research, C.S.I.R.O., who participated in both field trips.

Reptiles:

Although no deliberate trapping for reptiles has been carried out, a goanna was trapped in a National trap, several small lizards in Sheridan traps, three snakes were killed, and a skink collected by hand. Although all were processed as museum specimens, only two of these yielded satisfactory blood samples.

Sentinel suckling mice:

Pregnant mice were flown into Maprik from Canberra for both field trips, and their litters exposed to mosquito attack at selected sites throughout the area. There has been little evidence of mosquito attack, and an insignificant number of resting mosquitoes have been found in the sheltering hood.

As suggested by Professor Neville Stanley, the litters are now exposed without the mothers to eliminate the protection they afford. Litters survive without apparent harm for at least 14 hours under these conditions, and are readily accepted back by the mothers. This method also eliminates the tendency for mothers to eat their litters during transportation to and from distant stations.

Human material:

Through the cooperation of the Department of Public Health, Territory of Papua-New Guinea, four brain specimens from fatal cases of suspected viral encephalitis, and 24 blood or serum samples from acute or convalescent cases were received during the year.

Paired blood samples for serological investigations were obtained by Dr. Enders, Wewak, from 60 Australian Army personnel engaged in building a new road from Wewak to Maprik. During the November trip to Chambri, 102 village children were bled by Dr. R. MacLennan, and the results of the serological testing of this group will determine whether Chambri Lake will be included in future field trips.

Attempts at virus recovery:

During the year, material collected in November 1961 and May 1962 has been processed and passed at least once through suckling mouse brain and onto chick embryo fibroblasts under agar. No virus has been recovered from this or from the human material received during the year. The November 1962 material has not yet been examined.

Serological Investigations:

Human sera continue to show evidence of arbovirus activity, but with a lack of paired sera, it has usually been impossible to diagnose particular illnesses.

A start has only recently been made on HI tests of sera from wild birds and mammals. Of 43 samples tested to date against two antigens (Sindbis and M. V. E.), antibodies have been detected in seven.

REPORT FROM DR. R. L. DOHERTY
QUEENSLAND INSTITUTE OF MEDICAL RESEARCH
BRISBANE, AUSTRALIA

A number of virus strains recently isolated in Queensland have been inoculated intrathoracically into Culex fatigans (reared in the laboratory). About 100 mosquitoes were inoculated and taken in lots of 10 at intervals up to 10 days (in some cases to 15 days), ground up, and titrated. Multiplication was demonstrated with Kunjin (MRM61), Kokobera (MRM32), Edge Hill (C281), Stratford (C338), Sindbis (MRM 39), AMM2021 (N544), Koongol (MRM31), Wongal (MRM168), Mapputta (MRM186), and Corriparta (MRM1), but not with lymphocytic choriomeningitis virus included as a control. In some cases suckling mice were exposed to surviving inoculated mosquitoes and although mosquitoes did not take a blood meal, Kokobera, Sindbis, and Edge Hill viruses were transmitted by probing. Experiments on mosquito infection by feeding are in progress.

HI antibody to group B viruses has been found in a number of animal species, most frequently in cattle, horses, kangaroos, and wallabies. An attempt is being made to determine which of the group B viruses isolated in Queensland are responsible for this antibody by titrating HI-positive sera in neutralization test against MVE, Kunjin, Kokobera, Edge Hill, Stratford, dengue 1 and dengue 2. Sera from kangaroos collected in western Queensland in 1958 neutralized Kokobera to higher titre than the other viruses, and sera from wallabies collected in southeast Queensland in 1960 reacted best to Edge Hill. Results on cattle and horse sera do not as yet give any indication as to the infecting agent.

REPORT FROM DR. B. M MCINTOSH, ARBOVIRUS RESEARCH UNIT
SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH
JOHANNESBURG, SOUTH AFRICA

Susceptibility of baboon and domestic animals to chikungunya virus.

To investigate further the possibility that primates may be important hosts of chikungunya virus, this virus was inoculated intramuscularly into 2 baboons (Papio ursinus) and viremia levels determined daily for 5 days. One of the baboons died under anesthesia on day 1. The viremia levels encountered are shown in Table 1. No signs of illness were noticed.

High titer HI antibody was present in the blood at 21 days after inoculation. With regard to baboons as hosts to this virus, it is of interest to mention that of 3 baboon sera in a collection of sera from wild animals collected in Northern Rhodesia in 1962, 2 were strongly positive in the HI test to chikungunya.

Cattle, sheep, goats, and horses were inoculated intramuscularly with chikungunya virus. No viremia was detected in any animal on days 1 to 10. The blood 4 weeks after inoculation contained low titre HI antibody in all animals except one goat which was negative. None of the animals was positive for neutralizing antibody.

Olifantsvlei, Johannesburg Study Area.

Work in this highveld locality is continuing this summer. Trapping of mosquitoes at several sites is being carried out using the Californian lard-can trap baited either with dry ice or a fowl; a fowlpen trap baited with fowls; the Trinidad No. 10 trap baited with wild birds. The fowls in the fowlpen trap are being used as sentinels and blood specimens are being collected from them monthly. A collection of sera was obtained from 108 residents and these have been tested for antibody. Wild birds are also being collected and the bloods tested for virus and antibody. Most of these birds are being ringed and released after the blood sample has been collected.

1. HI results on human sera.

Of these sera, 78 were positive to West Nile and 23 to Sindbis. Although most of these donors had lived for part of their lives elsewhere, it appears likely that many infections with these 2 viruses has occurred at Olifantsvlei.

2. Wild bird sera.

From October 1962 through to January 1963, 348 bird bloods were collected. No virus was isolated. With the HI test, 5 were positive to Sindbis, 29 to Wesselsbron, and 51 to West Nile. Most of the Wesselsbron positive results appear to be the result of West Nile infection. The donors consisted mostly of red bishops, red-billed queleas, and masked weavers.

3. Mosquitoes collected and viruses isolated.

From September 1962 through to January 1963, 19,201 mosquitoes were collected at Olifantsvlei, as listed in Table 2. These mosquitoes were inoculated into mice for attempted virus isolation. Seven virus strains (3 West Nile, 2 Sindbis, 2 unidentified) were isolated from Culex univittatus in the months indicated in Table 3. No viruses were isolated from the other species. It is curious that virus was isolated only from Culex univittatus which was only the third most prevalent species.

4. Antibody conversions in sentinel fowls.

Three groups of a dozen fowls each have been exposed since October in the backyards of residents at Olifantsvlei. Positive HI antibody conversions to Sindbis and West Nile viruses have occurred in each of the groups.

Lake Chrissie Study Area.

This is another highveld habitat situated in the eastern Transvaal. A group of sentinel fowls is exposed there and mosquito collections are being made periodically. The mosquito fauna appears to be much the same as at Olifantsvlei. So far, from the 1,400 mosquitoes collected there this season, one strain of an unidentified virus has been isolated from Culex univittatus.

Ndumu, Northern Natal, Study Area.

Periodic collections of mosquitoes for virus isolation are being made at this locality. During 1962, 17 strains of virus were isolated. Interesting isolations from this collection include Ingwavuma virus from Culex univittatus, Mossuril virus from the same mosquito and two strains of the same virus, not yet fully identified but which would appear to be Ilesha virus from Aedes cumminsi and Mansonia africana.

Sindbis virus as a cause of illness in Man.

Dr. H. Malherbe of the South African Institute for Medical Research has recently isolated a virus from skin lesions in a European patient living in the northern suburbs of Johannesburg and who had not been away from Johannesburg recently. The virus has not yet been fully identified but it is a Group A agent and very probably Sindbis.

The acute stage of illness lasted for eight days. There was a low-grade fever, with headache, muscular and joint pains. On the 4th day, a maculopapular rash developed on the trunk and limbs with vesicles between the fingers and toes. One month after the onset, the patient is still listless and joint pains have persisted.

The virus was isolated in monkey kidney tissue culture and not in infant mice, from skin material collected on the 8th day of illness. Virus was not isolated from throat and rectal swabs and blood collected on the same day.

Table 1

Viremia in Baboons Following Inoculation Of Chikungunya Virus

	Logs. Days post-inoculation				
Baboon	1	2	3	4	5
1	6.4	6.2	5.7	2.9	Neg.
2	5.8	-	-	-	-

Table 2.

Mosquitoes collected at Olifantsvlei for virus isolation.

Month	No. of trap nights	Culex theileri	Culex univittatus	Culex p. pipiens	Aedes lineatopennis	Aedes mixtus/ microstictus	Other spp.	Total all spp.
1962								
September	14	38		1			3	42
October	24	245	15	3	1			264
November	17	1071	203	35	176	12	36	1533
December	23	2199	1698	597	356	166	432	5448
1963								
January	27	858	2335	7562	326	304	529	11914
Totals	105	4411	4251	8198	859	482	1000	19,201

Table 3.

Viruses isolated from mosquitoes collected at Olifantsvlei.

Month	West Nile	Sindbis	Unidentified
September, 1962.	0	0	0
October, 1962	0	0	0
November, 1962	1	1	0
December, 1962	2	1	0
January, 1963	0	0	2

REPORT FROM VIRUS RESEARCH UNIT
WEST AFRICAN COUNCIL FOR MEDICAL RESEARCH
YABA, LAGOS, NIGERIA

Virus Isolations

Only five arboviruses, including that of yellow fever on many occasions, have previously been isolated in Nigeria, the last being Ukauwa in 1960. Serological surveys suggest that man may be infected by many others. It is of some interest, therefore, to record the recent isolation of several further agents.

From the beginning of 1961 until the middle of 1962, mosquito catches, using human bait, were done in an area of rain forest 15 miles inland from Yaba. Although valuable entomological observations were made, the inoculation of over 20,000 mosquitoes, of different species, into day-old mice yielded completely negative results, as did the exposure of sentinel mouse groups. In August 1962, catching activities were removed six miles to the East to a more populated and cleared area. Catches off human bait at ground level and on a 50-foot tree platform have been supplemented by Trinidad No. 10 mosquito traps, baited with mice and lizards, and by the use of a sentinel M. rhesus monkey. Using the same techniques as before, some 40,000 mosquitoes were caught. The majority of these were Mansonia africana and were inoculated in batches into day-old mice. Three filter-passing, ether- and deoxycholate-sensitive agents have so far been recovered. Y1 was isolated and reisolated from a pool of 91 Culex mosquitoes of seven different species, caught in Trinidad No. 10 traps. It is highly lethal to infant mice by intracerebral and intraperitoneal injection, and to adult mice (up to 40 days old tested) by intracerebral inoculation. A hemagglutinin has not been prepared. Cross NT's with a limited range of viruses of groups A, B, C, and Bunyamwera have not shown any antigenic relationships. Y3 was isolated from a pool of 250 M. africana caught off human bait. It kills mice up to 20 days old by intracerebral and intraperitoneal inoculation. Y4 was isolated from a pool of 117 Anopheles of four different species caught off human bait. It is lethal only to mice up to three days old by intracerebral inoculation and is probably different from Y3.

Studies to characterize these agents, to determine their host range, and the incidence of antibodies in man are actively in train.

The growth of viruses in mosquitoes.

A series of experiments has been carried out to investigate the rates of growth of some viruses of groups A, B, C, and the Bunyamwera group in A. aegypti inoculated with small doses of these agents. Group B viruses (Uganda S and Zika) appear to multiply relatively slowly, requiring three or four days to reach a peak titer, whereas a group A agent (KMV strain of Semliki virus) reaches a peak only 24 hours after inoculation. The inoculation of mosquitoes with successively small amounts of virus suggests, however, that the insects may be susceptible to infection with a smaller amount of group B than group A agents. Mosquitoes do not appear to be as sensitive an indicator of the presence of small amounts of virus as mice.

The growth in A. aegypti of two Bunyamwera group agents (Ilesha and Ukauwa viruses) both isolated from febrile patients in Nigeria, has been investigated, and successful transmission to baby mice has been accomplished. Although serologically related, these two agents behave differently in the mosquito: infection and transmission of mosquitoes is difficult in the case of Ilesha virus, but easy with Ukauwa virus. It is possible that a species or genus of mosquitoes other than Aedes may be involved in the epidemiology of Ilesha virus.

Serological survey of East Nigeria.

During the years 1951 to 1953, that part of the eastern region of Nigeria lying roughly within the triangle of Onitsha-Nsukka-Enugu suffered a series of epidemics of clinical yellow fever, in which thousands of people are thought to have died. This was the last major epidemic to occur in West Africa. It was considered to be of some epidemiological interest to discover whether there was any evidence that the virus has been circulating in that area since that time. A visit was made in November and December 1962 and 1,400 sera were collected from children up to 12 years old in the same places that had been surveyed during the epidemics. The majority of these sera have been tested by NT for the presence of YF antibodies, and preliminary results show that there has been in all areas a continued activity of the virus, mostly at a low level but in a few places sufficient for 30 per cent of the 5 to 9 years age group to show apparent antibodies. The sera are also being tested for HI antibodies to several other arboviruses, of various antigenic groups.

Epidemiology of yellow fever.

No recorded outbreak of clinical yellow fever has occurred in Nigeria since the series of epidemics ten years ago, referred to above, although occasional sporadic cases, some of them verified, have been reported to this unit. Serological surveys, such as that above, have shown that the virus continues to circulate. One reason for this apparent latency may be due to partial protection afforded by previous infections with related group B viruses, which have been shown to be common in Nigeria. It is significant that yellow fever epidemics of any magnitude have usually occurred on the borders of the savannah zone, where arthropod-borne virus transmission is generally at a lower level than in the forest zones. It has been assumed that outbreaks have followed the introduction of yellow fever into communities whose immunity to the group B complex has fallen below a critical level at a time when climatic conditions have caused an abundance of vectors.

Some results have accrued from experiments designed to test part of this hypothesis. C. a. tantalus monkeys were inoculated at successive monthly intervals with the group B viruses, Dengue 2, Zika, Uganda S, and West Nile. Sera taken at each stage were tested for neutralizing antibodies to the different viruses by incubating serial four-fold dilutions of sera, after inactivation at 56°C for 30 minutes, with 100 LD_{50} of the challenge virus at 37°C for 60 minutes, and then inoculating the mixtures into mice intracerebrally. No accessory factor was added. This fairly severe test showed poor responses with neutralizing antibodies. However, hemagglutination inhibition (HI) tests with the sera showed significant rises in HI antibodies, with an increase in heterologous antibodies with successive virus inoculations. At each stage, some of the monkeys, in groups of four, were challenged by inoculation of viscerotropic yellow fever and the duration and height of their viremias compared with those in two non-immunized controls. In monkeys which had received two or more immunizing virus inoculations, the duration of the subsequent yellow fever viremia was significantly reduced by several days. This confirms what has been suspected from the well known serological relationships.

REPORT FROM DR. P. BRES, CHIEF, LABORATORY AND DR. L. CHAMBON, DIRECTOR, PASTEUR INSTITUTE DAKAR (REP. OF SENEGAL)

If the Dakar Pasteur Institute has already for many years carried out studies on yellow fever, it is only since 1962 that the work on arboviruses has really begun in this institute.

As a first step, it was found necessary to know the level of immunity against yellow fever in a population which had been regularly vaccinated every four years since 1939. A serological survey was performed with about 500 sera of all age groups, taken at random in urban and rural areas of Senegal, without asking people whether they had been vaccinated. Positive reactions to the seroprotection test in adult intracerebrally inoculated mice were found in 73% of the cases (P Bres et al, Bull. Soc. Path. Exot., 1962, in press).

The second step was a serological survey using the same sera tested against arbovirus antigens by means of the HAI test. The following results were observed (table 1).

1) The figures show a certain degree of variation (which is sometimes significant) according to the area where the sera were taken.

2) The possibility of heterologous reactions within the B group is carefully discussed with particular reference to the fact that the population had been vaccinated against yellow fever with a potent strain. A great number of sera yield inhibition with the yellow fever antigen only, although some of them reached a titer of 1/2560. This indicates that when an individual has, once or several times, been vaccinated against yellow fever with a potent strain, he does not possess any heterologous antibody if he has had no contact with another arbovirus. But many of the other sera yield positive reactions with two, three, or four antigens of the B group. These heterologous reactions demonstrate that the Senegalese have frequent opportunities to come into contact with one or several viruses belonging to the B group.

3) The Chikungunya virus is very often met with; the Bunyamwera, less often; but these viruses may be not very representative of the pattern in Senegal for the corresponding groups.

4) A further survey with local strains when available is necessary to confirm these first results.

The third step was the isolation of local strains. It was carried out by means of studies on bats, sentinel baby mice and captures of mosquitoes. Four strains were isolated, one of them from bats salivary glands. As to this latter strain, first attempts at identification seem to show that it belongs to the B group. It is certainly different from yellow fever, Uganda S and Zika viruses, but is perhaps related

to the West Nile virus. Further work is necessary for complete identification.

In conclusion, arboviruses are as frequent in Senegal as elsewhere. However local morbidity statistics do not mention hitherto any disease caused by these viruses. Maybe the program of mass vaccinations against yellow fever exerts some protective effect against B arboviruses.

Table I - Percentages of positive HAI reactions with sera Collected in different geographical areas of Senegal.

(2) is located 50 miles from (I)
(3) nomadic people

	Antigens	geographical characteristics						
		forest savannah (I)	forest savannah (2)	rain forest	important town	river side in subdesertic	sub-desertic (3)	mean
A	Chikungunya	74	65	48	46	46	44	53
B	Yellow fever	92	93	90	90	80	58	84
	Zika	34	I4	64	34	28	07	30
	Uganda S	53	33	32	25	32	I6	3I
	West-Nile	68	4I	32	30	26	23	36
	Bunyamwera	I8	I4	28	I5	3I	07	I8

REPORT FROM DR. A. J. HADDOW
EAST AFRICAN VIRUS RESEARCH INSTITUTE

Investigation of Burkitt's lymphoma syndrome.

Very active field and laboratory studies continue. To date, three strains of virus have been isolated from biopsies, but all have proved to be Herpesvirus hominis or a very closely related agent.

The sample builds up slowly, but we continue to find an unusually high incidence of Bunyamwera protective sera among patients and their relatives, standing at 24/62 (39 per cent) as opposed to 3-5 per cent in Uganda as a whole.

We have discovered an area in West Nile where three cases have occurred within a radius of 500 yards. An extensive screening of a large sample of sera from this area is planned with at least two comparable control groups from other areas for comparison.

Investigation of actual huts where cases have occurred has begun. To date, 14 have been studied. Only two common factors have emerged--the presence of permanent surface water in half a mile of the hut, and the presence of dense vegetation (forest in this area, thicket in the much drier northwest).

Clinical Studies.

Five natural infections with Sindbis virus in man have been studied. These are believed to be the first recorded. Apart from features common to many arbovirus infections, it is notable that 2 showed slight jaundice.

A case of Zika virus in a European (a laboratory infection) was interesting in showing a fairly widespread rash. A similar case of Bunyamwera infection in another European worker caused a severer fever, but there were no points of special interest.

Isolation of virus from bird-biting mosquitoes.

This year Mansonia aurites, a species preferring bird blood, has been unusually prevalent, particularly in catches made on the steel tower at Zika. Over 12,000 have been inoculated into mice. One strain of virus has been recovered. This belongs to group B and is very likely to be new to Africa.

Isolations from Aedes africanus.

One of the original objects of building the 120-foot steel tower was to find whether mosquitoes rise into the air above the forest after sunset, as at this time strong thermals develop, which could easily carry small insects to considerable height and distribute them widely.

During the first year's work at Mpanga forest, it was discovered that mosquitoes of certain species did enter this high level at the critical time. Unfortunately the species in which we are specially interested are not very common at Mpanga and little information was gained about A. africanus, the most important virus vector in our area.

The tower was moved to Zika, an excellent site, and almost at once five strains of chikungunya virus were isolated from A. africanus taken on the tower, and five from field staff working on it. This occurred early in 1961. It was also established that A. africanus is common in the zone above the canopy after sunset, and that specimens may be taken at least up to 50 feet above the canopy.

In November 1961, an intensive catching programme was begun, and this continued till December 1962. The catches were mainly 24-hour catches with man as bait, carried out simultaneously at all 7 levels (120, 100, 80, 60, 40 and 20 feet, and ground level).

At the end of May, an isolation of Zika virus was made, and thereafter further strains were picked up (all from A. africanus) till the beginning of November, the total being eleven. Unfortunately, this burst of virus activity coincided with very intensive work on the first isolate from a lymphoma biopsy and mice were so scarce that it was not possible to allocate enough to permit subdivision of the catches by levels. By mid-August, the situation had eased off somewhat and thereafter A. africanus catches were subdivided by level. Subsequently, the yield from each level was further subdivided by periods in the 24-hour cycle. Subdivision was considered of importance as it had been found, by means of dissection, that the parous females (which include all those which are potentially ready to pass on an infection) formed an increasingly high percentage of the night-biting population as one moved up from ground level to the platforms above the canopy.

After subdivision was begun, four isolations were made as follows:

1) Before complete subdivision. An isolation from A. africanus taken above the forest. On the night concerned, the pool consisted of 1 taken at 100 feet, 20-21 hours, and 8 from 80 feet, taken between 18 and 20 hours. The virus thus came from the 80-100 foot zone and was isolated from mosquitoes taken in the three hours after sunset (which is always 1800 hours in our catches).

2) The next strain came from a single mosquito, taken at 100 feet, in the period 19-20 hours.

3) The third came from a pool of two mosquitoes taken at 80 feet, in the period 19-20 hours.

4) The final isolation came from specimens taken at ground level, but in the afternoon, not by night. This fits the general picture well, as dissection has shown that by day there is a high percentage of older (parous) females at ground level.

Thus it has definitely been established that infected mosquitoes may rise into the warm air above the canopy in the hours after sunset.

REPORT FROM DR. C. SERIE
INSTITUT PASTEUR DE ETHIOPIE, ADDIS ABABA, ETHIOPIA

Yellow Fever in Ethiopia. Incidence of Arbovirus in the Investigated Zone.

Under the auspices of WHO, the Pasteur Institute of Ethiopia was in charge of a scientific investigation of the yellow fever epidemic which raged in Ethiopia and which at the end clarified certain epidemiological problems and which outlined the map of arbovirus distribution.

1) During the course of the 1961-62 campaign, it was possible to isolate 5 human sources of yellow fever:

The first in February 1961.
The second in June 1961.
The last three in January 1962.

The identification of these sources was made by the five institutes interested in this study: (Pasteur Institute of Ethiopia, Virus Laboratory of the Rockefeller Foundation in New York, Virus Laboratory of Entebbe, Pasteur Institute of Paris, Pasteur Institute of Dakkar).

It was also possible to isolate 12 sources of yellow fever virus from Aedes simpsoni which represents the main vector in the Ethiopian epidemic. One of these sources was isolated by direct bite of Aedes simpsoni on suckling mice.

2) The axis of the propagation of the epidemic is constituted by the Omo River.

3) The variability of morbidity and mortality incidence from one village to another makes apparent the importance of our fundamental knowledge of the microclimate in the development of a yellow fever focus.

4) The serological investigation gives us a percentage in the order of 35% positive YF sera in the Omo Valley.

5) The simian fauna studied, particularly in the Manera Region, indicates 80% of the primates serologically positive to yellow fever.

6) Investigation by HI of antibodies corresponding to certain arboviruses shows a different incidence according to the regions. Antibodies to yellow fever and Zika viruses predominate in the epidemic zone. The problem of double infection is posed in certain cases.

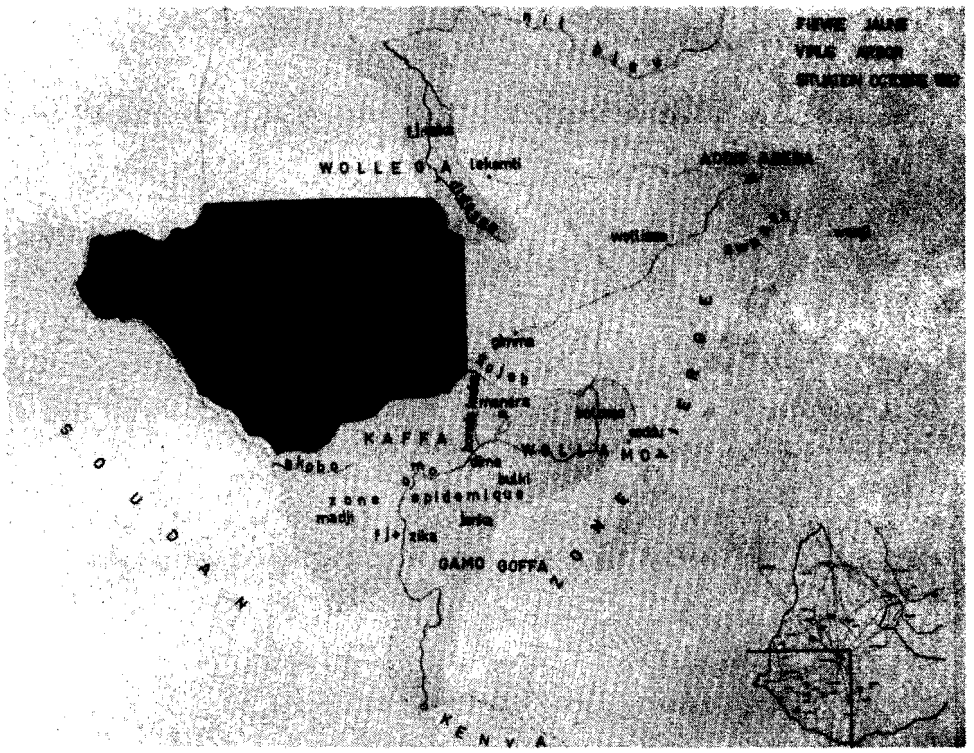
In the zone of western Ethiopia, there exists a large percentage in the 90% group of antibody rate to Casals group B viruses with net predominance of West Nile.

It would appear that in that zone we have not found the epidemic foci explaining crossed immunity between certain group B viruses and Amaril virus.

Towards the east, one notes a virgin zone to all antibodies.

The Chikungunya and Bunyamwera groups appear at a low incidence. These results are illustrated by the enclosed map.

Investigations which will be carried out at the Manera experimental station will permit us without a doubt to clarify the points remaining obscure at this date.


WOLLEGA
KAFFA
SOUDAN
KENYA

REPORT FROM DR. HARRY HOOGSTRAAL
MEDICAL ZOOLOGY DEPARTMENT, NAMRU-3, CAIRO, EGYPT

A major share of the effort of this department continues to be devoted to study of the epidemiology of kala-azar in the Sudan. Between this, however, some progress is being made towards clarification of the biology and systematics of tick vectors or potential vectors in various parts of the world. Reports on the Hyalomma fauna of Afghanistan, Pakistan, India, and Ceylon have recently been presented for publication. Studies of the tick fauna of Lebanon, Jordan, Nepal, and Formosa are nearing completion.

In conjunction with research of the Virus Research Centre, Poona, on Kyasanur Forest Disease, an extensive collaborative research project on the numerous Haemaphysalis ticks of India is underway. None of these tick species has been adequately described, immature stages were previously unknown, several new species have been discovered, and a considerable amount of new data on ecology, distribution, and host relationships are now available. Some Indian haemaphysalid species are known KFD vectors, others were previously easily confused with them or occur in the same geographical area.

Additional studies of tick parasites of birds and bats in several parts of the world have been completed or are nearing completion. A serial review of the mammals of Egypt, in press in the Journal of the Egyptian Public Health Association, is intended chiefly for information of epidemiologists, virologists, and microbiologists working in this area. A number of papers dealing with tick-borne diseases in Russia have been translated and distributed to colleagues.

REPORT FROM DR. NATAN GOLDBLUM
DEPARTMENT OF PREVENTIVE MEDICINE, HEBREW UNIVERSITY HADASSAH MEDICAL SCHOOL, JERUSALEM, ISRAEL

This is a preliminary communication of a survey of antibodies to Sindbis virus in a West Nile endemic area. This investigation is part of a collaborative research program on the prevalence, distribution, and etiological importance of arboviruses other than West Nile virus in West Nile endemic and other geographic areas. This research program is under the joint direction of Dr. N. Goldblum and Dr. J. Yofe, Department of Epidemiology of the Ministry of

Health, and is supported by a grant from The Rockefeller Foundation. The laboratory tests were carried out by Mrs. Edna Ben-Porath from this laboratory and the epidemiologic data collected by Mr. Z. Cochavi from the Department of Epidemiology.

Earlier studies carried out on the etiology, immunology, and epidemiology of arthropod-borne virus infections in Israel indicated that West Nile virus was the sole representative of the arbor group of viruses in the country. Recently, new findings suggested the presence of other members of the arbor group of viruses. Komarov and Kalmar (1960) succeeded in isolating a group B arbovirus from the brains of turkeys dying of encephalitis. This agent has been shown to be closely related to, though serologically distinct from, West Nile virus. In addition, indirect proof has been obtained through serological surveys carried out on wild animals and birds that a group A arbovirus is active in this area (personal communication from Dr. R. Goldwasser); this virus may be closely related to or identical with Sindbis virus which has been found to occur in Egypt and is probably indigenous in the Middle East. These findings have stimulated us to investigate the prevalence, distribution, and etiological importance of arborviruses other than West Nile.

Two hundred forty-six sera collected by venipuncture from children, 3 to 16 years of age, were tested for neutralizing antibody to West Nile and Sindbis viruses. Neutralization tests were carried out mainly by the plate plaque reduction technique on chick embryo cell monolayers. Virus stocks were prepared from brains of infected infant mice; 30-100 PFU were used in the neutralization tests. Inactivated sera were used at a 1 to 4 dilution. Ninety per cent or greater (sometimes complete) reduction in the number of plaques was considered evidence for the presence of neutralizing antibodies.

Table 1 shows the overall results obtained. Up to 18 per cent of sera from non-Jewish children were found to have antibodies to Sindbis virus; the per cent of positives for Sindbis virus antibodies among Jewish children from the same area was lower.

It was also observed that children that have antibodies against Sindbis virus are almost without exception positive for West Nile virus antibodies. The correlation between the presence or absence of antibodies to the viruses under study is brought out in Table 2. It will be seen that out of 15 sera with Sindbis antibodies, 14 were found in children with West Nile antibodies, whereas of 74 West Nile virus negative sera, only one had antibody against Sindbis virus. One wonders

what the reason for this curious correlation might be. Dr. R. M. Taylor, to whom we have communicated these findings, is of the opinion that: "Since these two viruses are not related antigenically, one might suppose that this correlation may be due to common experience in exposure since both viruses are transmitted by mosquitoes probably in most instance of the same species."

In order to determine the degree of sensitivity of the plaque reduction test used to detect antibody against the viruses under study, comparative neutralization tests were carried out using this technique and the intracerebral mouse neutralization test. In the latter, the same serum dilutions and virus challenge were employed. For Sindbis virus, infant mice, and for West Nile virus adult mice were used in the neutralization tests. It can be seen from Table 3, that there is a good correlation between the in vitro (PRT) and in vivo (mouse NT) tests. Moreover, certain sera were found positive in the plaque reduction test and negative in the mouse neutralization test while none vice versa. This would indicate at least a similar, if not higher, sensitivity of the method employed for antibody determinations.

Table 1.

Antibodies to West Nile and Sindbis viruses in a West Nile endemic area

Ethnic groups	Age Groups	Antibodies to West Nile virus		Antibodies to Sindbis virus	
		No. of sera tested	Percent positive	No. of sera tested	Percent positive
Non - Jews	6 to 9 years	114	30	44	14
	10 to 16 years	63	48	40	18
Jews	3 to 9 years	21	5	21	0
	10 to 16 years	48	31	52	4

Table 2.

Correlation between the presence and absence of antibodies to West Nile and Sindbis viruses

Ethnic groups	Age groups	Sera with antibodies to West Nile virus		Sera without antibodies to West Nile virus	
		No. tested	No. positive for Sindbis virus	No. tested	No. Positive for Sindbis virus
Non - Jews	6 - 16 years	51	12	23	1
Jews	3 - 16 years	16	2	51	0

Table 3.

Correlation between the plaque reduction (PRT) and mouse neutralization (NT) tests for West Nile and Sindbis Viruses

Antibodies to	No. of sera tested	Positive in PRT and in mouse NT	Negative in PRT and in mouse NT	Positive in PRT and negative in mouse NT
West Nile virus	39	22	15	2
Sindbis virus	19	4	14	1

REPORT FROM DR. A. SANNA
DIRECTOR OF THE INSTITUTE OF MICROBIOLOGY
UNIVERSITY OF PARMA, ITALY

Serological survey.

According to the programs previously referred to (Information Exchange No. 5, page 54), we have gathered a considerable number of human and animal sera from different parts of the Po Valley (Parma, Mantova, Piacenza) and from Sardinia.

During 1962, the presence of HI antibodies in 500 human sera of the District of Parma has been studied. All samples have been tested against antigens to the following viruses: Group A, Sindbis; Group B, West Nile and Ntaya. The following table gives the results of HI reactions in these 500 sera.

Serum Dilution	Sindbis	West Nile	Ntaya
1:10	10	30	16
1:20	3	10	10
1:40	2	9	5
1:80	0	7	7
1:160	1	4	3
1:320	0	1	4
1:640	1	1	2
1:1280	0	0	0
	17	62	47

The positive sera will be tested later by NT test.

At present a serological survey is being made on a number of human and animal sera of the District of Mantova.

Attempted virus isolations

From October 1962--in collaboration with Prof. A. Valle, Director of the Zoological Museum of Bergamo--we have begun attempts to isolate virus from arthropods associated with wild rodents (Rattus norvegicus) captured in the District of Bergamo. Till this moment about 1200 arthropods--representing the following species: Ceratophillus fasciatus, Echinolaelaps echidninus, Emolaelaps

glaskowi, Haemogamasus nidiformes bregetova, Polipax spinulosa, Euparasitus emarginatus--have been tested. Pools were inoculated intracerebrally in suckling mice, 2 blind passages being carried out. No virus has as yet been recovered.

Tissue culture studies

Some arthropod-borne viruses (Group A: Semliki Forest, Sindbis; Group B: West Nile, Uganda S.) have been tested in various tissue culture systems (pig, rabbit, and horse kidney). No one of the viruses tested has produced CPE. Of the tissue culture systems employed, pig kidney cultures were capable of supporting multiplication of Semliki Forest, West Nile, Uganda S viruses, but not of Sindbis virus; rabbit kidney cultures of West Nile and Uganda S viruses, but not of Sindbis and Semliki Forest viruses; horse kidney cultures of Sindbis and West Nile viruses.

Production of arthropod-borne viral hemagglutinins in tissue culture.

We have begun a series of researches on the preparation of hemagglutinating antigens in tissue culture. The production of hemagglutinins of Sindbis and West Nile viruses has been studied.

According to what was observed by other authors (Diercks et al, 1960) also in our tests "normal inhibitor" of the serum present in the nutrient medium was capable of masking HA in HKC fluids. Hemagglutinins could be recovered from inhibitor containing tissue culture fluid by acetone extraction. The addition of sucrose, as a protective one against the action of acetone, has not given important advantages. Hemagglutinating antigens have been obtained by means of bovine plasma albumin, instead of the serum. Better results have been obtained employing an inhibitor-free maintenance medium prepared by treating the inhibitor containing maintenance medium with kaolin (gr 1.25 of kaolin for 1 ml of serum present in the medium).

REPORT FROM DR. MIKA LIKAR
MIKROBIOLOSKI INSTITUT, LJUBLJANA, YUGOSLAVIA

The present communication describes the results of virological investigations of 50 wild birds shot in July 1962 in the village Trpjica known as an endemic focus of the infection with CEE virus in humans.

The organs of birds (liver, spleen, and lungs) were collected and immediately stored at -20°C. They were subsequently ground in mortars. A 20% suspension was prepared in Hanks balanced salt solution from a pool of all organs of one individual bird. The suspension was centrifuged at 2500 rpm in a refrigerated centrifuge for 10 minutes. The supernatant fluids were stored at -20°C and investigated later for the presence of antibodies.

Table 1
Birds Investigated

Species	Common name	No. of birds	Referred later as:
Parus ceruleus	Blue tit	10	B1-B10
Parus maior	Great tit	6	G1-G6
Motacilla flava	Yellow wagtail	1	W1
Falco tinunculus	Kestrel	1	K1
Emberiza citrinella	Yellow hammer	6	H1-H6
Pyrrhula pyrrhula	Bullfinch	3	BF1-BF3
Garrulus glandularius	Jay	6	J1-J6
Lanius collurio	Shrike	5	S1-S5
Aluada europea	Lark	3	L1-L3
Sitta europea	Nut hatch	1	N1
Pica pica	Magpie	1	M1
Turdus merula	Black bird	2	BB1-BB2
Columba palumbus	Wild pigeon	1	P1
Fringilla coelebes	Finch	1	F1
Picu viridis pinetarum	Woodpecker	2	WP1-WP2
Erithacus rubecula	Robin	1	R1

Table 2
Titers of Hemagglutinins

Extracts of bird organs	ANTIGENS FOR ARBOVIRUSES			
	Semliki forest	CEE	Calif. enceph.	Sandfly fever (Sicilian strain)
W1				1/30
B1	1/30			
B3			1/60	
B6		1/60		
S3		1/60		
BB1		1/30		
WP1		1/30		
H1		1/30		
BF1		1/60		

In the test, 8 units of hemagglutinins were used. In Table 2, reciprocal values of the dilutions of the extracts inhibiting 8 units of hemagglutinins are given.

Table 3
Titers of the reactive extracts of bird organs in the complement fixation test

Extracts of birds organs	ANTIGENS FOR ARBOVIRUSES				
	Semliki forest	CEE	Calif. enceph.	Sandfly fever (Sicilian strain)	EEE
W1				8/60	
B1					16/60
B3			8/60		
B6		32/120	8/60		
B7			4/60	16/60	
B9		32/60			
B10		16/30			
G2			8/60		
G4		32/60	4/120		
S1	16/120				
S2		32/30			
S3		16/30			
BB1		16/30			
BB2		32/120			
WP1		32/120		16/30	16/30
WP2	16/120	32/60			
R1			8/120		
H1		32/30			
H6		32/60			
BF1		32/60			
BF2		32/60			
BF3		16/30			
J1					16/30

The denominator in Table 3 shows the dilution of the extracts, the numerator the dilution of the antigens.

Table 4

Neutralization tests with some of the sera from Table 2 and Table 3

Virus and extract tested	VIRUS DILUTIONS 10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	Protection in $\log_{10}$
CEE virus (control)	4/4	4/4	4/4	4/2	4/0	
Calif. virus (control)	4/4	4/3	4/1	4/0	4/0	
Sic. virus (control)	4/4	4/3	4/1	4/0	4/0	
Semliki virus (control)	4/4	4/2	4/1	4/0	4/0	
CEE virus +B6	4/4	4/1	4/0	4/0		1.25
Calif. virus +B3	4/4	4/0	4/0	4/0		1.0
Sic. virus +W1	4/2	4/1	4/0	4/0		1.25
Semliki virus +B2	4/3	4/2	4/0	4/0		0.50

The numerator denotes the number of mice inoculated, the denominator shows the number of dead mice.

The extracts of the bird organs were tested also for the presence of arboviruses. The extracts were injected intracerebrally into 4-8 suckling mice. One of the isolates could be identified as a strain of CEE virus.

Table 5

Identification of the virus isolated from the extracts of the organs of a yellow hammer (1)

Mouse hyper-immune serum	ANTIGENS Hl	CEE	Calif.	Sic.	Psit.**
CEE	32/16*	32/32			
Calif.			8/16		
Sic.				8/16	
Psit.***					32/124

*The denominator denotes the dilution of the immune serum fixing 100% of the complement with the dilution of the antigen given in the numerator.
**Antigen for psittacosis-ornithosis group of viruses.
***Human immune serum.

REPORT FROM DR. HANS MORITSCH
INSTITUTE OF HYGIENE, UNIVERSITY OF VIENNA, AUSTRIA

During the past years, our CF antigens for the diagnostic work on tickborne encephalitis (TBE) were prepared from the brains of adult mice (8-10 g) by acetone-ether extraction. This method yields an antigen of low sensitivity. For example, out of 127 patients who presumably were cases of TBE (positive in NT and HI tests) only 34 developed CF antibodies which were detectable with this antigen. As a rule, CF antibodies could not be demonstrated before the 16th day following the onset of the clinical symptoms of the CNS (Ch. Kunz and H. Moritsch: Arch. Virusforsch., 11, 568-582, 1961).

In our recent work, an antigen made of infected (TBE strain, Type CEE, Vie 415 B) baby mouse brain by sucrose-acetone extraction proved to be clearly superior to the former preparations.

With this antigen studies were carried out in order to re-evaluate the immune response in cases of TBE as regards the development of CF antibodies:

1) CF tests with sera of clinical cases, hospitalized in Neunkirchen under the diagnosis of a virus infection of the CNS. Out of 69 patients who had neutralizing antibodies to TBE at a serum dilution 1:5, 67 developed CF antibodies during their illness. In 40 cases, the increase of titer was 4-fold or greater. Among these, 35 times CF antibodies were already present in the first serum sample, that has been drawn sometimes as early as the first day of the disease (phase II). The sera of 25 patients reacted to a titer of at least 1:64 but showed no significant rise during convalescence. Once the titer remained as low as 1:8. This case later was diagnosed as meningitis purulenta.

2) Persistence of CF antibodies in proved cases of TBE: From the results obtained with 45 proved cases (Table 1) it will be noted that in all but one case, CF antibodies were still detectable one year after the disease. In 43 cases a drop in titer to 1/4 or less of the initial value had occurred. These titers persisted at the same level in 16 out of 17 cases who were bled again in the subsequent year (i.e. 2 years after illness). Table 2 gives the results obtained with 7 patients who were tested for 3 to 4 years.

3) CF tests with survey sera: In 1961, a survey (1412 sera) had been carried out on the incidence of neutralizing antibodies to TBE virus in normal residents of the Neunkirchen district (approximately 100,000 residents). In 15% of the sera, antibodies could be demonstrated in the NT. The results of the CF tests with 102 of these sera are presented in Table 3. None of the donors revealed any indication of a previous infection of the CNS. It will be observed that 53 persons had CF antibodies in their serum. However, the titers were quite low, never exceeding values of 1:32. On the basis of our findings, it was concluded that in cases of TBE, a positive increase in titer occurs, provided the titer is 1:64 or greater and patients give no indication of a recent disease of the CNS.

Table 1

Reciprocal CF Antibody Titer in 45 Proved Cases of TBE

Titer in year of illness	Titer 1 year later	Fraction of initial titer	Numer of persons
512	256	1/2	1
256	64	1/4	1
	32	1/8	2
	16	1/16	2
	8	1/32	2
128	64	1/2	1
	32	1/4	6
	16	1/8	2
	8	1/16	3
	4	1/32	2
64	16	1/4	5
	8	1/8	8
	4	1/16	5
32	8	1/4	3
	4	1/8	1
16	<4		1

Table 2

Persistence of CF Antibodies in 7 Proved Cases of TBE

Titer in year of illness	TITER AFTER			
	1 year	2 years	3 years	4 years
64	9	n.d.	8	n.d.
128	8	8	8	n.d.
128	4	4	0	n.d.
512	256	32/64*	32/64*	n.d.
128	n.d.	n.d.	n.d.	16
n.d.	n.d.	16	16	16
32	8	0	0	n.d.

*Titer on repeat test

Table 3

Reciprocal CF - antibody titer of 102 persons positive in the NT but no Indication for TBE in their History

CF Titer	0*	4	8	16	32	32
Number of sera	49	18	20	9	6	0

0* - Titer = < 4

REPORT FROM DR. D. BLASKOVIC
DIRECTOR, INSTITUTE OF VIROLOGY
CZECHOSLOVAK ACADEMY OF SCIENCES, BRATISLAVA

Experimental infection of the great dormouse (Glis glis) with Central European tickborne encephalitis (TE) virus.

Ten specimens of dormice trapped in the Little Carpathian mountains were used in the experiment. They were kept in a room under natural conditions at a temperature about +20°C. Central European TE virus (strain Hypr M 49) was used. The titer of virus in 10% brain suspension was 10^8 LD_{50} per 0.1 ml i.c. for mice. The dormice were inoculated in the autumnal period (13 September) subcutaneously in the antebrachial area of the front left leg with 0.2 ml suspension in dilution 10^{-2}.

Viremia was observed after experimental infection of dormice. In one case the viremia lasted from the 3rd to the 7th day after the infection. The virus was isolated from the brain at 5th day and from pancreas of an animal which was killed at 7th day. No pathological changes were detected by histological examination of the brain, spleen, liver, pancreas, white and brown fat. Dormice produced neutralization antibodies against TE virus following infection.

On the basis of these results, we conclude that the great dormouse may play a role as a reservoir of TE virus in Central European natural foci of infection and because of their heterothermia they might serve as a long-term reservoir during the interepidemic period. The following study proves this posibility.

References:

Nosek, J., Kozuch, O., Lichard, M., Ernek, E., Albrecht, P: Experimental Infection of the Great Dormouse (Glis glis) with Central European Tick-Borne Encephalitis Virus. Acta. virol. 7, 1963 (in press).

Persistence of tick-borne encephalitis (TE) virus in hibernating hedgehog (Erinaceus roumanicus) and great dormouse (Glis glis).

The experiments were carried out during the winter period 1962-1963 with 4 hedgehogs and 5 great dormice in the Cave Driny in Little Carpathian mountains. The cages containing the hedgehogs and the dormice were placed in the cave near a small lake. The temperature during the hibernating period varied in the limits of +4°C and -2.5°C. The hedgehogs

and the dormice were inoculated at 4 December 1962 with the M 50 passage of strain Hypr of Central European tickborne encephalitis. The ic titer of virus in the brain suspension was 10^8 LD_{50}/.03 ml for mice. Both species were inoculated subcutaneously in the antebrachial area of the left front leg with 0.2 ml in dilution 10^{-4}. The infected hedgehogs and the subadult specimens of dormice fell into wintersleep on the third hour after inoculation. The adult dormice were in hypothermia which intermittently alternated with the longer or shorter wintersleep. The course of the infection was studied after hibernation.

When the animals were transferred to room temperature 29 days later (on January 2, 1963), virus was recovered from their blood. The virus was still detectable in hedgehogs up to the 8th day and in adult dormice on the 7th day. Viremia lasted for 35 days in infected hedgehogs, including the hibernation period, and 34 days in adult dormice. The hedgehogs and the adult specimens of dormice had no marked clinical signs of disease after hibernation. Two subadult dormice died during the hibernation period 25 days after inoculation. The third subadult specimen died on January 3, twenty hours after interrupted wintersleep. Remarkable in this case was that this specimen showed clinical signs of meningoencephalitis. TE virus was isolated from the blood of all subadult specimens taken from the heart, brain, pancreas, spleen, and liver. From 14th day antibodies against TE virus were present in hedgehogs and adult dormice.

These experiments confirm the results of Van Tongeren's experiments with hedgehogs (Van Tongeren, 1957, 1959).

Because of their heterothermia, hedgehogs and dormice might be long-term reservoirs of the virus in nature. This observation indicates that the possibility of these animal reservoirs during interepidemic periods exists.

References:

Kozuch, O., Nosek, J., Ernek, E., Lichard, M., Albrecht, P.: Persistence of Tick-Borne Encephalitis Virus in Hibernating Hedgehogs (Erinaceus roumanicus) and Great Dormouse (Glis glis). Acta virol., 7, 1963 (in press).

Isolation from ticks Ixodes persulcatus of an arbovirus different from tick-borne encephalitis virus.

During a study of natural foci of tick-borne encephalitis in Western Siberia, the Czechoslovak study group of the Institute of Virology of the

Czechoslovak Academy of Sciences, participating in a joint scientific expedition with members of the Institute of Poliomyelitis and Viral Encephalitides, USSR Academy of Medical Sciences, Moscow, under Prof. M. P. Chumakov, obtained first evidence on the presence of a virus substantially different from tick-borne encephalitis virus (TEV). The virus was isolated from suspension of unengorged females of Ixodes persulcatus ticks as well as from cerebrospinal fluids of 2 patients suffering from a febrile illness following tick bite. Neutralization tests with paired serum samples of these patients with the newly isolated virus showed conversion from negativity to positivity. Virus killed newborn mice and hamsters 2-5 days after inoculation, caused death in 7 day-old chick embryos, but was not pathogenic for adult mice and hamsters. The virus was not neutralized by hyperimmune sera against TEV, WEE, EEE, St. Louis and Jap.B. viruses. The virus was inactivated with 0.1% desoxycholate, ether and chloroform. Its resistance was more marked in alkaline ranges of pH. The presence of 1 M $MgCl_2$ enhanced its inactivation at 50°C and the action of trypsin and papain lowered its infectivity titer. The virus sedimented at 100,000 g/1 hr similarly to TEV, and is highly cytopathic for chick embryo cells as well as for cells of standard stable lines. The use of the fluorescent antibody method revealed the acumulation of antigen in the cytoplasm of cells during the exponential phase of virus growth.

References:

Chumakov, M.P., Karpovich, L.G., Sarmanove, E.S., Sergeeva, G.I., Bychkova, M.B., Tapupere, V.O., Libikova, H., Mayer, V., Rehacek, J., Kozuch, O., Ernek, E.: Report on the Isolation from Ixodes persulcatus ticks and from Patients in Western Siberia of a Virus Differing from the Agent of Tick-borne Encephalitis. Acta Virol. 7, 1:82-83, 1963.

Tick tissue cultures.

Attempts were made to study the problem of tick tissue cultures in order to find a suitable tissue for isolation of arboviruses from nature and to use this type of tissue for an eventual differentiation among viruses and to investigate the eventual changes in the properties of viruses which could be caused by their cultivation in tick tissues.

Investigations were carried out with tissues from developing adults within engorged nymphs of ticks undergoing metamorphosis. The nutrient medium used consisted of equal parts Eagles and Vago and Chastangs media and 0.1% fraction from dried calf serum. The cells were cultivated in Carrel flasks at 29°C. Under these conditions, cell monolayers were obtained. Subculturing was not attempted.

It was found that tick-borne encephalitis virus multiplied in the cell cultures described without causing a cytopathic effect. The titres of virus obtained ranged between 1-5 log. units. In many experiments, when the multiplication of virus in cultures was noticed the doses of virus used for the inoculation of cultures were so small that they were not detectable in intracerebrally inoculated mice; this fact possibly being of some importance for primary virus isolation.

References:

Rehacek, J.: An Improved Method of Tick Tissue Cultures. Acta virol. 6, 2:188, 1962.

REPORT FROM DRS. R. PANTHIER, C. HANNOUN, AND L. DE LOOZE YELLOW FEVER DEPARTMENT AND ARBOVIRUS LABORATORY PASTEUR INSTITUTE, PARIS, FRANCE

During the past year, new laboratories for arbovirus studies have been built and equipped. They have been in use since the beginning of 1963. The program includes epidemiological studies in France and in Africa, in connection with the overseas Pasteur Institutes. It is expected that the laboratory in Paris will work as a central reference laboratory for the different workers involved in arbovirus research in the Pasteur Institutes. In addition, basic virological studies are planned.

WHO Fellowship

From April 2, 1962, to December 13, 1962, Mr. Claude Hannoun visited laboratories in the USA (Rocky Mountain Laboratory, Hamilton, Montana; University of California School of Public Health, Berkeley, California; Communicable Disease Center, Atlanta, Georgia; New York State Department of Health, Albany, N.Y.; Rockefeller Foundation Virus Laboratory, New York) and stayed for several months at the Wistar Institute, Philadelphia, Pennsylvania, under a WHO Research Training Grant for Arboviruses.

Arboviruses and Diploid Cells

During this stay at the Wistar Institute, the susceptibility of human diploid cell strains (WI 38, LNG 418) has been studied. For the 17D vaccine strain of yellow fever virus, the inoculation at a multiplicity of infection of 0.5 to 0.05 per cell resulted in 3 days in a titer

of virus of 10^4 to $10^{4.5}$ mouse (IC) LD50 per 0.03 ml. A cytopathogenic effect was obvious in 4 days and complete in 7 to 9. By reading this cytopathogenic effect on the 7th day, the susceptibility seems to be one log below the susceptibility of mouse (IC). The effect of the medium constitution, of temperature, and of the addition of cortisone has also been studied. With other strains of yellow fever virus (French neurotropic, Manera-Ethiopia) no CPE was observed and the virus yield was lower (10^{-2} to $10^{-3.5}$).

The virus of WEE gives CPE in 3 days on the same cells giving, in the supernatant a titer of 10^{-5} at least (per 0.03 ml).

Survey of Antibodies in Monkeys.

A study of the antibodies present in the serum of monkeys captured in Guinea Forest has been started after preliminary results obtained at the Rockefeller Foundation Virus Laboratories in Dr. Casals' laboratory. On 10 serums collected in Guinea from Cynocephales, 6 had antibodies against Chikungunya virus, 1 against Zika, and 1 against several group B viruses. Only two were negative against the 13 strains used.

Effect of Trypsin on Hemagglutinins.

Confirmation on the observations of Cheng (1958) has been obtained concerning the inactivating effect of trypsin on the hemagglutinin of the B group, chiefly yellow fever virus. A standard technique has been designed to "titrate" proteolytic activity. Complete inactivation of HA is obtained at room temperature in 30 minutes with 8 µg. of our best sample of commercial trypsin, partial inactivation (50%) with 2 to 3 µg. This very sensitive technique is still under study.

Participation in the Study on Yellow Fever in Ethiopia

As a collaboration to the program designed by the Scientific Group on Yellow Fever Research in East Africa (WHO), 655 human sera and 88 animal sera have been examined by HAI (antigen prepared with Manera strain) and by CF (antigens prepared with Manera and Dakar strains). The results will be analyzed and compared by Dr. Serie, Pasteur Institute, Addis Ababa.

REPORT FROM DR. C. HALLAUER
UNIVERSITY OF BERNE, SWITZERLAND

It has been pointed out earlier (Information Exchange No. 4) that attenuated yellow fever virus variants can be isolated following long continued cultivation of yellow fever virus on human tumor cell lines (HeLa, KB). The infectivity, pathogenicity, and immunizing potency of these variants have been tested in monkeys with the following results.

The separately passaged strains Asibi I and II showed a highly diminished viscerotropism but an unchanged neurotropism in the 77th and 62nd KB passage, respectively. Subcutaneous inoculation of these two attenuated strains elicited the formation of antibodies and a strong immunity towards a subcutaneous and intracerebral reinfection with the pantropic virus. The degree of attenuation of the tissue culture passaged strains Asibi I and II was approximately equal to that of Theiler's vaccination strains 17E and 17AT respectively.

The continuous passage of two 17D strains (17D I London and 17D II Amsterdam) has not resulted in the reactivation of any viscerotropic qualities after 189 and 91 tissue culture passages respectively. The strains were, to the contrary, further attenuated and showed a progressive loss of their neurotropism. The pathogenicity of strain 17D I for cerebrally infected mice was in most cases restricted to undiluted virus samples (log 6.0 $TCID_{50}$), while strain 17D II was entirely non-pathogenic in a comparable high dosage (log 5.3 $TCID_{50}$). Both strains, however, immunized the mouse brain in a titer which nearly corresponded to their original tissue culture infectivity. Neither a febrile reaction nor evidence of virus in the blood could be found in monkeys which had been infected by the cerebral and subcutaneous route. In spite of the extreme blandness of the infectious process, the animals were reliably protected against a subsequent reinfection with the pantropic virus. The tissue culture strains 17D I and II thus showed a degree of attenuation which, to my knowledge, has not been seen earlier in a substrain of the 17D virus.

Comment: The continuous passage of yellow fever virus on human tissue culture cells (tumor cell lines) yields attenuated variants just as well as the passage on explanted mouse or chick embryo tissue. It follows that the physiological pecularities of the tissue culture cells are more important for the selection (or possibly neogenesis) of virus mutants than the taxonomic species from which the tissue culture was taken.

REPORT FROM PROF. S.R. PATTYN
HEAD, BACTERIOLOGY DEPARTMENT
INSTITUTE OF TROPICAL MEDICINE PRINCE LEOPOLD
ANTWERP, BELGIUM

1) Continuing our studies on the behavior of arboviruses in tissue cultures of HeLa cells, we try to determine for a certain number of viruses their multiplication in these cells, the appearance of plaques under agar overlay, interference phenomena, and HA antigen production.

Group A viruses:

SF produces CPE and plaques under agar (stoppered bottles with agar overlay described by Melnick for use with enteroviruses).

Plaques are much smaller than on CETC.

Very small amounts of HA antigen could be demonstrated using the serum-free maintenance medium described by Salminen (Lactalbumin hydrolysate - Hanks - Tris - Bovine albumin).

Group B viruses:

RSSE, no CPE, but fairly good multiplication.

From day 2 onward, the TC fluid titers 10^5/0.03 cc I.C. in the mouse -- no plaques could be demonstrated with the above mentioned agar overlay --HA antigen in small quantities: 1/8 to 1/32 after 4 to 6 days (as to 1/512 to 1/1024 in protamine treated baby mouse brain). No significant interference could be detected in fluid cultures between this RSSE infection of HeLa cells in tubes and superinfection with Polio I or Coxsackie A11 virus at different intervals.

YF - WN: no CPE and no important HA antigen production.

Bunyamwera: studies are in progress.

2) In view of the past activity of onyong-nyong virus in Uganda, neutralizing antibodies against the closely related chickungunya virus were searched for in sera from healthy persons originating from Katanga Province (Republique du Congo, Leopoldville) and Burundi. Thirty-two sera from Katanga and 84 from Burundi were examined by the Porterfield technique on CETC. No neutralizing antibodies were found.

3) A number of sera from children who had developed a meningo-encephalitic syndrome in a rural region around the city of Antwerp were tested for neutralizing antibodies (in mice) against RSSE. No such antibodies were found.

4) In the course of our studies of arbovirus infections in pregnant animals, we observed that newborn rats die after subcutaneous infection with 100-1000 (mouse) LD50 of SF virus, and that the virus is present in the brain. We are studying the possibility of using newborn rat brain as a source of HA antigen.

REPORT FROM DR. J.S. PORTERFIELD
NATIONAL INSTITUTE FOR MEDICAL RESEARCH
MILL HILL, LONDON, ENGLAND

As part of a WHO study on Trachoma, serum samples were collected from young adults living in different regions in Tunisia. The specimens were collected in January 1959 and were stored at -25°C after heat inactivation. In November 1962, 98 sera were received in London through the courtesy of Dr. A.C. Saenz of the WHO office in Geneva and Dr. M. L. Tarizzo, WHO Adviser in Tunisia. These sera have now been examined against a number of different arthropod-borne viruses. The present report is an interim one, since it is hoped to extend the survey by an examination of further viruses. The selection of virus was somewhat arbitrary, since no previous studies have been carried out in this area. Those tested to date include two Group A viruses, Sindbis and Chikungunya, two Group B viruses, West Nile and Israel Turkey meningoencephalitis virus, and two Bunyamwera group viruses, Bunyamwera and Ukauwa.

There were no positive results in plaque inhibition tests carried out with Chikungunya, Israel Turkey meningoencephalitis virus, or Ukauwa virus. Six sera out of 91 examined gave clearly positive results against West Nile virus and four were clearly positive against Sindbis virus. Thirty sera gave positive results against Bunyamwera virus, but the zones of inhibition produced in this system were much less definite than those seen with a control Bunyamwera immune serum.

Hemagglutination inhibition tests carried out using a West Nile antigen gave clearly negative results with 47 sera and the titres of the following sera were as follows: 1:10 = 8, 1:20 = 16, 1:40 = 11, 1:80 = 2, 1:160 or greater = 6. The six sera with titres of 1:160 or greater all gave clearly positive zones of plaque inhibition against West Nile virus.

Three of the four sera which were positive in Sindbis plaque inhibition tests were also positive in West Nile plaque inhibition tests. Since these two viruses are in different serological groups, this finding cannot be explained on the basis of a serological overlap; it may indicate that the persons from whom these sera were obtained came from an area in which the risk of exposure to mosquitoes was high.

At the present time, it may be concluded that West Nile and Sindbis viruses are present in Tunisia; this is not altogether surprising since both are known to be active in the Mediterranean region. The Bunyamwera results may indicate a fairly high rate of infection with a virus which is serologically close to Bunyamwera but which may be slightly different from it.

	West Nile		Sindbis
	P.I.	HAI	P.I.
Serum No.			
64	18 mm	>160	0
67	19 mm	>160	0
112	22 mm	>160	10 mm
115	24 mm	>160	0
146	23 mm	>160	14 mm
184	20 mm	>160	10 mm
153	0	20	12 mm

P.I. = plaque inhibition - zone diameter in mm.
HAI = hemagglutination inhibition titre against 8 units of antigen.

REPORT FROM DR. C.E. GORDON SMITH ARTHROPOD-BORNE VIRUS RESEARCH UNIT LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE LONDON, ENGLAND

Studies of louping ill in sheep and ticks in Ayrshire (S. W. Scotland)

1. Sheep*

Studies in 1961-62 were concentrated on Dalcairnie Farm. In sheep the hemagglutinin-inhibiting antibody is less persistent than the neutralizing antibody. Serological studies have shown that the incidence of infections varies markedly between 1 natural area of hill grazing

(hirsel) and another. Comparing 2 adjacent hirsels in 1961, the infection rate appeared to be 4-5 times higher on one than on the other as judged by antibody conversions in ewes (2 or more years old) and antibody conversions and deaths in exposed susceptible yearling sheep. Almost all the new infections in ewes occurred in 2 year old animals. Most of the antibody in lambs at the age of about 10 weeks was markedly correlated with that of their mothers and therefore probably represented maternal antibody. Only a few lamb deaths due to louping ill occurred during the 1961 and 1962 seasons. By exposing fully susceptible yearling sheep as part of an experiment in vaccination with Langat virus, infection rates on three farms (Camlarg, Dalcairnie, Knockgray, respectively) were found to be 100%, 39% and nil although tick infestation was heavy on all of them. Five out of six susceptible yearlings exposed on Camlarg in March died of louping ill, but 0/7 exposed from July to September showed any symptoms although 6 of them had serological evidence of infection. Whether this is due to the age of the sheep or to a lesser dose of virus is not at present known. Previous infection with Langat virus was shown to give significant protection against natural infection with louping ill. During 1961 several strains of louping ill were isolated from sheep brains and from blood-fed ticks in the study areas.

2. Ticks**

Ticks were collected from the ground by dragging: all of them were Ixodes ricinus. A total of 3981 larvae (11 pools), 368 nymphs (21 pools) and 22 adults (8 pools) were inoculated into suckling mice. Only one strain of louping ill virus was isolated: from a pool of 42 unfed nymphs collected from Camlarg in April. The area sampled in Camlarg was the most productive, Mossdale and Dalcairnie less so. Knockgray appeared to be the least infested. This correlated well with the infection rates found especially in exposed susceptible yearling sheep. There were two distinct nymphal peaks, one in April, about a month before the larval peak and the second in September. In all the farms, except Camlarg, the autumn peak was distinctly higher than the spring peak. Adults were taken in small numbers during all the months except March, although they were collected from all four farms only in April, May, June, and July.

*In collaboration with Mr. K. J. O'Reilly, Wellcome Research Laboratories, and Mr. A. L. Wilson, West of Scotland Agricultural College.
**In collaboration with Dr. M. G. R. Varma, Department of Entomology, and Mr. Wilson.

REPORT FROM DR. DONALD M. MCLEAN
THE HOSPITAL FOR SICK CHILDREN
TORONTO 2, ONTARIO, CANADA

During 1962, two strains of Powassan virus have been isolated from specimens obtained about 12 miles west of Powassan, Ontario. Strain #1427 was isolated from a pool of 35 Ixodes marxi ticks collected from a red squirrel, Tamiasciurus hudsonicus on 29 August, and strain #1828 was recovered from blood clot of a squirrel collected on 3 October. Powassan neutralizing antibody was found in 3 of 12 squirrels collected during October and 2 of those with antibody carried I. marxi ticks, but no antibody was detected in 16 squirrels obtained in April, July, and August. These findings strongly suggest that Powassan virus is maintained in nature by a cycle involving Ixodes ticks and squirrels.

A strain of Silverwater virus (#1081) was isolated from a pool of about 50 Haemaphysalis leporis-palustris (H. L. P.) ticks collected from a snowshoe hare, Lepus americanus, about 8 miles east of Powassan on 10 July 1962. Silverwater neutralizing antibody was detected in sera of 2 snowshoe hares captured during July and August, but not in 2 hare sera collected during April 1962. Silverwater complement fixing antibody was found in sera from 4 of 13 hares captured near Powassan during 1960. These findings suggest that Silverwater virus is maintained in the Powassan district as well as on Manitoulin Island, by a natural cycle involving H. L. P. ticks and snowshoe hares.

REPORT FROM DR. RICHARD O. HAYES
TAUNTON FIELD STATION OF THE ENCEPHALITIS SECTION
COMMUNICABLE DISEASE CENTER, USPHS
AND THE DIVISION OF COMMUNICABLE DISEASES
MASSACHUSETTS DEPT. OF PUBLIC HEALTH

A survey of migratory birds for EE and WE virus was conducted from March 30 to June 1, 1962. Blood samples from 187 birds were collected. All blood samples were tested and found to be negative for virus. Thus, no evidence was obtained from this sample that migratory birds from the south introduced EE or WE virus into Pine Swamp during 1962. Negative results also were obtained from similar spring surveys of 60 birds in 1960 and of 223 birds in 1961.

A total of 574 blood specimens were collected from wild birds during the period June 5 to October 9, 1962. Of this total, 390 were

collected from catbirds, 171 from chickadees and 13 from other species. Laboratory tests for virus have been completed on all of the samples. WE virus was isolated from the blood samples of 4 catbirds captured in Pine Swamp and from the blood of a chickadee captured at an upland study site. No EE virus was isolated.

Blood samples were taken 3 times per week during August 1962 from a swamp site sentinel chicken flock. A total of 415 blood specimens was obtained and tested for virus with negative results. Three of the previously mentioned WE virus isolations from catbirds were obtained from 193 catbird blood specimens collected in that swamp (Pine Swamp) during the same month. The negative results obtained from the sentinel chicken flock during August could be the result of reduced contact with Culiseta melanura for a review of the monthly mosquito population indices obtained in the shed traps at the sentinel flocks located in swamps revealed that the peak C. melanura populations during 1960, 1961, and 1962 occurred during June and then gradually declined each succeeding month.

Following the initial success reported last year (Arboinfoexchange No. 5) on control of C. melanura using granular insecticide formulations, a pre-season application of heptachlor granules was applied from an airplane to a 22-acre swamp at the rate of 2 pounds per acre. The pre-season application, made on March 28, 1962, was found to be unsuitable for controlling the mosquito breeding in the swamp for the entire summer, so the swamp was retreated on August 17, 1962. Larva control was attained for about a month following the second treatment. The adult C. melanura population indices, obtained during the study from shed-trap mosquito collections made at a sentinel chicken flock located at the edge of the swamp, were low except during the first 2 weeks in June. The mosquito larva indices in the swamp during May and early June were very low, so apparently the large numbers of C. melanura present the first 2 weeks in June migrated into the treated swamp from untreated areas.

REPORT FROM DR. ELINOR WHITNEY
DIVISION OF LABORATORIES AND RESEARCH
NEW YORK STATE DEPT OF HEALTH, ALBANY

Continuing Arbovirus Survey in New York

Isolations

Surveillance for the presence of arboviruses was continued in 1962 in Suffolk County. Arthropods were collected during July and August and birds were trapped during the spring migration and from the summer

resident population. One pool (25 insects) of Culiseta melanura of 116 arthropod pools yielded an agent which had properties similar to the six 1961 isolates. No agents were recovered from 212 spleens of birds from the spring migration and 164 spleens from birds in the summer population. Serologic evidence is being sought for these new agents by examining wild animal and human sera in neutralization tests.

Demonstration of Bunyamwera group antibodies in animal sera.

Two hundred and fifty-two animal and bird sera (2 bat pools representing 29 animals, 4 bear, 14 deer, 88 fox, 1 horse, 4 muskrat, 6 Microtus pennsylvanicus pools representing 30 animals, 2 opossum, 1 pheasant, 27 raccoon, and 103 rabbit) and three human sera were examined in neutralization tests with a strain (V62-7364) of Cache Valley virus, originally isolated by Holden and Hess[1] from Culiseta inornata collected in Utah. The Cache Valley virus belongs to the Bunyamwera group of arboviruses.

The technic of the test is similar to that previously described by us except that the intracerebral instead of the intraperitoneal route of inoculation was used. Although the samplings were small, 14 reactions were obtained: 8 (3.2%) gave positive results, 6 (2.3%) gave inconclusive findings. Antibody to Cache Valley virus has been demonstrated in animals trapped in Albany, Dutchess, Hamilton, Schuyler, Seneca, and Steuben Counties, as early as 1956 and as late as 1961.

Reference:

[1]Holden, Preston, and Hess, A.O. Cache Valley virus; a previously undescribed mosquito-borne agent. Science, 1959, 130, 1187.

REPORT FROM DR. JORDI CASALS
ROCKEFELLER FOUNDATION VIRUS LABORATORIES, NEW YORK

Antigenic Relationships Among Viruses Isolated from Ticks.

Under this heading are included viruses isolated from ticks and not belonging to the Russian tick-borne complex of group B. Twenty-one strains of such agents are under study, to determine possible antigenic relationships among themselves as well as with the remaining arboviruses. In Table 1 these agents are listed with their place or origin and the ticks from which the viruses were isolated.

The data for inclusion among the arboviruses vary with the strains, some being better studied than others; the viruses have all been isolated from ticks and, in addition, some of their strains, from man, other vertebrates and mosquitoes. They are all, or as many as have been tested, susceptible to the action of sodium desoxycholate; transmission by tick bite has been observed with a few of the viruses.

Only one of them, Colorado tick fever, has had any human medical importance to date. However, there is an increasing interest in these viruses for several reasons. Some have been isolated in ticks which transmit other viruses pathogenic for man or domestic animal, i.e., Dalcairnie in Ixodes ricinus which is also the vector of louping ill virus. It is not unlikely that in trying to isolate from ticks virus pathogens of man, an increasing number of strains similar to the ones studied here will be uncovered. It was considered, therefore, that it would be helpful to establish a frame of reference by studying the immunology of these tick-borne viruses. Another reason for their study is, of course, their potential role as cause of disease in man.

Determination of cross-reactions among these viruses has been done only by means of complement fixation, in this laboratory; no hemagglutinating antigen has been obtained with any of them using the standard procedures with the exception of Dalcairnie and IG 619. With these agents a low titer antigen has been obtained, 1:40 to 1:80; however, this has been a recent development and no use has as yet been made of the antigens for further study.

The immune sera derived from mice immunized by intraperitoneal inoculation of 10^{-1} suspensions of active virus, usually 0.5 cc. As much as possible the following schedule was followed for the injections and bleeding; injections were given on days 1, 25-30 and 50-60. The mice were bled 7 or 8 days after the second and third injection; the results shown in the accompanying tables were given by the sera obtained after the third injection of virus.

Exceptions to this schedule were with an occasional virus which caused high mortality of mice following the first injection of live virus, in which case inactivated virus was used. Also with Nairobi sheep disease virus with which no immunizations could be carried out in this laboratory owing to restrictions to importation of the agent; a mouse immune serum was supplied in this case by Dr. M. P. Weinbren with a stated titer of 1:256.

The plan of study was to test immune sera for each strain against all the antigens, both in serial two-fold dilutions beginning at 1:4. In addition, immune sera against the tick-borne viruses are tested against all hemagglutinating antigens available in the arboviruses.

The tests are not yet completed, but a number of results have been so far accumulated, as shown here.

An interesting thing is that among these agents not a single clear-cut instance of cross-reaction between viruses has been so far observed; whenever agents cross-reacted they did so to full or nearly full titer both of serum and antigen. Consequently, these cross-reactions proved that different isolates of the same virus were being compared. This statement applies only to the results of the complement fixation tests; whether strains which are identical by that test may be distinguishable by neutralization test will be investigated later on.

Twelve distinct, unrelated viruses have been so far established on the basis of these tests; they are listed here with the strains of each included in the survey: Quaranfil (EG AR 1095 used as prototype, SA AN 4671, SA AR 2339), Chenuda (EG AR 1152 prototype, SA AR 3441), EG AR 1304 (EG AR 1304 prototype, Nyamanini or SA AN 2526), EG AR 492 (EG AR 492 prototype, IG 673), IG 619 (IG 619 prototype, IG 3159), IG 690, IG 700 (IG 700 prototype, IG 758), Silverwater, Colorado tick fever, Hughes, Dalcairnie and Nairobi sheep disease.

Of these 19 strains, studies with IG 3159 have proceeded only to the point of indicating that it is probably another strain of IG 619; observations with Nairobi sheep disease are limited due to the fact that the virus may not be imported into this country. That SA AN 4671 and SA AR 2939 were closely related to or indistinguishable from Quaranfil and SA AR 3441 was similarly related to Chenuda had already been determined by the staff of the virus laboratory at Johannesburg. Also, Dr. Leo Thomas at the Rocky Mountain Laboratory, Montana, had found a close relationship between SAAN 2526 and EG AR 1304. Studies here with these strains confirmed these relationships.

Two additional viruses, Sogoto and Kaisodi (IG 14132) are being included in this study but no results are as yet available.

The results of this study are best seen in Table 2, in which it is observed that with serum titers as high as 1:512 and antigen titers as high as 1:1028, no cross reactions were observed. It is to be noted that each of the strains of the different viruses listed above was included in

the study; only the results with the prototype is reported in Table 2 for the sake of simplicity.

The closeness of the cross-reactions among strains of a given virus and on which the conclusions were reached that these were, at least by complement-fixation test, isolates of one virus can be judged by the results reported in Tables 3 and 4.

Determination of possible immunological relationships between these tick-borne viruses and the rest of the arboviruses has been done by testing immune sera of the tick-borne viruses for the presence of antibodies against hemagglutinating antigens. Thus far, no trace of cross reaction has been found. Immune sera for the following strains have been tested: EG AR 492, IG 473, EG AR 1095, SA AN 4671, SA AR 2939, EG AR 1152, SA AR 3441, EG AR 1304, IG 690, Colorado tick fever and Silverwater. The hemagglutinating antigens against which these sera were tested included 11 from group A, 13 from group B and in addition antigens for: Bunyamwera, Ilesha, Germiston, Tahyna, Marituba, Oriboca, Caraparu, California encephalitis, Bwamba, Sathuperi, AMM 2549, AMM 2325, Sicilian sandfly fever, Neapolitan sandfly fever, Akabane, Witwatersrand, Manzanilla, Koongol, Umbre, SA AN 4165 and Tacaiuma.

A final remark has to do with the relationship among the viruses Quaranfil, Chenuda, and EG AR 1304. Observations by other workers seemed to indicate the possibility that there was among them a distant relationship; however, it has been impossible in the present work to find any crossing by complement fixation between 3 strains of Quaranfil, 2 of Chenuda, and 2 of EG AR 1304. This is the reason why these viruses are listed among the ungrouped ones.

Table 1

Tick-borne Viruses

Name or number	Strain	Place of origin	Isolated from
Quaranfil	Eg AR 1095	Egypt	Argas persicus
	SA AN 4671	Union South Africa	Cattle egret
	SA AR 2939	Union South Africa	Argas persicus
Chenuda	Eg AR 1152	Egypt	Argas hermanii
	SA AR 3441	Union South Africa	Argas peringueyi
Eg AR 1304	Eg AR 1304	Egypt	Argas Persicus
	Nyamanini	Union South Africa	Cattle egret
Eg AR 492	Eg AR 492	Egypt	Rhipicephalus sanguineus
	IG 673	India	Hyaloma aegypticum
IG 619	IG 619	India	Haemaphysalis parva
	IG 3159	India	Mosquitoes
IG 690	IG 690	India	Haemaphysalis parva
IG 700	I 700	India	Hyaloma aegypticum
	IG 758	India	Culex fatigans
Silverwater	McLane	Canada	Haemaphysalis leporis-palustris
Colorado Tick Fever	Condon	USA	Man
Hughes	Hughes	USA	Ornithodoros capensis
Dalcairnie	Gordon Smith	Great Britain	Ixodes ricinus
Nairobi Sheep Disease		East Africa	Sheep
Kaisodi	IG 14132	India	Haemaphysalis spinigera
Sogoto	Sogoto	East Africa	Mixed ticks pool

Table 2

Complement-fixation test with viruses isolated from ticks

Antigen No.	SERUM 1	2	3	4	5	6	7	8	9	10	11	12
1. Quaranfil	512/16	0	0	0	0	0	0	0	0	0	0	0
2. Chenuda	0	128/32	0	0	0	0	0	0	0	0	0	0
3. EG AR 1304	0	0	256/512	0	0	0	0	0	0	0	0	0
4. EG AR 492	0	0	0	256/64	0	0	0	0	0	0	0	0
5. IG 619	0				256/64					0	0	
6. IG 690	0	0	0	0	0	512/256	0	0	0	0	0	0
7. IG 700	0			0	0		512/256			0	0	
8. Silverwater	0			0	0			512/512		0	0	
9. Colorado TF	0	0	0	0	0	0	0	0	64/128	0	0	0
10. Hughes	0			0	0					256/256	0	
11. Dalcairnie	0			0	0						256/1028	
12. Nairobi SD												256/?

Reciprocal of serum titer/Reciprocal of antigen titer; 0 indicates no reaction with dilutions 1:4 of serum and antigen, lowest used.
Blank spaces indicate combination not yet tested.

Table 3

Complement-fixation test with strains of Quaranfil virus

Antigen	Serum		
	1095	4671	2939
EG AR 1095	512/16	256/16	256/16
SA AN 4671	512/16	256/16	256/16
SA AR 2939	512/32		256/32

Footnote: see Table 2.

Table 4

Complement-fixation test with strains of IG 700 virus

Antigen	Serum	
	700	758
IG 700	512/256	256/512
IG 758	512/32	256/32

REPORT FROM DR. LORING WHITMAN ROCKEFELLER FOUNDATION VIRUS LABORATORIES, NEW YORK

As part of a study to explore in more detail the relationship of South American (Be Ar 7272) and North American (Holden 6V-633) isolates of Cache Valley virus, we were fortunate to have received from the CDC two isolates from Florida, A9-171b and Fl 100S, which had been shown by Dr. P.H. Coleman to be closely related to both Cache Valley and Chittoor (IG 20217) viruses by CF, NT, and HI. Initial HAI tests, as shown in Table 1, indicated that the two Florida isolates were closely related to one another, but sharply distinct from either the Be Ar 7272 strain of Cache Valley virus or the IG 20217 strain of Chittoor (Batai) virus.

Subsequent to these tests, hemagglutinating antigens were prepared by the sucrose-acetone method for the Holden 6V-633 strain of Cache Valley as well as for Fl 100S from Florida. These antigens were of low titer and were considered to be inferior in sensitivity to the antigens used in Table 1. Using these antigens, cross HAI test between A9 171b and Fl 100S indicated them to be the same. However, their sera were no more reactive with Holden 6V-633 antigen than with Be Ar 7272 antigen. Thus, the Florida strains are quite distinct from both of the listed strains of Cache Valley virus in HAI tests.

The relationship between Be Ar 7272 and 6V-633 requires further study. Table 2 gives results of cross HAI tests between the two which indicate that there is a difference, though slight, between them. Of particular interest is the result of tests using the serum of a rabbit immunized with 6V-633. As can be seen, it barely inhibited Be Ar 7272 antigen, yet inhibited the homologous antigen in a dilution of 1:160. This same specimen failed to inhibit A9 171b antigen in a dilution of 1:20.

CF studies on these agents confirmed the findings from CDC, namely, that Cache Valley virus (either Be Ar 7272 or 6V-633), Chittoor virus and the A9 171b were indistinguishable in this test.

A single attempt at cross neutralization testing was done by Dr. Buckley in HeLa cells. While this indicated measurable differences between Be Ar 7272 and 6V-633, and somewhat greater differences between these and A9 171b, further experiments will be necessary to confirm these findings and to establish more accurately the degree of differentiation between these agents.

Table 1

Cross HAI tests between A9 171b and F1 100S and Cache Valley virus (Be Ar 7272) and Chittoor (IG 20217)

Sera		ANTIGENS Be Ar 7272	A9 171b	IG 20217
Be Ar 7272	#1	80	20	20
	#2	1280	160	160
A9 171b	#1	0	2560	-
	#2	0	80	0
	#3	10	320	10
F1 100S	#1	10	320	10
	#2	10	320	20
	#3	20	320	10
Amm 2222 (Batai)	#1	40	40	320
	#2	160	40	1280
	#3	40	40	320

Table 2

Cross HAI tests between Cache Valley virus strains Be Ar 7272 and Holden 6V-633

Sera		ANTIGENS Be Ar 7272	6V 633
Be Ar 7272	#1	1280	160
	#2	320	80
	#3	160	10
	#4	160	10
6V 633	#1	0	20
	#2	80	320
	#3	80	160
Rabbit sera		10	160

REPORT FROM DR. W. MCD. HAMMON
DEPARTMENT OF EPIDEMIOLOGY AND MICROBIOLOGY
UNIVERSITY OF PITTSBURGH SCHOOL OF PUBLIC HEALTH
PITTSBURGH, PENNSYLVANIA

I. Live Attenuated Japanese B Encephalitis Virus Vaccine from Strain OCT-541

A. Intracerebral inoculation of monkeys

1. One monkey given undiluted vaccine pool died of encephalitis after a very prolonged incubation period and illness. The virus recovered had some markers of the virulent and some of the attenuated line. Partial reversion had thus occurred by direct i.c. inoculation of about 10^7 TCD_{50}.

B. Cancer Volunteers given the 1:100 and 1:1000 dilutions of vaccine, some combined with hyperimmune JBE gamma globulin failed to develop viremia and antibodies. The last two patients (35 days observation) given a 1:20 dilution have remained well, with no detectable viremia and no antibody on the 30th day. Dosage is to be increased in the next pair hoping to get again the response of the original pair given a 1:10 dilution.

II. Japanese B Encephalitis Vaccine, Inactivated Tissue Culture Type.

The potential of OCT-541 attenuated strain grown in hamster kidney tissue with formalin inactivation is being explored. Production titers in a synthetic medium plus human albumin are apparently adequate and inactivation with formalin at 1:4000 at 30°C has been selected for first antigenicity trials.

III. Duration of JBE Antibody Following Inoculation of Japanese B Encephalitis Hyperimmune Gamma Globulin.

A third volunteer given 0.13 ml/kg again showed antibody with peak titer at about 1 week (2.8 log N.I.) and persisting for 35 to 50 days (1.6-1.2 log N.I.), suggesting probable usefulness of passive protection for reasonable time periods.

IV. Philippine Hemorrhagic Fever.

Retesting of convalescent sera of 1956 epidemic demonstrated that chikungunya virus played no role in that epidemic. Inclusion of multiple dengue antigens in serological tests failed to determine infecting dengue virus types. Virus isolations alone determined this.

V. Thai Hemorrhagic Fever

A. Bangkok 1960.

1. Virus isolations from patients in a mission hospital in the Bangkok 1960 epidemic included dengue types 1, 4, TH-36 and several strains of mouse hepatitis related viruses, probably contaminants.

2. Serological tests of several types and with many antigens were completed on 78 serum pairs. Chikungunya virus played no important role in this series, probably none. HAI, CF, and neutralization gave excellent agreement. It is concluded that CF is the simplest and most useful diagnostic test.

B. Field Investigation 1961. Mosquitoes have been tested and several virus isolations made, some probably dengue and others mouse hepatitis related (? contaminants).

VI. Dengue Viruses.

A. Typing. This has been a major undertaking and is as yet incomplete. We are still uncertain whether TH-36 and TH-Sman prototypes represent types 5 and 6 or should be considered types 2 and 1 respectively.

B. Laboratory Infection.. Following 2 years after a probable TH-36 dengue infection, a type 4 subclinical infection occurred in the same person. Serological changes were of an unexpected type. Prior to infection CF had fallen to negative for all types except TH-36 (1:4) and HAI was at a level of 1:160 to 1:320 for all types. Then HAI only rose and only for type 4. A still later serum still showed no CF rise. Since the D-4 virus was at about the 26th mouse passage level, this may explain the failure to develop CF antibodies.

C. Fluorescent antibodies. Frozen mouse brain impression smears give positive and specific results by the indirect CF method, but non-specific reactions occur with the regular indirect method.

D. Tissue Culture.

1. Interference tests with EEE and JBE in CETC plaque and in hamster kidney cell (HKC) monolayer CPE tests were found to substitute for lack of good CPE by most dengue viruses. Neutralization tests can be performed.

2. Plaques were obtained with dengue 2 by HKC double agar overlay.

VII. EEE Virus in Thailand.

Strains believed isolated from Bangkok mosquitoes show no detectable antigenic differences from a New Jersey strain by several special tests. Reisolation had been obtained from one of the original frozen mosquito pools.

VIII. California Virus Purification

Terminal dilution purification did not significantly change the characteristics. Tissue culture with CPE was successful (hamster kidney and chick embryo). Immune ascitic fluid was produced in mice with a variety of procedures in order to select an optimal one.

REPORT FROM DR. DAVID E. DAVIS
PENNSYLVANIA STATE UNIVERSITY
DEPARTMENT OF ZOOLOGY AND ENTOMOLOGY
UNIVERSITY PARK, PENNSYLVANIA

With the assistance of a training grant, a manual is being prepared for the study of bird populations that are involved in outbreaks of arbovirus infection. The manual is in the first-draft stage, based on preliminary work last summer, and will be used during this summer and revised. At this stage, it has been sent to a few persons for their comments. It is hoped that a distribution of a mimeographed form can be made sometime in a year for more extensive criticism.

REPORT FROM DRS. HERBERT C. BARNETT AND FRED MCCRUMB
INTERNATIONAL CENTER FOR MEDICAL RESEARCH AND TRAINING
UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE, BALTIMORE

During 1962, over one-third of a million mosquitoes were collected and processed for virus isolation in West Pakistan. This material is being processed at the University of Maryland School of Medicine and at the Walter Reed Army Institute of Research, Washington, D.C., by odd and even numbered lots respectively. Virus isolation has not proceeded far enough to indicate results at this time.

Analysis of mosquito collections has been made for the period May to September, 1962. The only human biting mosquito collections undertaken on a regular basis were morning and evening collections made in Lawrence Gardens, a park in the center of the city of Lahore. The results indicate that Aedes (Stegomyia) mosquitoes were the predominant species attacking man in this situation. Resting collections made in houses in the city of Lahore presented quite a different picture as will be seen in Table 2 where Culex mosquitoes predominated. However, in resting collections made in houses in rural areas near Lahore, Table 3, still a different picture was found, with Anopheline mosquitoes predominating. The mosquito found resting in houses most frequently in rural areas is the important malaria vector Anopheles culicifacies. In cattle biting collections made in the same rural areas, mosquitoes of the Culex tritaeniorhynchus complex predominated and actually constituted about 84 per cent of all mosquitoes captured on cattle. It is of interest to note that while Anopheles culicifacies and Anopheles stephensi predominate in houses of this area, they were taken in relatively small numbers from cattle, thus possibly indicating strong anthropophilic tendencies. The importance of the Culex tritaeniorhynchus complex as vectors of Japanese encephalitis is well established not only in Japan but in many areas of Asia. The frequency with which these mosquitoes were captured in the Lahore area is, therefore of particular interest. As indicated in the previous report from the Division of Infectious Diseases, University of Maryland School of Medicine (Dr. McCrumb), over twelve per cent of serum specimens studied from human populations of Pakistan indicated the presence of neutralizing antibodies to Japanese encephalitis virus. The greater frequency of neutralizing antibodies to West Nile virus may simply indicate cross reactivity, but it could also represent independent viral activity. The results of virus isolation work with arthropod material should clarify these questions.

Table I. Human biting collections
Lawrence Gardens, Lahore, W. Pakistan
May-September, 1962
(1,948 man-hours collecting)*

Species	Number collected
Aedes albopictus	1,647
Aedes w-albus	1,135
Aedes unilineatus	651
Culex tritaeniorhynchus complex	352
Aedes thomsoni	189
Other spp.	25
Total	4,999

*Collections made five days each week during hours 0730-1000 and 1600-2000 inclusive.

Table 2. Resting collections in houses
Lahore, W. Pakistan, May-September, 1962
(147 collections)

Species	Number collected
Culex fatigans	2,496
Culex tritaeniorhynchus complex	582
Other species	32
Total	3,110

Table 3. Resting collections in houses of
two villages in the Punjab, near
Lahore, W. Pakistan, May-September, 1962
(140 Collections)

Species	Number collected
Anopheles culicifacies	14,295
Anopheles stephensi	10,112
Anopheles subpictus	6,728
Anopheles pulcherrimas	687
Other anopheline spp.	93
Culex tritaeniorhynchus complex	7,730
Culex fatigans	356
Other culicine spp.	6
Total	40,007

Table 4. Cattle biting collections in two villages in the Punjab, near Lahore, W. Pakistan, May-September, 1962 (139 collections)

Species	Number collected
Anopheles pulcherrimus	13,473
Anopheles subpictus	2,728
Anopheles stephensi	1,795
Anopheles hyrcanus	691
Anopheles annularis	273
Anopheles culicifacies	172
Culex tritaeniorhynchus complex	104,560
Culex epidesmus	329
Culex bitaeniorhynchus	98
Culex fatigans	40
Mansonia spp.	264
Other culicine spp.	343
Total	124,666

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

Serological evidence for Groups B and C arthropod-borne virus infections of man in Puerto Rico. (Dr. C. L. Wisseman, Jr.)

During the summers of 1960 and 1961, two medical students at this medical school, residents of Puerto Rico, were given summer fellowships from this department to collect human sera from residents of various parts of the island. Laboratory facilities for processing the sera were made available through the generosity of Dr. Oliver Gonzales of the Medical School of the University of Puerto Rico. Through their efforts, 529 sera were collected from different age groups in various localities. Because Dr. Alexis Shelokov and his group at the Laboratory of Tropical Virology, National Institute of Allergy and Infectious Diseases, had also acquired a collection of 149 adult sera from Puerto Rico collected in 1961 through a different mechanism, it was agreed to combine the collections, totalling 678 specimens, and to divide the work of testing them between the two laboratories. To begin with, this laboratory agreed to test the sera for HI antibodies to Groups B and C arthropod-borne viruses while Dr. Shelokov's laboratory would test for antibodies to Group A and the Bunyamwera Group. When completed, the work will be published jointly as a collaborative effort. One of the purposes has been to provide information regarding the general kinds of arthropod-borne viruses which might be affecting the human population of Puerto Rico and to identify areas of unusual virus activity. Dr. Paul Weinbren, who is developing a program for virus isolation there, has tentatively agreed to furnish some additional specimens, especially from the southern parts of the island, to improve the sampling of the different geographic and ecological regions and to aid in his selection of study areas.

We have recently completed testing of the entire collection at hand now with 10 Group B hemagglutinins (D-1, D-2, D-3, D-4, D-5, D-6, YF, Ilheus, SLE and Langat (TP-21)) and 3 Group C hemagglutinins (Marituba, Oriboca, Apeu) but have not yet analyzed them beyond simple age group distribution without regard to distribution on the island. Because even these results are of considerable interest, a brief account is given here. Dr. Shelokov's laboratory will report the results of their studies to this infoexchange at another time.

Most striking among the Group B viruses were the reactivity patterns with the dengue viruses. A high proportion of positive reactors with one or more dengue viruses was found in all age groups, beginning

with the 0-4 year group; however, the per cent positives with the different dengue viruses changed markedly with age. Thus, below 10 years of age, none were positive with type 1 and only a very few with type 2 and type 6 antigens. However, between 27 and 56 per cent were positive with types 3 and 4 dengue antigens and about half as many were positive with type 5. No positive reactions were detected with other Group B antigens (YF, SLE, and Ilheus) in this young age group, except for a single serum positive with the TP-21 antigen. These results suggest that types 1, 2, and 6 dengue viruses have not been active in the areas sampled in recent years, but that some other Group B virus, more closely related to types 3 and 4 dengue than to any of the other viruses tested, has been actively transmitted in very recent years.

The reaction pattern among the dengue antigens changes rather abruptly with increasing age above the 15 year age group, with a high proportion of reactors (up to 68 per cent in one instance) to all dengue antigens, including types 1 and 2 and types 5 and 6 (which cross-react strongly with types 1 and 2). This would coincide with clinical dengue recognized in the Caribbean area during the WW II period and before. Yellow fever antigen reactors begin with the 20-year-old group. Since yellow fever has not been recognized in the area for many years, it may be that these represent cross-reactions in most instances or possibly active immunization in some. Only 6 per cent of the entire set of 678 specimens were positive with the Ilheus antigen and these were in the older age groups which were highly reactive with the dengue antigens. Reactors with SLE and TP-21 antigens were sparse and scattered (2 and 0.7 per cent respectively).

Group C reactors were rare, contributing only 0.1 per cent of the entire set and found largely in children under 10 years of age. Nevertheless, these positive results establish the probable presence of Group C viruses on this island.

The influence of divalent cations on adsorption of West Nile virus to chick embryo monolayer cultures. (Dr. O.R. Eylar)

The in vitro assay of West Nile virus in chick embryo cultures has been modified with resultant sensitivities equal to or greater than those obtained with the suckling mouse system. Most significant was the recognition of an abnormally high divalent cation requirement for optimal adsorption of virus to host cells under the conditions of assay.

Initial studies with this virus indicated that maximal stability of virus occurred at approximately pH 8.5; however, plaque count assays carried out at that pH, using various buffer-diluent mixtures, resulted in maximal titers less than 1 per cent of those obtained in mouse titrations. Peculiarities in the results of assays at various pH's suggested that perhaps required cations were removed from solution in the reaction mixture. To investigate this possibility, the influence of divalent cation (barium, calcium, and magnesium) concentration on final plaque count was studied. Maintaining the pH and virus inoculum constant, while varying the cation concentration, it was found that 0.05-0.1 M magnesium ion concentration resulted in the highest plaque counts. Comparison of the various cations indicated that barium ion was the least effective of those tested, magnesium ion gave the highest counts, with calcium ion resulting in counts only slightly lower than those obtained with magnesium ion.

An increase in plaque counts could not be demonstrated when equivalent concentrations of monovalent cations, trivalent cations, monovalent anions or divalent anions, in the form of NaCl, KCl, $FeCl_2$, $ZnCl_2$, $AlCl_3$, $Na2SO_4$, K_2SO_4 salts, were added to the adsorption mixture.

Rate of adsorption of West Nile virus to chick embryo monolayers was markedly influenced by magnesium ion concentration. At a final concentration of $1x10^{-3}$M or $1x10^{-4}$M magnesium ion, attachment was very slow and had not plateaued by 4 hours incubation. An increase in attachment was noted at $1x10^{-2}$M magnesium ion; however, attachment was not complete for at least 3 hours. At $1x10^{-1}$M magnesium ion, attachment was rapid and a plateau attained in 45-60 minutes.

In view of the very high ionic strength of the buffer-cation diluent, it has been our experience that adsorption should not exceed 90-120 minutes (well into the adsorption plateau) since extended exposure of the cell monolayers to this mixture has a deleterious effect on the cells.

Once adsorption is complete, the monolayers are overlaid with a medium composed of 5 per cent calf serum, 0.1 per cent yeastolate, Hanks' BSS, 0.8 per cent Noble agar and sodium bicarbonate added to a final pH 7.4-7.5. After 3-4 days incubation at 37°C, a second agar overlay containing 1:15,000 neutral red is added and the bottles incubated at 37°C in the dark for 12-18 hours. Employing this technic, plaques, 2-4 mm in diameter, are readily counted enabling one to complete the assay in 4-5 days instead of the usual 14-21 days required in mouse titrations.

REPORT FROM DEPARTMENTS OF VIRUS DISEASES AND ENTOMOLOGY WRAIR, DEPARTMENT OF VETERINARY SCIENCE, UNIVERSITY OF MARYLAND, AND WILDLIFE DISEASE LABORATORY PATUXENT REFUGE

Recovery of Arboviruses from Mosquitoes of Assateague Island

Study of arbovirus ecology on the Chincoteague-Assateague Island complex was continued during Spring-Fall, 1962, with emphasis being placed upon identifying EEE and Cache Valley viruses in naturally occurring arthropods and establishing the natural vertebrate host range of Cache Valley virus in the area, and in the states of Maryland and Virginia. As in 1961, there was again little or no evidence for dissemination of EEE virus; none was recovered from over 90,000 mosquitoes collected between 27 April and 29 October (Table I), and there was no overt disease in man or equines during this interval. However, serological evidence of infection of a single catbird (Dumetella carolinensis) with EEE virus was obtained; this adult bird, first netted on the south end of Assateague Island, was found without antibody on 7 May 1962. When recaptured 8 miles north on 11 July, it was found to have developed neutralizing antibody (LNI $\geq$ 2.0). Of 1327 avian plasmas examined for neutralizing antibody to EEE virus, only 19 were found positive (LNI $\geq$ 1.7). These included Catbird (14), Blue Jay (2), Brown Thrasher (1), Black-polled Warbler (1), and Yellow-breasted Chat (1). Plasmas from 14 additional birds (8 species) gave equivocal tests. Serological study of mammalian and reptilian plasmas collected concurrently is in progress.

While no EEE virus was recovered from mosquitoes collected, 9 viruses, pathogenic for suckling mice, were recovered from Ae. sollicitans, C. salinarius and An. bradleyi-crucians complex (Tables I, II). It should be noted that Ae. sollicitans and An. bradleyi-crucians complex yielded Cache Valley-like viruses in 1961 (ABVIE #6, October 1962). Further, continued study suggests that our identifications of An. crucians in 1961 were not accurate. The adult females of 3 species An. crucians, bradleyi, and georgianus cannot be distinguished. Larvae reared from eggs obtained from adult anophelines collected on Assateague in late September, 1962, were identified as An. bradleyi. An. crucians has been reported from the area in the past, and for these reasons the term "Anopheles bradleyi-crucians complex" will be used to identify these potentially mixed collections of adult females.

While the 1962 viruses are as yet incompletely identified, at least 6 appear to have the propagation characteristics of Cache Valley virus and at least one other is different from the 1961 mosquito viruses from

Assateague. (Drs. F. Scheider, D. Gould, R. Byrne, and E. L. Buescher and Lt. Eugene G. Thompson).

Evidence for dissemination of Cache Valley Virus in Maryland and Virginia.

The occurrence of Cache Valley virus on Assateague Island prompted serological surveys of vertebrate populations of the area, and later of Maryland and Virginia for antibody to the Assateague Island strains of this virus. It was originally shown that the incidence of neutralizing antibodies was high in the wild ponies of Assateague (ABVIE, Oct 1962). This observation prompted investigation of equines and other ungulates resident in other parts of tidewater Maryland and Virginia, and eventually to other portions of the state, and other wild animals. The results of such surveys are summarized in Tables III and IV. Horses and cattle resident in tidewater areas apparently are regularly infected with this virus, and infection frequency appears to decrease in animals resident in higher country. Thus, fewest reactors are found in cattle reared in Garrett County, western Maryland. Goats and sheep of Montgomery County, Maryland, near Washington, D.C., also show evidence of infection with Cache Valley virus; pigs in Queen Anne's County are less frequently involved. From Table IV, it will be noted that wild carnivores of either tidewater or Piedmont, Virginia, are infrequently found with antibody; however, 4 of 10 white-tailed deer from mountainous Virginia possessed antibody.

Man in tidewater areas also possesses antibodies to this virus (Table V). The highest frequency of positive reactors has been found thus far in the residents of Chincoteague, Virginia. Like the data with domestic animals, the frequency of positive reactors appears to decrease in other than tidewater counties, the exceptions being Frederick and Montgomery Counties, Maryland.

Thus while disease in neither man nor animals has as yet been associated with Cache Valley virus, it would appear from preliminary tests that this agent has been rather widely disseminated among ungulates and man resident in tidewater Maryland and Virginia for several years. Evidence for natural infection of wild animals and birds tested thus far is minimal; this suggests, but does not establish, that the important natural transmission cycle involves saltmarsh mosquitoes and large vertebrates and man. Further investigation of the ecology of this virus is in progress. (Drs. F. Schneider, E. L. Buescher, and R. Byrne).

TABLE I

Virus isolations from mosquitoes collected on Assateague and Chincoteague Islands, Virginia, during 1962

	Total specimens	No. pools tested	No. pools positive
Aedes sollicitans	79,851	856	7
Culex salinarius	8,187	118	1
Anopheles bradleyi-crucians complex ↓	2,626	48	1
Aedes taeniorhynchus	2,024	42	0
Aedes cantator	1,167	23	0
Psorophora ciliata	9	3	0
Aedes vexans	9	1	0
Culiseta inornata	1	1	0
Total	93,874	1,092	9

↓ Adult females of the two species are indistinguishable; see text.

TABLE II

Arthropod Pools Yielding Viruses Collected
On Assateague Island, 1962

Mosquito Pool	Species	Pool Size	Date	Collection Type	Area
M784/62	Ae. sollicitans	100	7/11	Aspirated from man	Assateague Is.
M875/62	Ae. sollicitans	100	7/23	" "	" "
M911/62	Ae. sollicitans	100	7/30	Chicken house	Chincoteague Is.
M1014/62	Ae. sollicitans	100	8/13	Aspirated from man	Assateague Is.
M1035/62	Ae. sollicitans	100	8/15	" "	" "
M1410/62	Ae. sollicitans	100	9/25	" "	" "
M1439/62	Ae. sollicitans	47	10/1	Opossum-baited trap	" "
M1441/62	C. salinarius	108	10/1	" "	" "
M1533/62	An. bradleyi-crucians complex	100	10/12	" "	" "

TABLE III

Occurrence of Antibody to Cache Valley-like viruses in Domestic Animals of Maryland and Virginia

Species	Area [1]	Date	Frequency of Positive Reactors
Horses	Pungoteague-Keller, Va. (T)	June 1957	16/16
"	Onancock, Va. (T)	June 1957	6/10
"	Chincoteague, Va. (T)	June 1957	14/14
"	Assateague Is.,Va.(T)	June 1957	5/5
"	" " " (T)	May 1961	22/23
"	Chincoteague, Va. (T)	Oct 1962	20/20
Cattle	St.Mary's County,Md.(T)	Oct 1962	18/29
"	Talbot County, Md. (T)	Oct 1962	11/20
"	Carroll County,Md. (TP)	Nov 1962	6/20
"	Prince Georges Co., Md. (TP)	Nov 1962	12/15
"	Howard County, Md. (P)	Nov 1962	9/15
"	Montgomery Co.,Md.(P)	Nov 1962	3/4
"	Garrett Co.,Md. (M)	Nov 1962	2/15
Goats	Montgomery Co.,Md.(P)	Nov 1962	4/18
Sheep	" " " (P)	Nov 1962	6/22
Pigs	Queen Anne's Co.,Md.(T)	Dec 1962	2/10

[1] Counties arranged for each species in ecological order from Tidewater (T) through Piedmont (P) to Mountainous (M) country.

TABLE IV

Occurrence of Antibody to Cache Valley-like Viruses in Wild Mammals, Virginia [1]

Species	Place	Date of Bleeding	#+/TOTAL
Wild Rodents	Assateague-Chincoteague Area	Aug-Nov 1962	3/211 [2]
Raccoon-Opossum	" " "	Aug-Nov 1962	0/8
Red fox	Central Piedmont, Va.	1960	0/10
Gray Fox	" " "	1960	1/10
Opossum	" " "	"	0/4
Raccoon	" " "	"	3/10
Woodchuck	" " "	"	1/4
Squirrel	" " "	"	0/3
Cotton Rat	" " "	"	0/3
Cottontail Rabbit	" " "	"	0/3
White-tailed Deer	Eastern Appalachian Area, Va.	"	4/10
Avian Sera [3]	Chincoteague-Assateague Area	May-Oct 1961	0/61

[1] Many of these sera furnished by Dr. Ben Elisberg of this Institute.

[2] 2 Brown rats (Rattus norvegicus), 1 Meadow vole (Microtus pennsylvanicus) positive.

[3] 61 Avian sera pools species specific, representing 30 species, 239 individuals.

TABLE V [1]

Occurrence of Neutralizing Antibody to Cache Valley-like Viruses in Man: Maryland and Virginia

Area Source of Human Sera	Age Range (Years)	Date of Bleeding	#+/TOTAL
Talbot-Dorchester Counties	20-39	Dec 62-Jan 63	1/18
Wicomico County	20-36	" "	4/24
Anne Arundel County	20-40	" "	0/25
Prince Georges County	20-40	" "	0/25
Cecil County	20-40	" "	0/24
Montgomery County	20-38	" "	1/25
Frederick County	20-40	" "	3/22
Garrett County	21-39	" "	0/22
Chincoteague, Va.	8-85	May 1961	33/176 [2]

[1] Sera of Maryland residents provided by Dr. C. A. Perry, Chief, Maryland Bureau of Laboratories; sera of Chincoteague, Virginia residents provided by Dr. Martin B. Marx, State Department of Health, Richmond, Virginia.

[2] Age range of positives 13-67 years.

REPORT FROM DR. ROBERT J. BYRNE
DEPARTMENT OF VETERINARY SCIENCE, UNIVERSITY OF MARYLAND
COLLEGE PARK, MARYLAND

EEE Antibody Survey of Ponies on Virginia's Eastern Shore

A serological survey for eastern equine encephalomyelitis (EEE) antibodies was conducted in the summer of 1961 on herds of non-vaccinated ponies in the eastern counties of Virginia which lie between the Chesapeake Bay and the Atlantic Ocean (see map). Serum samples were obtained from 156 ponies in 3 different locations and tested by serum dilution neutralization (NT) and hemagglutination-inhibition (HAI) tests. Results are shown in the table.

Results of Serological Tests for Antibodies to EEE in Ponies in 1961

Location	No. of Sera Tested	No. of sera positive at 1:10 or >	
		NT (10^2-10^3 LD_{50}'s of virus)	HAI (4 units of antigen)
Chincoteague, Va.	91	25	10
Assateague, Va.	16	11	8
Pungoteague, Va.	49	4	1

All sera that were positive by the HAI test had an NT titer $\geq$ 1:80.

It is evident from these results and those obtained in the past[1] that there is a higher frequency of exposure to EEE in the Chincoteague-Assateague area than in an area a short distance inland.

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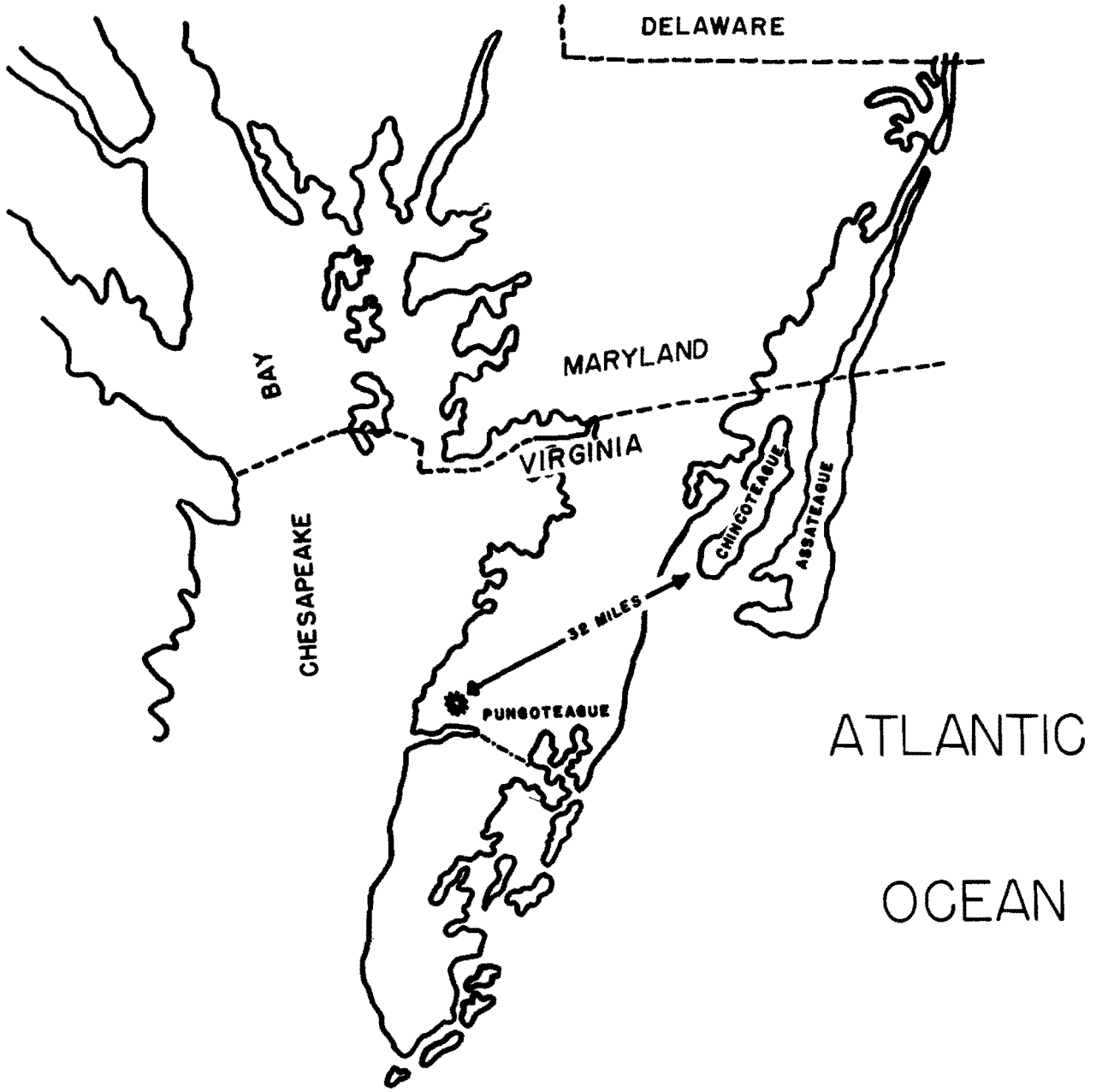
[1] Epidemiological Studies on Equine Encephalomyelitis in Maryland and Virginia, R.J. Byrne, F.S. Yancey, W.E. Bickley, and G. Finney, J.A.V.M.A., 135 (4):211-215, (August 15) 1959.

Location of Pony Herds Surveyed for EEE Antibodies in the Early Summer of 1961



CHINCOTEAGUE AND ASSATEAGUE ISLANDS NOT TO SCALE

REPORT FROM DR. FRANCIS B. GORDON
DEPT OF MICROBIOLOGY, NAVAL MEDICAL RESEARCH INSTITUTE
BETHESDA, MARYLAND

The susceptibility of monolayers of a fish cell line to arboviruses mentioned earlier (Arthropod Borne Virus Information Exchange No. 5, page 88), has been confirmed and additional information obtained. WEE and WN were carried in these cells, incubated at 24°C, through 11 and 14 passages, respectively. The series was started with inocula of mouse brain virus which was removed after 24 hours and the cells washed. Passages were continued using as inocula 7 day harvests of medium and frozen or sonicated cells. Titers of the harvests were consistently low, usually less than 10^2 mouse intracerebral LD_{50}'s. No CPE was observed. Total dilutions, incidental to passages, between the inoculum of passage 2 and the harvest of the final passage (both titers approximately equal) were 10^{-9} - 10^{-10} for WEE and 10^{-10} - 10^{-11} for WN, indicating an average of approximately 1 log increase of virus at each passage. Results with single passages of Sindbis, Semliki Forest and St. Louis, although not significant by themselves, were compatible with the results of the WEE and WN tests, i.e., slight increase in titer apparently occurred.

These tests always included controls of cell-free tubes of medium (MEM-10% fetal bovine serum with 1% vitamin mixture) inoculated and titered in the same manner as the cell cultures. It is noteworthy that the loss of titer in these controls at 24°C proceeded linearly and slowly; the rate varied from 1 log in 2.8 days (Semliki) to 1 log in 6.8 days (Sindbis), allowing detection of traces of virus in some tubes as late as 28 days.

There was no evidence for adaptation of virus to these cells nor for other change in the character of the viral strains. Cultures infected with WN were still susceptible to the CPE produced by infectious pancreatic necrosis virus of trout, although a 24-hour delay in its development was noted.

REPORT FROM DR. ALEXIS SHELOKOV
LABORATORY OF TROPICAL VIROLOGY, NIAID, NIH
BETHESDA, MARYLAND

(Combined operation of Middle America Research Unit, Balboa Heights, Canal Zone, and Arbovirus Section, NIAID, Bethesda, Maryland)

Epidemic of Hemorrhagic Disease in Bolivia

Between September 1959 and May 1962, about 360 cases of an epidemic febrile illness of unknown cause and unusually high mortality (120 deaths or 33%) were recorded in the northeast portion of Bolivia, the Department of Beni. This area extends from the Andes mountains to the border of Brazil and includes forests to the west and north and flat pampas from the center to the south. The rivers, forming a portion of the Amazon Basin system, flood the plains during the January and February rains.

Through the Interdepartmental Committee on Nutrition for National Defense, the Minister of Health of Bolivia invited the staff of MARU to investigate the epidemic. Dr. Mackenzie and Dr. Beye visited Bolivia in May, June, and July, 1962. During these investigations, it became apparent that cases were concentrated in 2 areas 70 miles apart: San Joaquin and Orobayaya. About 5 to 6 thousand persons was the estimated population in these two areas. On the basis of available reports, the disease incidence displayed a seasonal tendency with a peak in August and September of 1961.

In Orobayaya, a community of 257 persons, there had been 48 cases with 15 deaths. The clinical features were: slow onset, fever of 102 to 104°F and chills, generalized aching, headache, melena, epistaxis and hematemesis, gross tremor of tongue, intention tremor of hands, shock and coma, death in 7 to 10 days, and a striking alopecia in young girls during convalescence.

During the visits of May, June, and July, specimens obtained for laboratory study included acute and convalescent sera from observed cases; convalescent sera related to disease by history; sera from unaffected (control) populations; and sera from rodents and bovines in the area. Guinea pigs were inoculated at the bedside with blood specimens obtained from acute cases.

Neither virus nor rickettsiae were isolated from these animals or the acute case specimens examined by the conventional laboratory

procedures. Serological studies which did not contribute to etiologic identification included tests for plaque, typhus, rickettsial pox, leptospirosis, and groups A and B arboviruses.*

Complement fixing antibodies against Junin virus antigen were demonstrated in human sera. The distribution and concentration of CF antibodies were related to recent illness, and serologic conversions were demonstrated in the only three pairs of acute and convalescent sera available. As shown in Table 1, there was a low incidence of CF antibodies in the control populations selected from Riberalta, Magdalena, and some Orobayaya refugees (LEAST LIKELY on the basis of negative history). Relatively high frequency of positive CF results was obtained from the MOST LIKELY Orobayaya sera and the convalescent sera from Orobayaya and San Joaquin. When the latter were plotted according to CF titer and the number of months since illness, the curve shown in Figure 1 was obtained. CF titers were highest within 1 to 2 months after date of illness and fell to low levels from 5 to 13 months after onset. Table 2 contains the results of CF test on the 3 paired sera. All three converted from CF titers of less than 1:4 to 1:32 or 1:64.

The resulting hypothesis is that the epidemic was due to infection with Junin or a closely related virus. The clinical and epidemiological information at hand was consistent with that described for Argentinian epidemic hemorrhagic fever, reportedly caused by infection with Junin virus. Proof of this association in Bolivia depends upon recovery of the virus from cases; this is the primary objective of continuing field investigations. Attention is also directed to clinical and epidemiologic definitions, and demonstration of reservoir hosts and possible vectors. While re-infection has not been recognized, the value of naturally acquired immunity is not known. Current laboratory studies are directed to the development of possible vaccines.

*This laboratory is indebted to Dr. Charles L. Wisseman, Jr., University of Maryland School of Medicine, Dr. A.D. Alexander, Walter Reed Army Institute of Research, Dr. Dan Cavanaugh, Walter Reed Army Institute of Research, Miss Elizabeth Jackson, Division of Biologics Standards, NIH, and Dr. H. Mondragon, Gorgas Memorial Hospital, Canal Zone, for their assistance in the differential laboratory diagnostics.

Table 1

Hemorrhagic Fever, Bolivia: Complement Fixation Tests with Clinical Sera and Junin Virus Antigen

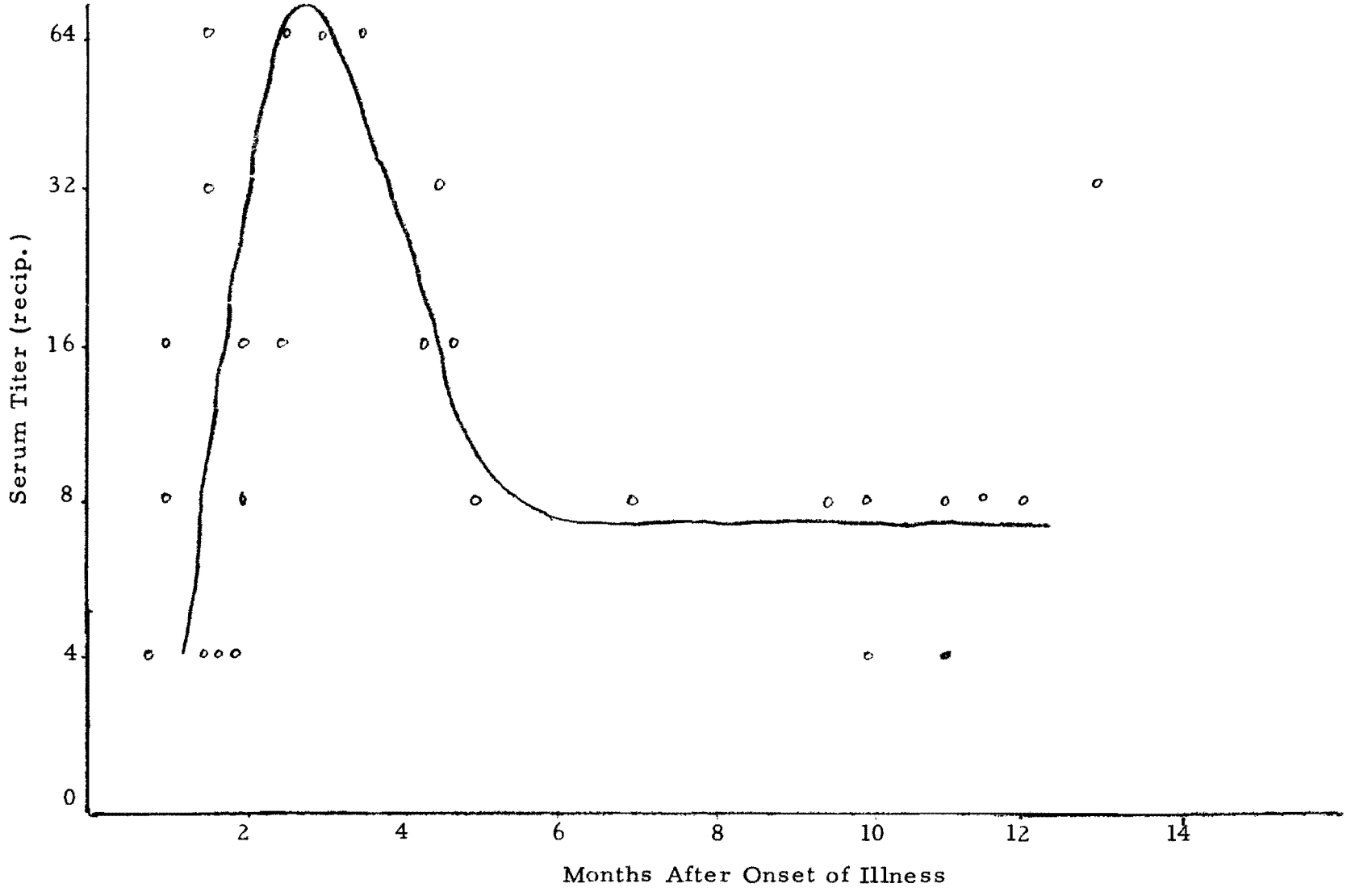
Serum Collections	No. Tested	No. Positive
Riberalta residents (control)	30	3
Magdalena residents (control)	32	4
Orobayaya refugees		
LEAST LIKELY	23	0
MOST LIKELY	21	5
Orobayaya convalescent sera	9	9
San Joaquin convalescent sera	26	17

Table 2

Hemorrhagic Fever, Bolivia: Complement Fixation Tests with 3 Paired Sera and Junin Virus Antigen

Patient Onset of Illness	Date of Bleeeding	CF Serum Titer
M. P. deS. 6V.62	20.V.62	<1:4
	29.VI.62	1:64
A. A. 3.VI.62	16.VI.62	<1:4
	30.VI.62	1:64
J. G. 20V.62	20.V.62	<1:4
	29.VI.62	1:32

Complement Fixation: Bolivian Sera and Junin Virus
Antigen Distribution of Serum Titers by Months after Illness



REPORT FROM THE ARBOVIRUS UNIT
VIROLOGY SECTION, COMMUNICABLE DISEASE CENTER
ATLANTA, GEORGIA

Tensaw Virus--A New Member of the Bunyamwera Group of Arboviruses.

In previous issues of the Information Exchange, mention has been made of a "Cache Valley-like" virus isolated on numerous occasions from mosquitoes (mainly Anopheles crucians) collected in Florida and Alabama. This agent was found to be antigenically distinct from Bunyamwera, Chittoor, Cache Valley, and Wyeomyia viruses. Subsequently, an Alabama and a Florida isolate were sent to the Rockefeller Foundation Virus Laboratories for final identification. Results obtained at RFVL confirm that this virus is a new member of the Bunyamwera group. The virus has now been designated Tensaw virus--the term referring to the Tensaw River that borders the Alabama study site.

Table I
Isolates of Bunyamwera Group Related Strains of Tensaw Virus
1960-1962

	MONTH AND AREA*										
	June		July		August		Sept		Oct		
Mosq. Species	Ala	Fla	Ala	Fla	Ala	Fla	Ala	Fla	Ala	Fla	Total
Anopheles crucians	-	-	5	39	3	-	8	27	-	8	90
Psorophora confinnis	-	6	-	7	-	-	-	-	-	-	13
Culex nigripalpus	-	-	-	1	-	-	-	1	-	-	2
Anopheles quadrimaculatus	-	1	-	-	-	-	-	-	-	-	1
Mansonia perturbans	-	-	-	-	-	-	1	-	-	-	1
Totals	0	7	5	47	3	0	9	27	0	8	107

*Florida collections represent 2 areas. Isolates in June and July were made from mosquitoes collected in the Big Cypress Indian Reservation. Isolates in September and October were from the Tampa Bay area. Other Florida collections made at Big Cypress in August, September, and October have not yet been processed.

Isolations of a Hart Park-related Virus from Mosquitoes

Laboratory studies have been continued in an effort to identify a number of virus isolates obtained from mosquitoes collected during the past three years in Alabama, Florida, and New Jersey. Fifteen of these isolates appear to be related to each other and to Hart Park virus by complement fixation testing. The number of isolates, mosquito species involved, area and year of collection are recorded in Table II.

Table II

Virus positive Mosq. pools	Mosquito species	Area of collection	Year of Collection
2	Culiseta melanura	S. Alabama	1960
1	Culiseta melanura	S. Alabama	1962
9	Culiseta melanura	New Jersey	1960
1	Culex restuans	New Jersey	1960
1	Culex restuans	S. Alabama	1960
1	Culex nigripalpus	S. Florida	1961

Isolations were all made in 1 to 2-day-old mice by intracerebral (i.c.) inoculation. On original isolation, mice died on the 7th to the 10th day with typical signs of encephalitis. Increasing the number of suckling mouse passages decreased the mouse survival time; i.e., after the 4th or 5th i.c. passage, mice died in 3 to 4 days, and after the 10th such passage, mice died in 2 to 3 days.

One isolate was selected as a prototype strain and studied in detail. This virus failed to produce signs of infection in suckling mice inoculated intraperitoneally or subcutaneously and failed to produce clinical disease in adult mice by any route of inoculation. Subcutaneous inoculation of rabbits, guinea pigs, and 1/2-day-old chickens did not produce illness nor could virus be detected in the blood during the first 6 days post-inoculation. The virus was not adapted to primary cultures of hamster kidney cells, monkey kidney cells, chicken embryo cells, or duck embryo cells grown either as fluid cultures or as agar overlays. Eggs inoculated via the yolk sac, allantoic cavity, and the CAM survived and no visible evidence of infection was detected.

The virus was inactivated after 15 minutes at 56°C but not after two hours at 37°C. Filtration studies showed that the virus passed through a 450 mμ APD membrane filter, but only 10% of the virus passed through

a 220 mμ filter and no virus through a 100 mμ filter. Eighty per cent of the virus activity was lost from the supernatant fluid after centrifugation for 1 hour at 10^3 g, 99% after 1 hour at 10^4 g, and 99.9% after 1 hour at 10^5 g. Chemically, 2 to 3 logs of virus were destroyed by 1% sodium deoxycholate after 1 hour of incubation at 37°C.

Complement fixation antigens have been prepared from sucrose-acetone extracted suckling mouse brains. Sucrose-acetone extracted brain antigens of the 4, 5, 6, 7, and 16th suckling mouse passage failed to hemagglutinate at pH's 5.75 to 7.0. Serologically, complement fixation tests indicated the virus was not related to any of the following viruses: arbovirus groups A, B, C, Bunyamwera, and California; Theiler's virus, mouse hepatitis virus, LCM and EMC viruses. However, the new isolate and Hart Park virus appeared to share a common complement fixing antigen. In cross box titrations, Hart Park antiserum titered 1:32 with the homologous antigen and 1:8 with the unknown virus antigen. Similarly, serum prepared against the unknown agent titered 1:32 with the homologous antigen but only 1:8 with Hart Park antigen. These preliminary results indicate that perhaps a "Hart Park group" of arboviruses occur with a wide geographical range of activity in North America.

Isolation of California Group Viruses:

Recently, three viruses were isolated from mosquitoes and tentatively identified as members of the California group of arboviruses. One isolate was obtained from a pool of Anopheles crucians collected in New Jersey in 1960. Two other isolates were obtained from Aedes atlanticus-tormentor and A. infirmatus collected in the Tampa Bay area of Florida in 1962.

These viruses produce a fatal infection in suckling mice by intracerebral, intraperitoneal, and subcutaneous inoculation and in adult mice by intracerebral inoculation. A febrile response and a viremia were detected in rabbits and guinea pigs inoculated peripherally. All three agents are sensitive to 1% sodium deoxycholate.

The results of preliminary complement fixation studies are given in Table III. (From work done by Dr. Dora Tan, WHO fellow to the Virology Section of CDC):

Table III

	Antigens			
Serum	CEV	NJ-94f	Fla-Cl 14ee	Fla-TBM 273g
CEV	32/128*	8/128	8/32	0/0
NJ-94f	64/128	128/512		
Fla-Cl 14ee	8/128		16/32	
Fla-TBM 273g	8/128			64/128

*32/128 = Titer of serum 1:32, titer of antigen 1:128.

FINAL EDITORIAL NOTE

ORGANIZED ITINERARY OF VISITS TO LATIN AMERICAN ARBOVIRUS ACTIVITIES IN CONNECTION WITH TRAVEL TO THE INTERNATIONAL CONGRESSES OF TROPICAL MEDICINE AND MALARIA IN RIO DE JANEIRO, BRAZIL

The directors of the arbovirus laboratories in Caracas, Venezuela; Port-of-Spain, Trinidad; Belem, Brazil; Buenos Aires and La Plata, Argentina; Bogota and Cali, Colombia; and Panama, have enthusiastically encouraged the itinerary of scheduled visits to their activities in order to make most effective use of the visitors' time.

As described previously, plans for two groups have been scheduled. One group from the Pacific area and western North America will gather in Panama no later than August 20 to visit laboratories in Panama, Colombia, and Argentina prior to the congresses, and to Belem, Trinidad, and Caracas, following the congresses, completing the trip on September 18. The other group from the eastern United States, Europe, Africa, and Asia will gather on August 24 for an itinerary in the reverse direction, completing the visits in Panama, September 22.

The itinerary has been planned so that no more than a calendar month is required for the arbovirus laboratory visits and full attendance at the congresses. A simultaneous schedule of sightseeing and other activities of interest to wives and friends accompanying the arbovirus investigators is also planned. For obvious reasons of advantageous group accommodation and transportation arrangements, participants must gather and continue the itinerary with the group. Specific details can be obtained by addressing inquiries directly to: Room 1611, Fulton National Bank Building, Atlanta, Georgia.

Having previously carved out visits to these activities at the cost of time and inconvenience, this opportunity for a comprehensive look at the considerable and diverse activity in arbovirus investigations in Latin America is a unique scientific opportunity for the limited period of one month. The sooner responses are received from participants, the better will be the plans for the trip.

Telford H. Work, M. D.
Editor