ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

Number Six October 1962

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REPORT OF THE SUBCOMMITTEE ON INFORMATION EXCHANGE

This subcommittee, which is responsible for issuing the Arbovirus Information Exchange and Catalogue, continues to receive requests for the Catalogue and to be placed upon the mailing list for the Exchange. Although explained in previous issues of the Exchange, many of these requests manifest a lack of understanding of the purpose and limitations, and particularly of the restrictions that are placed upon both the Exchange and the Catalogue.

To clarify and avoid misunderstanding, the following form letter has been prepared in answer to requests to participate in the Exchange:

Dear Dr. _____:

In answer to your inquiry concerning participation in the "Arthropod-Borne Virus Information Exchange", it is appropriate to inform you of the nature, purpose, and limitations of the Exchange and the qualifications for participation.

It is important to emphasize that the Exchange, which is distributed periodically by the Subcommittee, is a privileged communication. It is not a publication and cannot be quoted or otherwise used as a reference. The limitations are outlined in the "Important Notice" inscribed on every issue:

> "This Exchange is issued for the sole purpose of timely exchange of information among investigators of arthropod-borne viruses. It contains reports, summaries, observations, and comments submitted voluntarily by qualified agencies and investigators. The appearance of any information, data, opinions, or views in this Exchange does not constitute formal publication. Any references to or quotation of any part of this Exchange must be authorized directly by the person or agency which submitted the text."

Failure to adhere to this understanding or other unethical use of knowledge obtained from the Exchange, which is the basic principle for this type of free exchange of information, will result in immediate elimination of the violator from any further participation.

It should be stressed that participation is a two-way affair, requiring that the participant acknowledge all communications from the Subcommittee

and contribute significantly to the Exchange as his current research progresses. If, in the opinion of the subcommittee, an investigator fails to participate or to respond to two consecutive communications from the subcommittee, his name will be dropped from the list of active participants.

If, after consideration of these conditions, you desire to apply for participation in the Exchange, you should submit a brief account of the sphere of interest, past work, and current activity in the field of arthropod-borne virus research that you or your institute is engaged, specifying under what aegis, grant, or sponsorship the work is being conducted. Your application will be submitted to the subcommittee and you will be informed of its decision.

Yours sincerely,

R. M. Taylor, M. D.

To further circumvent improper reference to the Exchange and Catalogue, the following letter was sent to editors of journals likely to receive manuscripts on arboviruses for publication:

Dear Sir:

The American Committee on Arthropod-Borne Viruses is conducting an exchange of information among investigators actively engaged in research in this category of viruses. This information is embodied in a "Catalogue of Arthropod-Borne Viruses" and in the "Arthropod-Borne Virus Information Exchange". However, neither is considered to be a formal publication and the subcommittee directly responsible for the issue of these two information exchanges wishes to notify you of the limitations and restrictions placed upon their use as a source of reference.

The Catalogue includes the following statement: "The distribution of the Catalogue and the associated current information and abstract service is limited to laboratories or institutes actively engaged in research related to the isolation, classification, or the natural behavioral characteristics of arthropod-borne viruses. Registration of a virus in the Catalogue does not constitute formal publication but only serves as a notification of the isolation and characteristics of a virus to other investigators receiving

the Catalogue. Use of the Catalogue for the purpose of general reference in publications in restricted and it is not permissible to use it as a source of reference to a virus that has not been described in a formal publication without the explicit consent of the person making the registration."

The Arthropod-Borne Virus Information Exchange (formerly referred to as a "Newsletter") carries the following notice: "This exchange is issued for the sole purpose of timely exchange of information among investigators of arthropod-borne viruses. It contains reports, summaries, observations, and comments submitted voluntarily by qualified agencies and investigators. The appearance of any information, data, opinions, or views in this exchange does not constitute formal publication. Any reference to or quotation of any part of this exchange must be authorized directly by the person or agency which submitted the text."

We are, therefore, soliciting the aid of members of your editing department in helping to prevent misuse of these vehicles of information exchange. There is no objection to a person referring to the Catalogue and Information Exchange in the text, as described in the Charles Franklin Craig Lecture (Purpose and Progress in Cataloguing and Exchanging Information on Arthropod-Borne Viruses. Amer. J. Trop. Med. and Hyg. 11: 169-174, 1962); however, they should not be listed in a bibliography as original sources of reference for any scientific fact or any particular virus name mentioned therein.

Yours sincerely,

R. M. Taylor, M. D.

Catalogue:

The issue of the revised catalogue in March of this year contained registrations of 110 viruses. Since then, seven new viruses have been registered and two re-registered that were in the old but not in the revised edition, making a total of 119 viruses presently registered in the new revised edition. Notably lacking are members of the tick-borne viruses of the RSSE complex. Registration of these related viruses is being held up until registrations of all the known prototype strains are received.

Notwithstanding repeated requests, the re-registrations of the four Malayan viruses are lacking. Also we are aware from personal correspondence and from annual reports of several field laboratories that a number of new viruses have been discovered but registration is being deferred pending further study. It is anticipated that registrations of these viruses will be coming in shortly.

The issue of the revised catalogue in March included coded abstracts reproduced on 3 x 5 slips from Biological Abstracts covering the years 1959 through 1961. The June and September supplementary distributions have brought the total number of abstracts to approximately 1, 500. These abstracts were selected from Biological Abstracts from 1958 to date and from the Bulletin of Hygiene and Tropical Diseases Bulletin from 1959 to date.

With the object of keeping the catalogue current, the responsibility of supplying new and especially unpublished information on the registered viruses was assigned to selected collaborators. So far, thirty slips coded for filing with the abstracts have been issued, giving additional information supplied by these collaborators on viruses registered in the catalogue.

It is now the intention to distribute new registration cards and the abstract and current information slips to the recipients of the catalogue at quarter year intervals.

To date, 84 catalogues have been distributed, 33 within the continental United States and 51 overseas, representing 32 countries.

Subcommittee on Information Exchange

Richard M. Taylor, M. D., Chairman
William F. Scherer, M. D., Catalogue
Telford H. Work, M. D., Editor

EDITORIAL NOTES

The sixth issue of the Arthropod-borne Virus Information Exchange continues to show gain in quantity, quality, timeliness, and extent of distribution. With such continued growth, the problems and complexities

of editing increase. One of the most evident problems is documentation of the origin of the contributions. In the earlier issues, it was possible to state the name of the person who actually submitted the item as an address to which interested readers might write for further information. The number of multiple contributors of one contribution, which may be of size no larger than the list of collaborators, has posed an editorial and mechanical difficulty which must be dealt with in some more standard way.

Where it is important to extend recognition to more than three individuals, and institutions or agencies, this can be done by inclusion of an additional short paragraph in the text. Another mechanism which can be observed in this issue is the bracketting of names associated with particular sections of the reported effort. Careful consideration of this problem by those who submit contributions for future issues will save arbitrary editorial changes which may miss the intention of the contributor. It should be remembered that the purpose of this mechanism is to facilitate exchange of information. The simpler and more direct the reference point, the simpler the requirements for exchange.

Increased content of the Information Exchange now requires that it be prepared in pieces rather than sequentially from beginning to end as in early issues. This leaves space gaps which must be filled. There is growing general and lay interest in the accomplishments in the arbovirus field of science. Newspaper and magazine coverage on this subject increases and it is of interest to follow how this information and misinformation is disseminated.

The space gaps can be filled with such items of interest and, as in editing any other timely vehicle of information dissemination, an editor chooses from an accumulation of items those he thinks best fitted to the readership and space. This will explain the origin of the item from the "authoritative" New York Times on the O'Nyong-Nyong epidemic in East Africa in issue five of the Infoexchange. This had actually filtered down from the press clipping service of the Public Health Service as a representation of one of the mechanisms by which the responsible authorities keep aware of new health problems. That it was inaccurate in certain respects was obvious. That it represented the type of information reaching the public by summary of interview, official reports, or scientific papers should be of particular interest to those who work directly with the virus and its problems. Unless there is strong objection from the readership, this editorial practice will continue, as much for the edification of the reader

as the filling of space. Correspondence discussing such issues is invited for it becomes substance for consideration by the Subcommittee on Information Exchange.

It should be noted that the term arbovirus as a single word has now been accepted by the International Committee, as presented in Dr. Hammon's report in this issue. If this change in spelling and terminology can be adopted in future manuscripts and contributions, it will save editorial time.

Appended is a recent analysis of distribution of and participation in the Information Exchange. Dr. Taylor, the Chairman of the Subcommittee, continues to receive requests from many agencies and some individuals who are not eligible under terms the letter reproduced earlier spells out. Of more concern is the small number of participants who have never contributed, and seldom acknowledged inquiries, announcements, and issue of the Information Exchange. A careful record of this is maintained and after review at the next subcommittee meeting, those who have not fulfilled the minimum stated requirements for participation will be deleted from the list.

This next meeting will be held on October 30 in Atlanta, Georgia, in conjunction with the annual general meeting of the American Committee on Arthropod-Borne Viruses, which is an affiliate of the American Society of Tropical Medicine. Headquarters for the meetings is at the Atlanta Biltmore Hotel. On the program of the society on November 1 and 2 are papers of interest in half-day sessions on Arboviruses and Immunization against Arbovirus Infections. A detailed announcement with summaries of papers has been contributed to this issue of the Infoexchange by Dr. Joseph Smadel, convener of the symposium.

We hope to see many of you in Atlanta at the end of October.

Telford H. Work, M.D.
Editor

DISTRIBUTION OF THE INFORMATION EXCHANGE

Continental United States	62		
Africa	9		
		West Africa	4
		East Africa	2
		South Africa	2
		Ethiopia	1
Australia and New Zealand	7		
Canada	3		
Latin Americas	21		
		Venezuela	2
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		Argentina	4
		Mexico	2
		Brazil	4
		Colombia	3
		Panama	1
		West Indies	1
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Europe	26		
		Austria	1
		Denmark	1
		Czechoslovakia	2
		Yugoslavia	4
		Ireland	1
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		Italy	1

Distribution of Information Exchange (continued):

Israel	1		
Far East	9		
		Thailand	1
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		Japan	3
		Malaya	2
		India	1
Foreign Service	11		
		Formosa	2
		Egypt	3
		Japan	1
		Malaya	1
		Thailand	1
		APO 180	1
		APO 851	1
		APO 205	1
Total	150		
		(Overseas 88, representing 46 countries)	
		USA 62	

ABSTRACTS OF PAPERS TO BE PRESENTED AT THE SYMPOSIUM ON IMMUNIZATION AGAINST ARBOR VIRUS INFECTIONS CONVENED BY DR. JOSEPH E. SMADEL, TO BE HELD ON 2 NOVEMBER 1962 AT THE ELEVENTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE, ATLANTA, GEORGIA

Immunological Relationships Among Arbor Viruses (Jordi Casals, The Rockefeller Foundation Laboratories, New York, N. Y.)

Information will be presented on the antigenic relationships within the different groups of arbor viruses as determined by means of cross immunity and serological tests. Observations on intra-group resistance will be reported.

Studies on Attenuated VEE Virus Vaccine (Robert W. McKinney, United States Army Medical Unit, Fort Detrick, Maryland)

The material to be presented will summarize the studies dealing with the development and use of the attenuated VEE virus vaccine. The discussion will cover the derivation of the virus, its characterization as to infectivity, host range and the response of several species including man to infection with the virus. Also included will be a discussion of cross-protection studies in animals immunized with the attenuated virus and challenged with 5 different strains of VEE virus.

Selection of a Variant of Western Equine Virus of Low Pathogenicity for Study as a Live Virus Vaccine (Harald N. Johnson, Viral and Rickettsial Disease Laboratory, California State Department of Public Health, Berkeley, California)

A paper describing the early studies of this virus was published in Virology in November, 1961. Cloning of this virus has been continued and at clone 15 a variant was selected for study as an attenuated vaccine. The average survival times (AST) for infant mice inoculated intracerebrally or intraperitoneally with this variant are the same, i.e., > 4 days. As with all clones tested subsequent to that reported in the paper, this clone is not pathogenic for young adult mice inoculated intracerebrally with any dosage of virus. However, intracerebral inoculations of >10 infant mouse LD_{50} induces a solid immunity against subsequent intracerebral challenge with neuro-adapted Western equine virus. Each clone has been routinely tested for antigenicity by immunizing young adult mice and challenging them a month later by intracerebral inoculation with neuro-adapted Western equine virus. The key to obtaining minimum pathogenicity appears to have been selection of clones based on the relative AST for infant mice inoculated intracerebrally and intraperitoneally, that is, when the AST became the same for both routes of inoculation, it was evident that neurotropism played no part in the pathogenicity of the virus.

Rift Valley Fever Virus Vaccine (Raymond Randall, L.N. Binn, and V.R. Harrison, Walter Reed Army Institute of Research, Washington 12, D.C., and the Department of Medicine, University of Maryland Medical School, Baltimore 1, Maryland)

A formalin-inactivated Rift Valley fever (RVF) virus vaccine prepared from rhesus monkey kidney cell cultures, infected with the pantropic or field strain of the virus, has been used to immunize more than 1000

individuals without causing any untoward reactions. A number of those immunized have worked in laboratories where pantropic RVF virus was under investigation. None of the vaccinated persons whose sera neutralized a hundred LD_{50} of virus or more developed signs or serologic evidence of infection during the 24 months of observation.

Being cognizant of the fact that rhesus monkey kidney cell cultures usually contain one or more spontaneous virus contaminants, we used a 1:1000 concentration of formalin for inactivating the vaccine instead of the usual 1:4000 concentration.

Following the report of Sweet and Hilleman on vacuolating virus (SV_{40}), we tested retained aliquots of six lots of RVF vaccine of the rhesus cell type for this virus. Three of the six contained viable SV_{40}.

As a result of these findings, the vaccine is now being prepared from African green monkey cell cultures with no further evidence of contamination with SV_{40}.

Studies are being made on the immunogenic capacity of the vaccine following lyophilization. The addition of 2% USP human serum albumin has been found to stabilize the potency of the product so that it can be stockpiled or shipped without refrigeration.

Progress Report on Japanese B Encephalitis OCT-541 Attenuated Virus Strain (W. McD. Hammon and Johng S. Rhim, Department of Epidemiology and Microbiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, Pennsylvania)

Data will be presented on the stability of the strain at various culture passage levels and when grown at different temperatures. The attenuated strain grows well at 24° C and at 37° C, but fails to multiply at 40° C. This is in contrast to the original mosquito isolate from which it was derived and to other strains of Jap B virus which grow equally well at 37° C and 40° C. Plaques are formed under double agar overlay. These are smaller than those of the virulent strains. Monkeys and chimpanzees inoculated subcutaneously on one occasion with the 24° C cultured strain OCT-541, failed to develop antibodies and the former developed viremia on challenge with the parent strain. Two or three injections produced antibodies in monkeys. Observations on terminal cancer volunteers are in progress. The first two, given a 1:10 dilution, responded with viremia after 10 and 12 days, were asymptomatic, and one developed antibodies.

Viremia was detected only by i.c. mouse inoculation. Possible oncolytic effect of virus is also being evaluated. Two subsequent volunteers inoculated with 1:100 dilution plus high titered anti-Japanese B gamma globulin and 2 others with 1:1000 dilution without gamma globulin have run asymptomatic courses. Viremia and antibody results pending at this time (mid-July).

Attenuated Living Type 1 Dengue Virus Vaccines (Charles L. Wisseman, Jr., Benjamin H. Sweet*, Edward C. Rosenzweig and Ollie R. Eyler, Department of Microbiology, University of Maryland Medical School, Baltimore, Maryland)

Beginning with partially attenuated lines of the Hawaiian strain of type 1 dengue virus developed previously in Sabin's laboratory, four new variants were produced in mice, purified by serial terminal dilution passages and characterized in suckling and weanling mice and in rhesus monkeys following intracerebral inoculation. None produced paralytic signs in monkeys, though occasional neuronal degeneration was observed in histological sections. Each strain, subsequently inoculated into a separate group of 3 or 4 human volunteers, in doses ranging from about 2000 to 10,000 suckling mouse LD_{50}, produced greatly modified infection. Three of the strains produced mild to moderate systemic signs, slight elevation of temperature and rash. The remaining strain, designated as the MD-1 strain, produced no detectable symptoms, fever, incapacitation or rash. Yet it elicited the production of neutralizing antibodies just as the other three strains did. Two additional groups of 10 volunteers each were subsequently inoculated with 20,000 and 200,000 suckling mouse LD_{50} doses of the MD-1 strain. Again, no significant symptoms, fever, incapacitation or rash occurred. Neutralizing antibodies were detectable by the fourteenth day after vaccination. HI antibodies appeared irregularly and, when present, were of low titer. CF antibodies were not detected.

*Present address: Research Associate, Merck, Sharp, and Dohme Research Labs, West Point, Pennsylvania.

An Immunization Procedure Against Certain Group B Arbor Viruses (Winston H. Price, Ralph Lee, Walter O'Leary, James Parks, and James Ganaway, Department of Epidemiology, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland)

Using the serologic relationships that exist between members of the group B arbor viruses, an experimental immunization procedure

against certain group B arbor viruses has been studied in primates that involves a triple inoculation procedure in which 17D yellow fever vaccine is followed subsequently by injections of attenuated strains of West Nile virus and TP21 virus respectively. This procedure will protect primates to a great extent when the vaccinated animals are challenged subcutaneously with over 1000 infectious doses of any of the following virulent group B viruses: dengue, types 1, 2, 3, or 4; St. Louis encephalitis; Japanese B encephalitis; West Nile; Russian spring summer encephalitis; Kyasanur Forest Disease (KFD); Omsk; Central European tick-borne encephalitis; Wesselsbron; and 3 recently isolated new group B arbor viruses.

The attenuated strain of TP21 alone will protect primates against subcutaneous challenge with many infectious doses of all 7 other viruses of the Russian spring summer group. Efficacy of the vaccine is based on the height and duration of the viremia of vaccinated animals as compared to the viremias found in control animals when both groups of animals are challenged as well as on histopathologic signs of encephalitis in the brain and spinal cord of control and vaccinated animals using Smith's intracerebral starch technique. The value of the triple vaccination procedure will be discussed particularly in relation to protection against Powassan virus as this is an illuminating example of how the principle of serologic overlap acts in the vaccination procedure.

Oral immunization of spider monkeys with the attenuated strain of TP21 will protect them against subcutaneous challenge by Kyasanur Forest Disease.

In order to obtain the maximum and broadest protection against the greatest number of group B arbor viruses, using the principle of serologic overlap, it is important to select the right group B viruses and to inoculate them in the proper temporal sequence. Inactivated viruses, at least under our experimental conditions, could not be substituted for the attenuated strains. The reasons for this will be discussed as well as data relating to the duration of immunity produced by the triple vaccination procedure.

Data on the safety and permanence of the attenuated characteristics of West Nile virus and TP21 virus for possible human use will be presented along with problems of attenuating these viruses. For example, studies of plaque purified isolates of TP21 virus have shown that the majority of such isolates increase in virulence after chick embryo passage as measured by the encephalitogenic ability in rhesus monkeys and peripheral infectivity in mice. Such a phenomenon does not occur in TP21 virus isolates grown in hamster kidney tissue cultures.

SUMMARY OF MEETING ON SCIENTIFIC GROUP ON YELLOW FEVER RESEARCH IN EAST AFRICA

SUBMITTED BY DR. ARTURO SAENZ, MEDICAL OFFICER FOR ARBOVIRUSES, WORLD HEALTH ORGANIZATION GENEVA, SWITZERLAND

The second meeting of the Scientific Group on Yellow Fever Research in East Africa took place in Geneva, 30 May-1 June 1962.

The results of the studies carried out in Ethiopia with the support of WHO during the 1961/62 epidemic season under the direction of Dr. C. Serie, Director, Institute Pasteur, Addis Ababa, were reviewed and analyzed by the Group. Epidemiological and entomological surveys have been carried out in several areas of the country. Studies on the sera collected are not yet complete but high immunity rates have been found in the population of some of the areas. A complete report will be published as soon as the studies are finished.

An active epidemic focus was found in a small valley (Tchabera) in the southwest of the country. Three yellow fever isolations were made from human cases seen in January 1962 at Manera, in the Tchabera Valley. In March 1962, twelve strains of yellow fever virus were isolated from Aedes simpsoni in the same region. In one series of experiments, field-captured Aedes simpsoni were permitted to bite baby mice and transmission of yellow fever was obtained.

Ninety per cent of the mosquitoes captured in the Tchabera area were Aedes simpsoni and a small dark and a large pale variety of this mosquito were recognized. These two varieties occurred together in approximately equal number and behaved similarly. The two varieties were pooled for inoculation for virus isolation but one or more mosquitoes from each pool were saved for later taxomonic verification. Taxomonic studies are being carried out now. Aedes aegypti was not found in the Tchabera region.

The strains of virus as well as samples of the sera collected during the serological surveys have been sent to collaborating laboratories in Dakar (Institut Pasteur), Paris (Institut Pasteur), New York (Rockefeller Foundation), and Entebbe (East Africa Virus Research Institute). All virus strains from humans and mosquitoes thus far adequately examined by the collaborating laboratories have been confirmed as yellow fever. A high proportion of sera from monkeys (Colobus and baboons) caught in the forest near the epidemic area had been found to be positive.

The studies to be carried out during the coming season were discussed. Serological and entomological surveys will be undertaken in areas not yet explored, and intensive epidemiological, entomological, virological, and mammalogical studies will be continued in the Tchabera area.

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

The recent isolations of vesicular stomatitis from arthropods in nature (MARU, Belem Virus Laboratory), their artificial transmission by A. aegypti in the laboratory (IVIC), and other properties they share with the arboviruses make it likely that they will be encountered by workers in the field. It will be interesting to make it known, therefore, that the Pan American Foot-and-Mouth Disease Center in Rio de Janeiro will accept virus samples for identification and differential diagnosis with Foot-and-Mouth viruses as well as paired serum samples for CF tests.

Materials to be tested should be addressed to:

Dr. William M. Henderson
Centro Americano de Febre Aftosa
Caixa Postal 589
Rio de Janeiro, Brazil

Dr. William C. Reeves and Dr. William F. Scherer, acting as Consultants of the Pan American Health Organization, surveyed the current research activities in the countries of Latin America and made recommendations for their support and coordination. Their report was submitted to the Pan American Health Organization's Advisory Committee on Medical Research, which met for the first time in Washington on June 18-22, 1962.

REPORT FROM DR. W. McD HAMMON ON THE AUGUST 1962 VIRUS NOMENCLATURE SUB-COMMITTEE MEETING, MONTREAL, CANADA

I attended the meetings of the International Subcommittee on Virus Nomenclature (C. H. Andrewes, Chairman) at the International Congress for Microbiology at Montreal and as chairman of the Subgroup on Arthropod-borne Viruses gave a report which is presented below. Jordi Casals, Pierre Lepine, and George Dick were other members of the subgroup. Jordi Casals made a number of helpful suggestions in the writing of the report. Lepine approved the recommendations, without comment on the descriptive portion and no comments have been received from Dick.

The action of the committee was complete endorsement of all the recommendations. Therefore, arbovirus, as a single word, spelled without an "r", has now become the officially approved name of this virus group. It may thus be used alone and is no longer a mere abbreviation of Arthropod-borne animal viruses. The actions of the Subcommittee on Nomenclature of Viruses will be published, along with the subgroup report given here.

In respect to taxonomy, the Subcommittee noted that this group is unified only by a common type of epidemiologic cycle, but is otherwise a heterogeneous group in respect to size and a few other known characteristics. The group will not be granted the firm type of taxonomic position given such others like the poxvirus or myxovirus groups, but is approved as a group temporarily since this classification serves a very useful purpose. Eventually, these viruses will probably be subdivided into several groups with separate group names, each composed of viruses with similar size, probably similar numbers of capsomeres, each with or without cubicle or helical symmetry, with or without an envelope, the virus formed in the nucleus or in the cytoplasm, etc. Data of this type are badly needed to bring more order into the taxonomy and classification of our group.

Report of the Arthropod-borne Virus Subgroup:

The Arthropod-borne Animal Viruses are a large group of about 150 recognized viruses, members of which find natural hosts in one or more of a number of vertebrate species including man, other mammals, birds, and possibly certain reptiles and amphibians. Presumably, their unique characteristic is naturally occurring biological transmission (involving multiplication and an extrinsic incubation period) through the bite of an arthropod to a vertebrate. Viremia in a vertebrate serves as a source of vector infection. Thus, this classification is based on biologic criteria related to the life cycle of the agent.

Vectors are known to include mosquitoes, sand flies, ticks, mites, and midges (Culicoides). In no instance is pathology recognized in the arthropod vector, while a wide spectrum of diseases, or infection without disease may be produced in vertebrates.

Unfortunately, demonstration of the complete biological viral cycle postulated as the criterion of classification is time consuming and difficult and has been demonstrated for only a limited number of viruses now considered members of the group. In most instances a virus has been included as a probable member on the basis of less stringent but nevertheless highly suggestive evidence, i.e., antigenic relationship to an established member combined with similarity of certain biological properties, ability to be experimentally propagated in series in certain bloodsucking arthropods when ingested or inoculated parenterally, or isolation from a field collected suspect vector which did not contain visible blood. Several of these criteria have been met by most viruses now classified as arthropod-borne animal viruses.

Physical and chemical characteristics of most of the viruses now included in the group have not been well studied. The one characteristic examined for the greatest number (about two-thirds) is sensitivity to ether or desoxycholate. With rare exceptions all have been found relatively sensitive, although the degree of sensitivity varies considerably. In the exceptions (like Mag 115 & P-581) to this general characteristic, classification of the virus in the arthropod-borne group remains uncertain, though the agents were isolated from mosquitoes. All those tested (few) contain RNA. Sizes of the few tested vary greatly, ranging from about 20 mu to possibly as large as 150 or 180 mu (Blue tongue, Ilesha and Turlock), but most are at the small end of the spectrum. Most of those examined for shape are spherical, although rods have also been described. Stability to heat varies considerably but in general they are relatively unstable at 37° and 56°. The range of pH stability has been tested for only a few and in these it appears to be in a relatively high alkaline range. All tested so far retain infectivity when lyophilized or when frozen at about -70°. Many can be preserved well in 50% glycerine at pH 7.2 or above at 5° C for many months or years. The few tested in the presence of 1 M $MgCl_2$ are rendered more labile to heat at 50° C. Protein digesting enzymes readily inactivate some (Group B) and not others (Group A). A few have been tested for sensitivity to certain sulfhydryl reagents and again important differences were noted between those of groups A and B, the former not affected. Most of the viruses, when properly treated, will agglutinate erythrocytes of one or more species of vertebrate.

Most viruses in the group produce encephalitis when inoculated intracerebrally in suckling mice, or suckling hamsters, while great variation occurs in their ability to produce disease by other routes of inoculation or in older animals. Susceptibility to disease of other animal species varies greatly. In tissue cultures many viruses multiply readily in one or many types of primary or continuous cell lines, some with cytopathogenic effects. Some viruses produce plaques readily under agar even though they do not produce cytopathogenic effects in regular monolayer cultures.

At present, final identification of these viruses depends almost entirely on antigenic structure as revealed through one or more types of immunological tests. Pathogenicity of a number of viruses has been altered through laboratory manipulation, but important antigenic changes do not appear to be readily produced in the laboratory if at all.

No natural or useful taxonomic subclassification, based on physical or chemical properties, pathogenicity, tissue culture susceptibility or range of vertebrate or arthropod hosts, appears possible at present. Certain groups and subgroups are apparent on the basis of antigenic structure. Since at present these same characteristics are essential for identification of individual viruses, this type of subclassification appears to serve a useful role, although with further knowledge of other physical and chemical properties a better basis may well be found.

At present, almost all of the large number of newly described agents in the group are being given local place names of the area of original isolation. A few of the viruses long recognized are named for the disease with which they were etiologically associated such as yellow fever, blue tongue, louping ill, etc. Some, like dengue types 3 and 4, have been so named because of their close immunologic relationship to previously recognized dengue-producing viruses type 1 and 2.

"Arthropod-borne viruses" has come to be a well-accepted name through usage for this group of agents, beginning with usage for a group causing encephalitis--the arthropod-borne virus encephalitides (Hammon and Reeves). Two abbreviations for arthropod-borne are in current use, "arbor" (Casals) having priority and "arbo" (Hammon) suggested as a modification. These are usually used as separate words to avoid the connotation of officially accepted nomenclature as represented by adenovirus, myxovirus, poliovirus, etc.

Committee Recommendations

It is recommended that:

1. This group of agents be recognized as a major group.

2. The group be named Arbovirus. The abbreviation arbor virus has been used by many in this field. This minor change in spelling is recommended to avoid any connotation that arbor might have with the plant virus group.

3. Authors be encouraged to give place names rather than disease names to new viruses except within a group like dengue where another numerical pattern has already been established.

4. Subgrouping be continued as at present by antigenic relationships as they are recognized.

5. No official nomenclature be applied to these subgroups, now known as groups A, B, C, Bunyamwera, Guama, Bwamba, California, etc., since shifting is occurring and new relations are appearing between and within certain of these groups.

6. Workers be encouraged to explore the physical and chemical characteristics of the rapidly growing group in order that better taxonomic classification be realized.

REPORT FROM THE TROPICAL MEDICINE SURVEY, NATIONAL ACADEMY OF SCIENCES, NATIONAL RESEARCH COUNCIL

A study of needs and resources for research in the broad field of tropical health has been undertaken by the Division of Medical Sciences, National Academy of Sciences, National Research Council. The project had its inception in discussions within the American Society of Tropical Medicine and Hygiene in 1953 and 1954. In January of the latter year, Dr. Frederick Brady, president of the Society at that time, appointed an ad hoc committee to consider ways and means of implementing such a survey. The committee, under the chairmanship of Dr. Albert Sabin, functioned for a number of years and was responsible for enlisting the aid of several agencies in support of the program that was eventually proposed. In 1958, necessary financial support was obtained from the National Institutes of

Health, the U.S. Army Research and Development Command and the Rockefeller Foundation. Under the direction of Dr. Willard H. Wright, a small staff was recruited. Guidance was provided by an advisory committee under the chairmanship of Dr. Sabin. The actual work was begun in 1959 and was completed early in 1962.

The findings of the staff have been incorporated into a report which will be published in hard covers late in 1962. Because of this anticipated delay, a summary has been prepared, printed by offset, containing the analytical and critical substance of the full report but without maps or figures presenting the major part of the statistical detail.

The report is divided into seven parts. Part I presents an analysis of significant human infectious and nutritional diseases of tropical and certain near-tropical areas. Part II performs the same task with regard to important animal diseases of the tropics. Part III surveys existing resources for health and medical care in the tropics. Part IV is concerned with economic aspects of tropical health. Part V examines existing research programs in tropical health as carried out by United States federal agencies, by medical schools and schools of public health, and by certain industrial and private laboratories. Foreign research is also scrutinized. Part VI discusses programs of domestic and foreign research grants, domestic fellowship programs, the teaching of tropical medicine and training facilities in the United States and abroad, current career opportunities, and future manpower needs in the field of tropical health. Part VII represents an assembly of informed opinion regarding the most pressing current research needs in medical and hygienic problems of the tropics. Contributions to this part were received from an international group of 89 individuals, each expert in his field.

In addition, there are incorporated seven recommendations made by the Advisory Committee which, if adopted, would promote a larger and more effective national effort in tropical health.

Queries regarding the availability of the full report, entitled "Tropical Health" may be addressed to the Manager, Publications Office, National Academy of Sciences - National Research Council, 2101 Constitution Avenue, Washington 25, D. C.

A limited number of copies of the Summary Report are available on request from the Chairman, Division of Medical Sciences, National Academy of Sciences - National Research Council, 2101 Constitution Avenue, Washington 25, D. C.

REPORT FROM THE TAMPA BAY REGIONAL ENCEPHALITIS LABORATORY, TAMPA, FLORIDA, ON THE EPIDEMIOLOGY OF ARBOVIRUS GROUP B INFECTIONS IN THE TAMPA BAY AREA OF FLORIDA*

*A summary of joint investigations carried out by the Florida State Board of Health, the Communicable Disease Center, U.S. Public Health Service, and the Health Departments of Pinellas, Hillsborough, Sarasota, and Manatee Counties.

Prior to the summer of 1962, the virus of St. Louis encephalitis had been isolated only three times in Florida. There had been no confirmed outbreaks of human disease due to SLE virus and only occasional reports of sporadic human infections scattered over the southern peninsular portion of the state.

The first outbreak of presumed St. Louis encephalitis occurred in Pinellas County during the fall months of 1959. There were 68 clinical cases with five deaths. Of these, 45 were considered to have adequate blood specimens for diagnostic serologic studies; 23 gave evidence of recent or past infection with SLE or a closely related group B arbovirus. The brain tissue of two fatal cases was studied virologically; there was no viral isolation.

Following an interval of 22 months, there was a smaller outbreak of encephalitis beginning in the same area. From late October to early December, 1961, 25 cases occurred--9 in Pinellas County, 10 in Manatee County, and 6 in Sarasota County. Chronologically, the infections occurred first in Pinellas. The disease was uniformly more severe than in 1959, there being 7 deaths in the 25 cases. Five of these were autopsied and all revealed the typical histopathological findings of acute viral encephalitis. Again the serological evidence indicated infection with SLE or a closely related group B arbovirus. Of the 25 cases, the serological findings in 13 were considered as confirmatory and 12 presumptive for this infection.

During the period July 1 through September 27, 1962, a total of 350 cases of suspected viral infection of the central nervous system were brought under observation in the four counties surrounding Tampa Bay-Pinellas, Manatee, Sarasota, and Hillsborough. At the time of preparing this summary, 172 of these had had at least one specimen examined serologically for evidence of viral infection. In 54 of these, the findings

were considered as confirmatory of SLE infection, in 58 as presumptive, while in 60, the laboratory evidence to that date was negative. As yet, there are no serologic findings on the remaining 178 cases. Under the epidemic conditions which prevailed, a wide variety of disorders with mild CNS symptoms were reported. However, in Pinellas County, of 125 reported suspects, 97 (78%) have serologic evidence of recent or past infection with SLE and the proportion undoubtedly will be increased with later serologic findings. Also, on the basis of clinical observations, of the first 190 cases occurring in that county, 129 (68%) presented findings consistent with classical relatively severe acute encephalitis. Although the total number of cases presumably due to SLE infection cannot be determined at this time, it is apparent that the number will be substantial.

There have been 20 deaths presumed due to encephalitis in the 1962 epidemic. Ten of these came to autopsy; to date, six have been found to have the gross and microscopic findings consistent with acute viral encephalitis. Five had virological confirmation of recent infection with SLE virus. Specimens from one (and probably two) of the autopsies inoculated into suckling mice yielded a virus presumptively identified as SLE.

The cases occurred first in Pinellas County during the late weeks of July. There was a rapid extension from an initial central focus in St. Petersburg until eventually the whole county was involved. The initial case in the Manatee-Sarasota area had its onset on August 18, but the peak occurrence followed that in Pinellas County by some three weeks. The 40 suspect cases in these two counties closely resembled those in Pinellas County. There have been only three confirmed and four presumptive SLE infections in Hillsborough County and, of these, three had substantial exposure in Pinellas County. In Hillsborough County an exacting surveillance of CNS infections was initiated early in 1962 as a part of an investigation of trivalent oral polio vaccination. A variety of mild disorders with possible CNS involvement occurring chiefly in the younger age groups was reported for exacting study. This continued with little change through the encephalitis epidemic period. All were studied for evidence of arbovirus infection. Less than 20% of these suspect Hillsborough County cases examined to date have had confirmatory or presumptive evidence of SLE infections. Although 56 suspect cases were brought under surveillance in this county, these continued to involve chiefly children and young adults. In those with onsets in July and August, the infections were characteristically mild. These cases did not conform to the picture of the epidemic cases elsewhere.

In the three county area (Pinellas, Manatee, Sarasota) in each epidemic, those at greatest risk were the elderly of either sex and commonly those living in homesites with gardens or patios surrounded by heavy vegetation. The infection rate was low in the colored. It is higher in elderly females than in males. In 1961, there appeared to be some concentration of cases in areas adjacent to ponds, lakes, or slow-moving streams where fresh water mosquito breeding was evident. In the recent epidemic, cases have been widespread. A relative lack of evidence of spread of similar epidemic infections in Hillsborough County is as impressive as the number and wide distribution of cases in Pinellas County.

Limited epidemiological and ecological studies have been possible. Following the 1959 outbreak, a random serological survey in Pinellas County revealed an inapparent infection rate with SLE or a closely related virus of 1.5% of the human population. Extensive serological surveys are planned for late 1962.

Beginning in October 1959, avian bloods were collected for serologic and virus isolation studies. In 1960, 886 birds were captured by mist nets, were bled, banded, and released. There were 176 recaptures. Virus isolation studies in wet chicks and suckling mice were performed by the CDC laboratories. EE virus was isolated from 2 birds and WE from 1. There was no isolation of SLE virus. The capture and bleeding of birds was resumed during the 1961 outbreak and extended to a flock of chickens on the premises of one of the cases. Of the 66 chickens bled, 12 had HAI antibodies against EE and 10 against SLE-MVE complex. Of 61 birds collected on the grounds of a small zoo, 18 were positive for SLE antibodies and one for WE. Most of the birds found positive for SLE were ducks and parakeets. Concurrently, in 1961, sera were collected from small mammals and primates in the zoo. There were positive findings for SLE infections in opossums, monkeys, a yellow rat snake as well as in chickens, peafowls, parakeets, and pigeons. Attention was again directed to birds in 1962. The early observations on avian specimens failed to yield positive findings in over 300 examinations for virus isolation. Serologic findings in chickens and ducks were consistent with high preceding infection rates with SLE virus. Tests of wild birds are in process.

Observations by a professional ornithologist were initiated during the recent outbreak. In Pinellas County, strikingly high bird populations were found in the urban areas presumably due to an abundance of food placed in artificial feeders and scattered by bird lovers. The abundant species included mourning doves, english sparrows, mocking birds, blue jays,

redbellied woodpeckers and cardinals. There were also localized large flocks of pigeons in the downtown area. The most unusual observation was the finding of three large flocks of free living parakeets. It was estimated that the largest of these contained more than 10,000 birds. Some 600 ducks are maintained on 31 lakes in the St. Petersburg area. Very few chickens were found in the urban area although a few commercial flocks are maintained in the northern part of the county. Essentially no migratory birds were observed.

There have been efforts to identify the arthropod vector(s). Four pools of mosquitoes collected in 1959 were inoculated into weanling mice with negative findings. During 1960 a total of 11,505 mosquitoes were collected and pooled as 21 different species. These were tested in suckling mice and one-half day old chicks. No EE, WE, or SLE isolations were made. However, there were two isolations of a virus described as Cache Valley like.(These were obtained from pools of A. crucians). Collections were continued during the summer and fall of 1961. Due to drought conditions, mosquito counts in the sentinel traps were extremely low. The predominant species was C. nigripalpus. From a mixed pool of Culex species collected in October, a virus identified as SLE was obtained. Beginning on August 14, 1962, collections were made for viral examinations. Over 12,000 mosquitoes have been collected from widely scattered areas in Pinellas County. From these, a very significant number of viral isolations have been obtained. These include to date, seven isolations of presumed SLE from C. nigripalpus and a substantially greater number of Cache Valley virus, these from A. crucians. Further isolation studies are in progress.

As a part of general mosquito control operations, measurements of mosquito density are taken routinely. In 1962, the mosquito population in Pinellas County was unusually high in July but, by August, this had dropped to levels below the average. The predominant mosquitoes found in the urban areas were C. nigripalpus, A. crucians, P. confinnis, and A. taeniorhyncus.

In summary, the evidence at this time appears to point with reasonable certainty to St. Louis encephalitis virus as the cause of the three observed outbreaks of human disease in this area. The preliminary serologic findings of the first two outbreaks are now supported with ten or more isolations of presumed SLE virus, two (probably three) from human and eight from mosquito sources. The clinical and epidemiological observations fully support this etiologic hypothesis. If the virus was present in the area before 1959, it was circulating with few if any tangential infections of humans. The virus now appears to be endemic in the region and circulating

at such a level that seasonal outbreaks of human infection can occur. The most probable vector, especially in the mosquito-to-human transmission chain, is the species Culex nigripalpus. Other vectors such as C. quinquefasciatus, A. aegypti, and A. crucians cannot be ruled out at this time. Supporting the C. nigripalpus hypothesis are the total population counts during epidemic periods, the observed association of this species with birds, the domestic breeding habits and zoophilic characteristics of the species. The isolation of SLE virus in wild-caught specimens in an epidemic period leaves only the need for evidence from transmission studies to firmly incriminate this vector. The vertebrate reservoir of the virus in this area remains completely unknown. No virus isolations have yet been accomplished from birds and serological studies have indicated a variety of species may be infected. These include the pigeon, duck, parakeet, peafowl, and chicken. Opossums, snakes, monkeys, and man have also been demonstrated to have infection with this virus. Opinions on the amplifying host or hosts for this virus in this area are only speculative at this time. Unknown also are those interesting complexes of factors which produced the large amount of tangential infection and disease in man in one area of the Tampa Bay region and not in another. Hypotheses to explain this phenomenon are at present more plentiful than factual. Finally, it is evident that there are not one but four arboviruses circulating in nature in this area. Only one of these, SLE, has been observed to produce epidemic human infection with disease. Sporadic human infections with EE have been observed. The others, WE and Cache Valley, remain at the moment epidemiologic curiosities. The need for intensive, multi-disciplinary, year-round studies of arbovirus infections in man and in nature in the Tampa Bay region is self-evident.

RESEARCH OBJECTIVES

1. To determine the basic biologic cycle of SLE virus in arthropod and vertebrate hosts.

2. To describe as accurately as possible those factors which lead to tengential infection and disease in man.

3. To develop and initiate control measures based on the observations made in relation to the first two objectives, and evaluate their effect in modifying the occurrence of disease in man.

4. To study the apparent variations in the prevalence of SLE infection in Pinellas and Hillsborough Counties and to seek causes for observed differences.

5. To provide favorable opportunities for coordinated auxiliary studies of arbovirus infections by appropriate groups or individuals.

The study therefore has two major interrelated though differing purposes. It is designed to use the exceptional opportunities for field studies to gather basic information concerning the ecology of SLE and other arbovirus infections in the Tampa Bay area. The ultimate aim is to assemble the information which will provide effective direction of control measures required to prevent human infections, and to establish the effectiveness of indicated control procedures.

REPORT FROM DR. NATHAN J. SCHNEIDER, DIRECTOR, BUREAU OF LABORATORIES, FLORIDA STATE BOARD OF HEALTH, JACKSONVILLE, FLORIDA

Arbovirus isolation attempts in mosquitoes - Vero Beach, Florida:

Mosquitoes were collected in the vicinity of Vero Beach during July through October, 1961, to determine which arboviruses exist in the area. Collections were made with light traps and truck traps. In general, light traps were more productive in the collection of mosquitoes than truck traps operating in the same general area. A total of 36,797 mosquitoes were placed in pools by species and collection sites. No viral agents were detected among 883 pools of mosquitoes inoculated into suckling mice. The only evidence of arboviral activity in this area during this period was a human case in which there was demonstrated a rise in HAI antibody titre against SLE antigen during the course of an illness.

Identification of a Group B viral agent from the Tampa Bay area:

Laboratory studies of an agent isolated from a pool of mosquitoes, culex sp., during November 1961, have been completed. It has been identified as the etiologic agent of SLE. Identification of this agent has been confirmed by two reference laboratories (WHO Regional Reference Laboratory for Arboviruses at CDC, Atlanta). This isolation, together with serological findings among human cases of encephalitis during 1959 and 1961, lends additional evidence of SLE activity in the Tampa Bay area.

Serological Surveys:

Serological studies for the presence of HAI antibody among residents of three south central Florida counties were conducted. Preliminary findings on 363 sera indicate little group A and some group B arbovirus activity in this area. (Table 1)

Table 1
Frequency of Residents of Three South Central Florida Counties Presenting Evidence of Past Arbovirus Infection (366 residents tested)

HA antigen	No. of sera 1:10 or >	Per cent of total
EEE	1	0.3
WEE	0	0
SLE	21	5.7
MVE	32	8.7
Ilheus	18	4.9
Dengue 2	20	5.5
Total	92	25.1

Serological Survey - Seminole Indians:

Similar serological studies were conducted on sera from Seminole Indians residing on the Dania and Brighton reservations (Table 2). Findings seem to indicate a low group A activity, similar to individuals residing in the three south central Florida counties. However, group B arbovirus activity is undoubtedly higher among the Seminole Indians surveyed.

Table 2
Frequency of Seminole Indians Presenting Evidence of Past Arbovirus Infection (226 Indians tested)

HA antigen	No. of sera 1:10 or >	Per cent of total
EEE	1	0.4
WEE	1	0.4
SLE	54	24
MVE	51	24
Ilheus	13	6
Dengue 2	4	2
Total	124	54

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

Isolations of Bunyamwera group viruses from south Florida (Chamberlain Sudia, and Coleman):

Laboratory work has been continued on mosquitoes collected at the Big Cypress Seminole Indian Reservation in south Florida, 1961 (see Information Exchange No. 4, October, 1961). Thus far, collections from March through July, 1961, comprising a total of 194,447 mosquitoes of 25 species, have been tested in suckling mice in 3,178 pools. These have yielded 65 virus isolations. Only 15 of these isolates have been studied to date, and have been tentatively identified as belonging to the Bunyamwera group and being closely related to Cache Valley virus. Most of those still unidentified appear to be of this same type, based upon the AST in suckling mice. Table 1 shows the species infected, dates of collection, and number of isolations.

The sera of the Seminole Indians themselves have not yet been tested against this agent. However, a number of other animal species from the area have been checked, with some, particularly the dogs, possessing HI antibody (Table 2).

The same or a similar virus was taken twice from Anopheles crucians collected in Pinellas County, west central Florida, in 1960, 8 times from this same mosquito species collected in south Alabama in 1960, and from Psorophora species (probably confinnis) taken in Indiana in 1961. In view of this apparent wide distribution, additional serological studies were performed on human and animal sera collected in the past year from areas other than south Florida, and are presented in Table 3. It is of interest that 14 of 150 south Alabama human sera were positive. This was the same area which yielded 8 Cache Valley-like virus isolations from Anopheles crucians.

The negative serologies on chickens and birds from south Florida and other areas suggest that avian species may be unimportant as natural hosts. This suggestion is supported by laboratory inoculation studies. Chicks inoculated subcutaneously when 1/2-day old with the Cache Valley-like virus, failed to develop a viremia over a 7-day period, or to demonstrate HI antibody at 1 or 2 months. Other animals tested were hamsters, laboratory rabbits, wild cottontail rabbits, guinea pigs, grey squirrels, cotton rats, opossums, raccoons, eastern chipmunks, and

Rhesus monkeys (Table 4). The rabbits, hamsters, and cotton rats produced the highest viremias, up to titers of $10^{4.0}$ or greater, measured intracerebrally in suckling mice. This is suggestive evidence that wild rodents or rabbits may be natural hosts.

None of the experimental hosts appeared to be affected adversely by the subcutaneous inoculation of the virus, with the possible exception of the wild cottontail rabbits. Only 3 of this species were on test and all died, but these deaths could have resulted from daily blood-taking and handling rather than from virus effects.

Table 1
Virus Isolations from South Florida Mosquitoes, 1961

Mosquito Species	Number of Isolations	
	June	July
Anopheles crucians		47
An. quadrimaculatus	1	
Culex nigripalpus		4
Mansonia indubitans		1
Psorophora confinnis	3	9
Totals	4	61

Table 2
Sera of Animals from the Big Cypress Reservation
Tested for HI Antibody Against the Cache-Valley-like Isolate

Animal species	No. Tested	No. positive	No. Equivocal
Cotton rat	22	0	
Rice rat	3	0	
Cotton mouse	76	0	
Grey squirrel	2	0	
Dog	14	10	
Raccoon	7	1	1
Opossum	13	0	
Cow	16	1	3
Chicken	97	0	
Snake	15	0	1
Totals	265	12	5

Table 3
Miscellaneous Sera Tested for HI Antibody Against
Cache Valley-like Virus

Animal Species	Source	No. Tested	No. Positive	No. Equiv.
Man	S. Alabama	150	14	
Man	Pinellas Co., W. Central Fla.	58	1	
Raccoon	N. Florida	6	2	
Raccoon	Georgia	5	0	1
Wild Birds	S. Alabama	74	0	
Chicken	Arizona	185	0	
Water moccasin	Texas	35	0	
Water moccasin	Kentucky	6	0	

Table 4
Results of Subcutaneous Inoculation of Various
Animal Species with Cache Valley-like Virus

Animal Species	Viremia	HI Antibody
Chicks	-	-
Hamsters	+	1/1280 to 1/10, 240
Guinea pigs	-	<1/10 to 1/640
Lab rabbits	+	1/1280 to 1/2560
Cottontail rabbits	+	To be done
Grey squirrels	-	To be done
Cotton rats	+	To be done
Opossums	-	1/80 to 1/160
Raccoons	+	To be done
Eastern chipmunks	+ (1 of 2)	Not done
Rhesus monkeys	+ (1 of 2)	1/640 and 1/2560

Attempts to determine antigenic differences between EE strains (Morgante):

An effort was made to determine definite antigenic differences between seven strains of EE virus, and to establish which of the strains was the best antigen for lab use in human and animal serology. The strains used were Arth 167 M_2SM_1; Florida 08-3, 08-55 M_1SM_2; Trinidad

2-4443 M_6; New Jersey 1960 M_1SM_1; Massachusetts catbird 369 M_1SM_1; Wisconsin horse 984 E_1SM_1; and standard M 2047 P470. Each was given two additional SM passages. Antigens and antisera were prepared in suckling and adult mice, respectively, and used in cross CF, HI, and N tests.

No differences appearing significant were found except that the Trinidad antigen showed consistently a haemagglutination-inhibition titer higher in the homologous system than in the heterologous. The arth 167 strain, already at use in the laboratory, still appeared to be the strain of choice in performance of HI, CF, and N tests.

REPORT FROM DRS. LOUIS S. GRANT AND EDWARD A. BELLE, DEPARTMENT OF MICROBIOLOGY UNIVERSITY OF THE WEST INDIES, MONA, JAMAICA

An intensive serological survey of residents in Jamaica was initiated with the help of the Rockefeller Foundation and the Trinidad Regional Virus Laboratory in 1953, after a large number of sera collected from different parts of the island showed high antibody titres to two well-known arthropod-borne viruses--St. Louis encephalitis and dengue. Since that time, over one hundred cases suspected of having encephalitis were admitted to the University Hospital. Twenty-five of these cases form the basis of a paper to be published.

Paired serum specimens from three of these cases demonstrate a rise in specific antibodies to St. Louis virus in haemagglutination-inhibition and complement fixation tests. The sera showed complete protection against the same virus in baby mice neutralization tests.

REPORT FROM DR. N. H. WIEBENGA, HEAD ARTHROPOD-BORNE VIRUS SECTION, LABORATORY OF TROPICAL VIROLOGY, NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES, NATIONAL INSTITUTES OF HEALTH

Combination Antiserum Pools:

Studies have continued with type specific group A antisera which have been combined into pools that might be used as serological grouping reagents. (See Exchange No. 4) Each type specific antiserum and four pools containing different combinations were tested by NT and HI tests against EEE, Sindbis, Uruma, WEE, AMM 2354, Chikungunya, VEE, and Una virus antigens. The broadest reactivity was obtained from a pool of EEE + Sindbis + Uruma + WEE antisera; however, a pool of Sindbis + Mayaro seemed to be almost equally cross-reactive, while pools containing EEE + Mayaro + WEE or EEE + WEE cross-reacted with fewer antigens of the group.

Serum titers apparently were not significantly reduced by the dilution resulting from the mixing of type specific sera; tests against representative group B, C, and Bunyamwera antigens demonstrated that all four group A antiserum pools were group specific.

Bunyamwera Group Virus Plaques under Agar:

Continued studies of Bunyamwera group viruses in monkey stable (MS) cell cultures demonstrated that only Germiston, Guaroa, and Wyeomyia produced plaques under agar. Optimal conditions required 5 days incubation under 1.5% Nobles' Agar in Eagles' Plaque Medium before the addition of neutral red; plaques were easily observed and counted 24 hours later. Plaques formed by Germiston and Guaroa were small, from 1 to 2 mm in diameter, while those of Wyeomyia were larger, from 2 to 4 mm in diameter.

In view of the possible activity of Takemoto's agar inhibitor, viruses of this group were tested for plaque production under agar media containing DEAE-Dextran in concentrations graded from 20 to 60 mgm % in 5 mgm increments. As summarized in Table 1, all viruses tested produced plaques in the presence of from 30 to 40 mgm % DEAE-Dextran, with reasonable agreement between the resulting PFU titers and their known TC CPD_{50} titers. Incorporation of DEAE-Dextran did not modify the requirement for 5 days incubation under agar prior to the addition of neutral red.

TABLE 1

BUNYAMWERA GROUP VIRUSES IN MS CELL CULTURES

COMPARISON OF PLAQUE FORMATION UNDER AGAR,

WITH AND WITHOUT DEAE-DEXTRAN IN EAGLES' PLAQUE MEDIUM

VIRUS	TC CPD_{50} [1]	PLAQUES: 5 DAYS INCUBATION + NEUT. RED			
		UNTREATED		DEAE-DEXTRAN TREATED	
		SIZE[S]	PFU[1]	SIZE[S]	PFU[1]
BUNYAMWERA	6.7	NEG.	-	LARGE	6.9
BATAI (CHITTOOR)	5.8	NEG.	-	LARGE	6.3
CACHE VALLEY	5.7	NEG.	-	LARGE	5.4
GUAROA	6.5	SMALL	5.9	MIXED	6.3
GERMISTON	8.3	SMALL	8.5	LARGE	7.9
WYEOMYIA	6.7	LARGE	5.3	LARGE[X]	5.9
ILESHA	5.3	NEG.	-	LARGE	5.7
KAIRI	5.7	NEG.	-	MEDIUM	5.1

1 = LOG_{10} PER ML.

S = PLAQUES ARBITRARILY DIVIDED INTO SMALL (LESS THAN 2 MM DIAM.) AND LARGE (MORE THAN 2 MM DIAM.)

X = UNUSUALLY LARGE PLAQUES - UP TO 5 MM.

REPORT FROM DR. BENNETT L. ELISBERG
DEPARTMENT OF RICKETTSIAL DISEASE
WALTER REED ARMY INSTITUTE OF RESEARCH
WASHINGTON, D. C.

One of the consistent characteristics of the arthropod-borne viruses is the ability of ether and sodium desoxycholate to inactivate these agents. Because of certain undesirable features of the ether sensitivity test, investigators studying respiratory and enteric viruses have explored the suitability of chloroform for use in place of ether. Their reports indicate that the respiratory and enteric viruses which have been tested have the same response to both ether and chloroform; viz. ether sensitive viruses were inactivated by chloroform and ether resistant agents were resistant to chloroform.

It was not previously known whether all ether sensitive arthropod-borne viruses could also be inactivated by treatment with chloroform. In the course of completing the characterization of viruses isolated from mosquitoes in Malaya, parallel sensitivity tests employing sodium desoxycholate and chloroform were carried out. Due to limited supplies of suckling mice, it was not possible to include an ether test in the experiments.

Each virus was tested for its sensitivity to chloroform and sodium desoxycholate at the same time. Standard seeds composed of 20% infected mouse tissue suspensions in 3.2% bovine albumin in buffered saline stored at -65° C, were diluted 1:10 with 0.8% bovine albumin borate buffered saline pH 9.0 (BAB) and centrifuged. Following this, serial 10 fold dilutions of the supernate were made. Equal portions of the serial virus dilutions under test were added to two sets of tubes containing 0.8% BAB (which served as controls for each of the treatments), and to other tubes containing sodium desoxycholate (final concentration 0.1%) in the 0.8% BAB diluent. Similar additions were made to tubes containing chloroform (5% final concentration) in the same BAB diluent. In a few of the tests the concentration of the chloroform was increased to 10%.

The series of tubes containing sodium desoxycholate and virus, and the corresponding tubes in the control sequence were incubated in a water bath at 37° C for 1 hour and then inoculated intracerebrally into 1-3 day suckling mice. Two litters of suckling mice were inoculated with each dilution of virus.

In the chloroform test, the contents of the tubes containing the chloroform, and the control diluent, were mixed after the addition of the appropriate virus dilution by applying the tubes to a Vortex mixer for 15 seconds. This produced a very fine emulsion of chloroform in the aqueous phase which was then kept in suspension by shaking for 10 minutes at 37° C on an Eberbach Shaking apparatus. The chloroform was sedimented by centrifugation of the tubes at 1, 500 rpm for 5 minutes. The supernatant fluid was removed and inoculated into suckling mice as described above. Extreme caution must be used in this step of the procedure for chloroform accidentally removed with the supernatant fluid makes the inoculum toxic and an inordinately high mortality occurs in the suckling mice during the first three days following injection. It should be noted here that the conditions of mixing, shaking, and incubation of the two test systems had no detectable different effect on viral activity inasmuch as the control titrations for each of the reagents under test gave almost identical results.

The effectiveness of sodium desoxycholate in the inactivation of sensitive viruses can be influenced by the protein concentration of the menstruum. Several tests were carried out where the concentration of the bovine albumin was reduced from 0. 8% to 0. 2% to determine if lower concentrations of protein similarly increased the effectiveness of chloroform.

Table I presents the results of the sensitivity tests on 10 arthropod-borne viruses. Among these are included members of the Casals Group A, Group B, and Bunyamwera Group, and a new group comprised of viruses which have been isolated only in Malaya. It is apparent from the results that chloroform is capable of inactivating the arthropod-borne viruses studied. Reduction of the bovine albumin concentration from 0. 8 to 0. 2% permitted the sodium desoxycholate to more effectively inactivate Getah, Tembusu, and Batai viruses. The reduction of protein content of the diluent, or increase of the chloroform concentration from 5 to 10% did not significantly influence the inactivating capacity of the chloroform in tests with Tembusu, Getah, Sindbis, Batai, and Ketapang viruses.

Three viruses known to be ether resistant have been tested so far. A strain, each of Coxsackie A-4 and B-4, and encephalomyocarditis viruses were not inactivated by either sodium desoxycholate (1:1000) or chloroform (1/20) in the presence of the bovine albumin diluents.

TABLE I

Inactivation of Certain Malayan and Other Arthropod-Borne Viruses with Sodium Desoxycholate and Chloroform

Classification	Strain Designation	Percent Bovine Albumin in Diluent	Reduction in Titer (LOD LD_{50}) Sodium Desoxycholate 1:1000	Chloroform 1:20
	Getah (AMM-2021	0.8	1.4	≥4.9
		0.2	≥3.8	≥5.0
CASALS GROUP A	Bebaru (AMM-2354	0.2	≥4.3	≥4.1
	Sindbis (Gelam, AMM-2215)	0.2	≥5.0	≥6.4
CASALS GROUP B	Jap. Enceph. (Nakayama)	0.8	≥4.8	≥4.9
	Dengue-1 (Hawaiian)	0.8	≥5.2	3.5
	Dengue-2 (N. Guinea C)	0.8	3.3	≥3.8
	Tembusu (AMM-1775)	0.8	2.8	≥5.9
		0.2	≥4.6	≥6.0
CASALS BUNYAMWERA GROUP	Batai (AMM-2222)	0.8	2.2	≥6.3
		0.2	≥3.1	≥5.5
CASALS NEW GROUP	Bakau (AMM-2325)	0.8	≥4.0	4.0
	Ketapang (AMM-2549)	0.2	3.2	≥4.9

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

Cache Valley-like Viruses from Chincoteague-Assateague Island Mosquitoes:

Collaborative investigation of the ecology of arthropod-borne virus infections was continued on Assateague Island during 1961. These studies which seek to determine the mode, extent, and chronology of dissemination of EEE virus in a coastal mid-continent habitat, failed to reveal any significant activity of this virus during the 1961 season. Thus, no EEE virus was recovered from over 32,000 collected mosquitoes (Table I) or 1800 samples of plasma obtained from summer resident birds. Further, there was no apparent EEE among wild ponies on Assateague, or the herds on Chincoteague. However, six strains of virus, ultimately shown to be related to Cache Valley virus, were recovered in suckling mice from pools of Aedes sollicitans and Anopheles crucians collected from late August to early October. This comment summarizes the identity and behavioral characteristics thus far known for these agents.

Collection histories for the 6 recovered strains are summarized in Table II. Mosquito collections were ordinarily held at 70-80° F for 24 hours before identification, pooling and freezing at -20° C as approximately 10% suspensions in saline containing 0.4% bovine albumin, penicillin, and streptomycin. Frozen suspensions were transferred from the field laboratory to WRAIR weekly, where isolation attempts in suckling mice were made. Certain specimens, found to be contaminated with Gram negative organisms, were retreated after chloramphenicol (500 mcg/ml) was added.

Each of the 6 strains killed the majority of suckling mice upon initial inoculation after 5 to 6 days incubation; each was rapidly established in suckling mice by serial passage. Infectivity titers of 1st and 2nd passage mouse brain ranged from 10^{-} to 10^{-}/0.02 ml; however, by 5th passage, titers attained 10^{-} to 10^{-}. Weanling mice were also susceptible, but approximately 100 times less sensitive than suckling mice to virus in 5th to 10th mouse passage. M273/61 and M303/61 killed upon subcutaneous inoculation of suckling mice to approximately the same titer as when inoculated intracerebrally. Wet chicks inoculated subcutaneously with 10-100 suckling mouse $ICLD_{50}$ developed no apparent illness, no viremia,

and no detectable antibodies by the 23rd post-inoculation day. No obvious disease followed intraperitoneal injection of adult mice, or rabbits, although both developed high titered antibodies after inoculation. The agents also propagated in continuous cultures of grivet monkey kidney cells, but not in hamster kidney monolayers or embryonated eggs. Agents M273/61, M291/61, M303/61, and M400/61 were destroyed or markedly reduced in titer by shaking with chloroform for 10 minutes at room temperature.

Attempts to produce hemagglutinin from strain M273/61 in 7th passage using the standard acetone-sucrose method have not been successful. The only evidence for hemagglutinin was obtained at pH 6.4 and 37° C from brain material; titers were 1:20 or less. No hemagglutinin was shown with antigens prepared from liver. Attempts to obtain an HA antigen from M303/61 similarly failed.

The agents were ultimately shown to be related to one another and to Cache Valley virus by neutralization and complement fixation tests. Potent neutralizing antisera to each of the unknown viruses were readily made in rabbits. Antisera to M273/61 and M303/61 neutralized between $10^{3.2}$ and $10^{5.7}$ LD_{50} of each of the 6 strains, but failed to neutralize Bunyamwera virus. Both antisera however neutralized between $10^{1.5}$ and 10^{2} LD_{50} of Cache Valley virus (strain Be Ar 7272) in intracerebral tests. Antisera to Bunyamwera virus failed to neutralize M273/61 virus; attempts to neutralize the newly recovered viruses with Cache Valley virus antiserum are in progress. Reciprocal CF tests with mouse antisera made to each of 4 strains of the Assateague Island viruses (M273/61, M291/61, M303/61, and M400/61) showed that they were essentially identical (Table III); further, these antisera reacted to significant titer with the Belem strain of Cache Valley virus.

Preliminary serological surveys indicate that all of 12 adult wild Assateague ponies, bled in July 1961, possessed significant amounts of neutralizing antibody to strain M273/61. Thus, without further study, it appears that this agent has been present, and circulating among equines on Assateague Island in years past. Additional observations on the ecology of Cache Valley-like virus in the Chincoteague-Assateague Island area are in progress and will be reported at a later date. (Submitted by Dr. E. L. Buescher)

Table I

Virus isolations from mosquitoes collected on Assateague and Chincoteague Islands, Virginia, during 1961

Species	Total specimens	No. pools tested	No. pools positive
Aedes cantator	37	3	0
A. sollicitans	25,363	288	5
A. taeniorhynchus	994	59	0
A. vexans	172	3	0
Aedes spp.	34	1	0
Anopheles crucians	396	31	1
An. quadrimaculatus	57	4	0
Anopheles spp. *	51	4	0
Culex salinarius	5,398	4	0
Culiseta melanura	1	1	0
Psorophora confinnis	3	1	0
Total	32,506	519	6

* Species not determined

Table II

Collection Histories, Arthropod pools yielding Assateague Island Viruses, 1961

Mosquito Pool	Species	Pool Size	Collection Date	Collection Type	Area
M273/61	A. sollicitans	100	8/23	aspirated from personnel	Assateague
M291/61	A. sollicitans	100	8/23	raccoon baited trap	Chincoteague
M303/61	A. sollicitans	98	8/24	raccoon baited trap	Assateague
M400/61	An. crucians	10	9/13	heron baited trap	"
M424/61	A. sollicitans	100	9/26	aspirated from personnel	"
M515/61	A. sollicitans	57	10/2	heron baited trap	"

Table III

Immunologic Homogeneity of Assateague Island Viruses, 1961.

CF Antigen *	Reciprocal CF Antibody Titers with Indicated Mouse Antisera			
	M273	M291	M303	M400
M273	64	128	64	64
M291	64	128	32	64
M303	64	64	32	64
M400	32	64	32	64
Cache Valley (Be Ar 7272)	32	32	16	32

* Used in antigen excess (5% mouse brain suspension clarified high speed centrifugation)

REPORT FROM DEPARTMENT OF ENTOMOLOGY WALTER REED ARMY INSTITUTE OF RESEARCH WASHINGTON, D. C.

A program of studies on mosquitoes and mosquito-borne viral agents was initiated jointly by the Walter Reed Army Institute of Research and the University of Maryland School of Medicine in West Pakistan. Studies begun in Lahore in May will be continued until October of this year, and than reinstituted the following year. Earlier serologic surveys on young adults conducted by Dr. Fred R. McCrumb, Jr. of the University of Maryland School of Medicine, International Center for Medical Research and Training had indicated that Group B agents were apparently quite common in West Pakistan and that Group A agents were less common. Antibodies to West Nile virus appeared to be predominant. The present studies have as their objectives the delineation of the mosquito fauna, study of the host range and feeding habits of mosquitoes, study of mosquito population dynamics, and collection of mosquitoes for virus isolation. In collections made through June, Culex pipiens fatigans was the only mosquito found resting in large numbers in houses in the city of Lahore, whereas Anopheles culicifacies and Anopheles stephensi were the predominant species resting in houses in rural areas. Human biting collections yielded very few mosquitoes, but Stegomyia species predominated in these small catches. Culex tritaeniorhynchus was the predominant species in cattle biting and light trap catches. Onset of the monsoons in July is expected to materially alter the mosquito picture. Intermittent operation of electrical power has been playing havoc with the maintenance of frozen material.

Characterization and identification of virus isolates obtained in Pakistan and Iran which were reported on previously, have been temporarily suspended because of problems stemming from their instability. Most of these isolates underwent marked reduction in titers when stored as mouse brain serum-saline mixtures at -70° C. Recently it was found that virus prepared in a sucrose-gelatin stabilizing medium retained titer to a far greater extent than aliquots of the same virus prepared in the conventional serum-saline mixtures, regardless of the temperature at which they were stored. Peculiarly, the pH of this stabilizing medium is on the acid side and currently studies are underway in the effects of pH on virus degradation. At the same time, the sandfly isolates are being adapted to adult mice to facilitate further characterization and identification.

REPORT FROM DRS. FRANK M. HARDY AND DAVID ARBITER
VIROLOGY DIVISION, U.S. ARMY MEDICAL UNIT
FORT DETRICK, FREDERICK, MARYLAND

Studies dealing with the cellular changes associated with Venezuelan equine encephalomyelitis (VEE) virus infection in the L-cell have been initiated in this laboratory. Findings thus far have correlated certain changes with virus replication.

Cytochemical Examination: Cells fixed with osmium tetroxide and stained with Sudan Black were found to contain minute black granules in their cytoplasm. The number of cells containing these granules as well as an increase in their concentration was found to occur as early as 8 hours after infection of the cultures. Titration of infected culture supernatant fluids in mice via the IC route related these increases to virus growth.

Chemical Analysis: Extraction of fixed cells with either cold acetone or boiling ether-alcohol mixture (3:1) did not reduce the concentration of the granules. These results suggested that the inclusions were composed largely of phospholipid.

To further characterize the inclusions, various lipid solvents were employed in the extraction procedure. While no one solvent completely extracted the sudanophilic material, its solubility was found to increase as the polarity of the solvent decreased. These results suggest that the lipid granules are comprised of more than one type of phospholipid.

Further work is in progress to more fully characterize the phospholipid components with the possibility that specific VEE virus associated changes may be demonstrated.

REPORT FROM DR. FRANK G. FAVORITE
CHIEF, MEDICAL ENTOMOLOGY DIVISION
ARMY CHEMICAL CENTER, MARYLAND

Our program continues to be essentially one of surveillance. We have continued in the main with collections of blood from birds and other animals. Most of the blood samples have been screened for virus in 21-day-old white mice. (See Table 1. Table 1 is a continuation of the data reported in the fourth issue of the Information Exchange.) Two of the bloods taken

from Yellow-billed cuckoos have produced mortality patterns in mice suggestive of EE virus. No identification has been made yet. It is also hoped that our data may provide linkage information to workers both north and south of our peninsula where discrete outbreaks of EEV are not uncommon.

Listed below are some observations which have been made during the course of our program:

a. A mature male cowbird, collected locally on 13 March 1962, bled, and released, had band number 60/125192.

b. Random ectoparasite collections reveal one species which was taken from 18 out of 118 birds; the rabbit tick, Haemaphysalis leparispalustris

c. In the fourth issue, we remarked upon attempts to separate serum from whole blood using a seamless cellulose tubing technique reported in the literature. After considerable work with this method, it was determined unsuited for processing bird bloods.

d. We have found some distinct advantages in converting to vacuum tubes for collection of blood samples in the field. This system allows for a higher percentage of uncontaminated specimens and is less traumatic to the animals, particularly birds.

e. We are about to extend our blood collecting program to larger wild animals, as deer, using a remote-drug delivery system. We are interested to learn if others have found the narcotizing agent, nicotine alkaloid, to interfere with Neut. and HI tests.

f. Blood feeding arthropods have been collected periodically and are being held in a frozen state until time permits processing. Processing will include determination of blood meal source and screening for virus, if indicated. Antisera to the following animals have been prepared or are in the process of preparation:

1. human
2. gray squirrel
3. opossum
4. herring gull
5. cottontail rabbit
6. gray fox
7. cat
8. white rat
9. dog
10. goat
11. sheep
12. black rat snake
13. common grackle
14. yellow-shafted flicker
15. crow
16. ring-billed gull

Table 1. Blood samples collected and screened ( ) for viral agents in 21-day old white mice, 18 August 1961 to 7 August 1962. (All samples reported in the 4th Issue of the "Newsletter", adjusted total 213 (100), have been screened.)

FAMILY	SPECIES	NUMBER OF SAMPLES	
Birds:			
Falconidae	Sparrow Hawk	1	(1)
Charadriidae	Killdeer	1	(1)
Scolopacidae	Common Snipe	1	(1)
Laridae	Herring Gull	2	(2)
	Ring-billed Gull	1	(1)
Cuculidae	Yellow-billed Cuckoo	1	(1)
Picidae	Yellow-shafted flicker	1	(-)
	Hairy Woodpecker	2	(1)
	Downy Woodpecker	3	(2)
Tyrannidae	Eastern Wood Pewee	1	(1)
Carvidae	Common Crow	1	(1)
Paridae	Carolina Chickadee	1	(1)
	Tufted Titmouse	2	(2)
Sittidae	White-breasted Nuthatch	2	(2)
Mimidae	Mockingbird	1	(1)
Turdidae	Robin	10	(5)
	Wood Thrush	1	(1)
	Hermit Thrush	4	(4)
Sylviidae	Golden-crowned Kinglet	1	(1)
Sturnidae	Starling	148	(142)
Parulidae	Myrtle Warbler	2	(2)
Ploceidae	House Sparrow	25	(24)

Table 1 (continued)

FAMILY	SPECIES	NUMBER OF SAMPLES	
Icteridae	Red-winged Blackbird	56	(56)
	Rusty Blackbird	3	(3)
	Common Grackle	5	(2)
	Brown-headed Cowbird	32	(31)
Fringillidae	Cardinal	3	(1)
	Slate-colored Junco	8	(7)
	White-throated Sparrow	3	(1)
	Swamp Sparrow	1	(-)
	Song Sparrow	2	(1)
Reptiles:			
Colubridae	Black-rat Snake	3	(1)
Mammals:			
Didelphidae	Opossum	2	(2)
Sciuridae	Gray Squirrel	9	(9)
Leporidae	Eastern Cottontail	1	(1)
Sub-total		340	(311)
Cumulative Total		553	(524)

REPORT FROM DR. DAVID E. DAVIS, PROFESSOR OF ZOOLOGY, PENNSYLVANIA STATE UNIVERSITY, UNIVERSITY PARK, PA.

Under a PHS grant, a three-month training program on the techniques for study of birds and arboviruses was undertaken during the past summer. The four senior-level students were assigned different projects on the University Woodlots. Three students were trained in the use of mist nets for capturing birds and also how to bleed and handle the blood. Two methods were employed to describe the vegetation on the area. One student was assigned to collect and key out the mosquitoes present on the area.

At the time of writing this report, approximately 150 birds were banded and 60 bloods taken to the laboratory for analysis. Due to post breeding wandering, a large number of nomads were captured and only a few subsequently recaptured. For this reason, an estimate of the population on the area could not be determined.

REPORT FROM DRS. MARTIN GOLDFIELD AND OSCAR SUSSMAN NEW JERSEY DEPARTMENT OF HEALTH, TRENTON, N.J.

As reported in a previous issue of this publication, the New Jersey group has set up four study sites where mosquitoes are trapped one day and birds are netted, banded, and bled four days of every week during the summer and fall. Three of the sites are located in the Atlantic shore area where human EE infections are now known to have occurred for many years. The Great Swamp area in Morris County, situated in the northern part of the state (distant from the shore) was chosen as the fourth study site. Studies of more than 500 blood samples collected from residents of a municipality adjoining this site indicated no detectable evidence of past infection with EE or WE in man.

The 1961 season was marked by exceedingly low mosquito populations, as reported by County Mosquito Extermination Commissions and as compared with trapping data of our own in previous years. This was particularly true with respect to A. sollicitans in the shore areas. Our surveillance system of horses and pheasants during 1961 revealed only one episode of EE involving unvaccinated breeders in a flock of pheasants in the northernmost county of the state. A total of only 18,446 unengorged mosquitoes were trapped, pooled, and tested. Five thousand wild birds were banded and bled, of which 1300 have been tested thus far.

All primary virus isolations have been made in 1/2 day-old chicks observed for 96 hours. Subsequent passages have been made in recently weaned mice, and viruses have been identified by NT in this host. Virtually all virus strains have been successfully reisolated.

As seen in Table 1, 25 isolations were obtained from 21 pools of mosquitoes. Of great interest is the fact that, excepting A. vexans, all isolations from C. melanura were obtained later in the season than those from other species. It is difficult, therefore, to attribute infection of these other species to a "spill-over" from a bird-C. melanura cycle. The isolation of EE from a pool of three specimens of U. sapphirina represents the first such report, to our knowledge, regarding this species, whose feeding habits are virtually unknown. The eight arboviruses isolated from mosquitoes of the Great Swamp study site (together with five isolates from wild birds) indicate clearly that epizootic activity can be detected in an inland area where no evidence of involvement of man can be found.

Twenty-one isolations of WE have been made from the blood of 1300 wild bird bloods thus far tested. It is noteworthy that three of the birds with detectable viremia were among 101 birds netted during the week of June 12th

at one study site (Brigantine National Wildlife Refuge), an area that yielded no WE in mosquitoes throughout the season. This represented the first full week of bird netting at this site during the 1961 season.

During the 1961 season, surveillance of a flock of chickens was maintained at each of the four study sites. During the first half of the season, randomly selected chickens were bled at approximately two-week intervals. It was later possible to band chickens in each of the four flocks and to obtain sequential blood samples on the same birds. In all, 666 blood samples were collected between May 23rd and October 23rd, 1961, and tested for EE and WE HI antibody levels. Of 26 sera demonstrating HI activity (21 for EE and 5 for WE) only one disclosed significant EE antibody in mouse neutralization tests. The positive sample was collected at the Brigantine site on July 31st and showed an HI titer of 1:80. All other chicken sera yielded HI titers between 1:10 and 1:40 on repeated testing but were uniformly devoid of significant neutralizing activity. Thus, HI testing of chickens would appear to be misleading unless substantiated by neutralization tests. During the 1961 season in New Jersey, these chickens appear to have failed as a sentinel of significant EE and WE epizootic activity. However, it is still possible that infection of chickens may be dependent on those factors promoting epidemic activity.

Thus far in 1962, there have been no authenticated cases of arbovirus infections in horses, pheasants, or man. The spring and summer seasons have been marked by alternating periods of drought and cold weather that appear to have been very effective in keeping mosquito activity at a low level. A total of 3,729 wild bird bloods, 4,199 unengorged mosquitoes, and 125 miscellaneous specimens have been tested for the presence of virus. Only two isolates have thus far been authenticated, both WE from catbirds. Significantly, these two were again obtained in the 1st two weeks of netting activity at the Brigantine Refuge in the early part of June. A third isolate, from a Downy woodpecker bled on July 18th at Forked River, has not as yet been identified.

Table 1
1961 Mosquitoes
Arboviruses Isolated

Great Swamp:

7/18-8/1	Culex pipiens	EE & WE
7/18-8/1	Culex restuans	WE
8/1	Culex salinarius	EE & WE
9/19-9/26	Culiseta melanura	WE
9/26-10/3	Culiseta melanura	WE
10/3-10/17	Culiseta melanura	EE

Table 1 (continued)

Forked River			
8/3-8/31	U. sapphirina	EE	
8/10	Culex salinarius	EE	
9/24	Culiseta melanura		WE
9/24-10/8	Culiseta melanura	EE	
Brigantine			
7/6-7/25	Culex territans	EE	
Mays Landing			
8/10	Culiseta melanura		WE
8/17-8/24	Culiseta melanura		WE
8/31	Culiseta melanura	EE	
8/31	Culiseta melanura		WE
8/24-9/7	Culiseta melanura	EE &	WE
8/31-9/7	Culiseta melanura	EE &	WE
9/7	Culiseta melanura	EE	
9/14	Culiseta melanura		WE
9/14-10/3	Aedes vexans		WE
10/3	Culiseta melanura		WE

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

Attenuated Strain of Japanese B Vaccine (OCT-541) as a Vaccine:

See abstract of paper for symposium at Atlanta, included in this same issue of the Information Exchange.

Mouse Hepatitis Virus and Possible Relationship to Supposed Arbovirus Isolations:

While testing mosquitoes collected in Bangkok in 1961, an apparent series of isolations of viruses was made. A few of these became of high titer after a few passages. They were not chikungunya as expected. No HA

antigen could be prepared but a good CF antigen reacted with antisera prepared in mice against several strains. No primary or continuous cell line in use showed any suggestion of CPE. Other laboratory animals were not tested but no spontaneous disease was observed.

While this was still in progress, spontaneous deaths began to occur in all our suckling mice in all animal rooms regardless of inoculum or dilutions. Control uninoculated sucklings when exposed in the same rooms became ill within a few days. Adult mice were resistant. These illnesses in sucklings were due to the same virus supposedly isolated from mosquitoes, as proved by antigen prepared from their brains.

All mice were purchased from another city as "pathogen free". They were delivered in special filter cages. Pregnant females were received as our source of sucklings. These adults had no antibodies until after their litter became infected. Isolation precautions had been maintained in all mouse rooms because these were pathogen free and no mice were received from other sources. No wild mice are present. At no time was there any evidence that mice arrived infected.

We stopped all work and stopped all mouse orders and did an elaborate scrubdown and sterilization. More than a month later, mice from the same source were obtained, the other Bangkok mosquito suspensions were inoculated and all other work resumed. No virus was present in the mosquito suspensions and no unusual spontaneous deaths occur.

On the basis of all data, mouse hepatitis virus was suspected. Dr. Wallace Rowe at NIH confirmed this by showing our prototype virus to be antigenically closely related to other viruses in the complex.

The next chapter in this story is possibly the most significant. The epizootic itself may not have too much significance. We were convinced by our tests and the use of these same mice by others that the mice probably became infected in our environment and by the air-borne route. But where did the original virus come from?

As we have previously reported, we have a number of low-titered mosquito and blood "isolates" from the Far East that never could be antigenically studied. They formed no usable antigens even after 20 to 75 passages in suckling mice. Antisera prepared from them reacted against no established viruses in our hands. A few mouse antisera from such isolates were than tested against the CF antigen of our epizootic hepatitis virus. Three reacted! Some of these isolates had been made

originally in mice from another dealer. Dr. Rowe informed us that he has found latent infection by these hepatitis viruses in essentially all of several large colonies tested. It therefore appears quite probable that low-titered CNS virus isolates in suckling mice now in the hands of several workers and defying identification are hepatitis virus and that many of the virus stocks of known agents which we all have may contain a low-titered undesirable passenger virus belonging in this group. This latter may account for unexpected low-titered immunologic cross reactions (other than HAI) which crop up between viruses not previously recognized as crossing.

We thought the above experience might be helpful to others and therefore record it in some (but not all) detail. By the way, liver pathology by our strain is essentially negligible. It produces encephalitis! Virus is present in the liver of mice but in comparatively low titer.

Bangkok Symposium on Hemorrhagic Fever (1961):

Colonel Felsenfeld, who has been ill, informs me that the final proof has been returned to the printer in Bangkok and the monograph will soon be printed and copies delivered to Pittsburgh. It will be mailed to the distribution of the Information Exchange.

Reeves-Hammon Monograph:

"Epidemiology of the Arthropod-borne Viral Encephalitides in Kern County, California, 1943-1952," University of California Publications in Public Health, Volume 4, Public Health Monograph Series, is now out by the California Press--hope you all get to see it.

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

Chicken embryonic cell cultures were compared with weanling mice in neutralization tests for antibody to Japanese encephalitis virus. Virus-dilution tests yielded similar results whether weanling mice inoculated intracranially or chicken embryonic cell cultures under agar medium were used to detect unneutralized virus. Exceptions occurred only with chicken

plasmas (neutralization indices were greater in mice than in cultures) and with a few sera of several species showing log neutralization indices of 4-5.5 (results were higher in cultures than in mice). Results with microcultures of cells grown in wells of leucite hemagglutination trays were similar to those in customary macrocultures in bottles, yet required less serum. Serum-dilution tests were readily performed in microcultures of cells under agar medium. Sera, positive or equivocal by virus-dilution test, had serum-dilution titers > 1:4, suggesting that in microcultures, sera could be screened at 1:4 initial dilution for antibody to JE virus. Rises in antibody titers were detected in cell cultural serum-dilution tests without addition of accessory factor. The concentration of yeast extract employed for growing chicken embryonic cells before addition of agar medium, influenced number and clarity of JE virus plaques; inhibitors for arthropod-borne viruses were found in agar.

A method was developed for sterilizing Aedes aegypti eggs and rearing sterile larvae, pupae, and adults.

Viral interference occurred between influenza and a variety of arthropod-borne viruses in embryonated chicken eggs. Non-pathogenic Escherichia coli given intraallantoically to embryonated eggs prevented brain damage from neurotropic influenza virus given intravenously.

JE, but not WE virus, was inactivated by chicken bile or pure bile salts despite presence of plasma or serum from a variety of animal species. In the presence of serum, JE virus was approximately three times more sensitive to sodium deoxycholate than WE virus, but in the absence of protein, it was only twice as sensitive. Apparently plasma proteins bind some deoxycholate and that remaining unbound is enough to inactivate JE but not WE virus. Sodium deoxycholate had an effect on viral hemagglutinin similar to its effect on infectivity, but it had no inactivating effect on JE or WE RNA. To date, 21 arthropod-borne viruses have fallen into two groups based on their inactivation by 1000 µg per ml of sodium deoxycholate in chicken plasma. Four group A, Colorado tick fever and Sicilian sandfly fever viruses were not inactivated whereas 6 group B, two group C, two Bunyamwera, Guama, California, Bwamba, Anopheles A and Naples sandfly fever viruses were inactivated.

Continuing studies on capturing technics of birds (shooting vs netting) as factors in surveys for arthropod-borne viral neutralizing antibodies showed that in up to 5% of North American Blackbirds, shooting brought

about appearance in plasma of neutralizing substances specific for WE or SLE viruses. Of 15 Blackbirds developing WE neutralizing substance after shooting, only 2 concomitantly developed SLE neutralizing substance; of 7 birds developing SLE neutralizing substance after shooting, only 2 of 6 tested showed even equivocal changes with WE virus.

Mag 115 virus, recovered from mosquitoes in Japan during 1956, is between 50 and 100 mμ in size. No hemagglutinin has yet been produced and attempts to identify the virus immunologically continue to fail. Neutralizing antibody was found in plasmas from Japanese pigs bled during August and September 1956 and 1957; of 57 pigs less than a year of age, 11 (.19) showed LNI >2.2 and 16 (.28) showed LNI 1.7-2.2. One pig in a mosquito trap at Sagiyama developed antibody between late June and mid-July 1957. No neutralizing antibody was found in 29 humans residing in rural areas or 13 humans from urban areas near Tokyo, and only 1 of 54 nestling and juvenile ardeids bled in August and September 1956 and 1957 was positive in neutralization test (a Black-crowned Night Heron bled on 3 September 1957 at Shinhama).

Studies have continued with other unclassified viruses from Japan (M7/270 from Anopheles sinensis collected at Sagiyama in 1957; Tsuruse from a Blue Magpie and BCNH K622 from a Black-crowned Night Heron near Tokyo in 1954). M7/270 virus remains difficult to work with because its known host range is limited to suckling mice, and it produces only low titers of virus in infected tissues. Tsuruse virus fell among the arthropod-borne viruses that are inactivated by sodium deoxycholate and ether, is apparently between 50 and 100 mμ in size and did not react in CF test with psittacosis antiserum nor in neutralization test with GD VII or EMC antisera.

During July and August 1961, mosquitoes and plasmas from humans, pigs, cows, and wild birds were collected at two locations in central Mexico; these specimens are currently being examined for their content of arthropod-borne viruses and/or antibodies.

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

The Division of Laboratories and Research, Albany, New York, instituted an arthropod-borne virus program late in 1959. Evidence of

arbovirus activity in New York State has been sought and found in wild animals and in persons by two serologic methods, hemagglutination-inhibition and neutralization tests in mice. Isolation of infectious agents from various arthropods and birds has also been attempted by intracerebral inoculation of 1-day-old mice and by inoculation of primary hamster kidney cell cultures. The results have been prepared for publication.

Qualitative neutralization tests were carried out in 1-3 day old mice. Undiluted serum was mixed with an estimated 100 LD_{50} of virus. Homologous hyperimmune serum for the test virus, and virus titrations in normal serum were in each test. The four virus strains used were group A, Eastern, and Western encephalomyelitis (EE, WE), and group B, Powassan (POW) and St. Louis encephalitis (SLE). The technic of the hemagglutination-inhibition (HI) test was that of Clarke and Casals. All sera were treated with 25 per cent kaolin and adsorbed with goose red blood cells.

Data obtained in 1960-61 indicated that arbovirus activity was present in wild animals in 12 of the 15 counties in New York State from which sera were obtained. The predominant antibody detected was group B, closely related to Powassan virus, which Casals showed was a member of the tick-borne Russian spring-summer complex.

One hundred and fifteen sera (7 bear, 6 cottontails, 70 deer, 9 foxes, 1 opossum, 22 raccoons) that had been collected for other projects from 1955-60 and stored at -20° C were tested in vivo. Thirteen animals (1 bear, 8 foxes, and 4 raccoons) neutralized POW virus and 5 of these also neutralized SLE. Three sera (2 deer and 1 raccoon) had some detectable WE antibody.

During December 1960 and June-August 1961, sera from 84 foxes and 16 raccoons trapped in Schuyler County with the cooperation of Mr. Samuel Linehart of the New York State Conservation Department were examined. Forty-two per cent of these sera had neutralizing antibodies for POW and 27 per cent for SLE. Group A antibodies were relatively rare, 11 per cent neutralized WE virus and 4 per cent EE virus.

The HI tests confirmed in general the neutralization tests. Twenty-four of 51 sera from the preliminary survey reacted with the group B antigens. Of these, 20 sera (1 bear, 6 foxes, and 13 raccoons) inhibited both POW and SLE, and 4 sera (3 cottontail and 1 bear) reacted only with SLE. Group A HI antibodies were found in 3 fox and 6 raccoon sera.

Two pools of bat sera collected in Schoharie County had high HI titers for SLE, 160 and >320. These reactions may have been due to bat salivary gland virus, another member of the B group of arboviruses.

Inhibition of POW agglutination was shown in 42 per cent of the Schuyler County fox and raccoon sera and 37 per cent inhibited SLE. Only 11 per cent had HI antibodies for WE and 6 per cent for EE.

Seven pools of meadow mice sera and 2 cottontail sera collected from Suffolk County in 1961 were tested and 6 of the pools had HI titers of 10-40 with SLE. One cottontail serum had antibodies for WE.

In contrast to the animal sera, group B antibody was seldom encountered in the sera from 463 persons examined using the same methods.

Blood samples were collected from 385 persons admitted in 1961 to three of the State hospitals for the mentally retarded, Letchworth Village, Rome and Newark State Schools; 49 sera were also collected from the resident population in Newark State School during September-October 1960. Tested also were 58 sera from 28 persons who had clinical histories suggestive of encephalitis.

Powassan neutralizing antibodies were detected in 2 persons. One was an 11-year-old boy, who had lived exclusively in Albany County prior to entering Rome State School. The second, an 18-year-old male, had lived in Iowa, Texas, and Pennsylvania before his admission to Letchworth Village. The serum from the young man also neutralized SLE virus. Sera from 2 other persons gave inconclusive neutralization results with SLE. Neutralizing antibodies for WE were detectable in 2 sera.

A greater number of the human sera reacted in the HI tests than in neutralization tests. Confirmatory results were obtained with duplicate sera from the 11-year-old boy and 18-year-old male. The former inhibited POW antigen only, with a titer of 10, while the latter inhibited hemagglutination of both POW and SLE with titers of 10 and > 320, respectively. Seven other sera had low POW titers. These sera and 19 others reacted with SLE antigen.

Group A reactions were observed in 5 persons, one of whom had an HI titer with WE of 320, which was strongly suggestive of an infection, but there was insufficient serum for a neutralization test. This serum was from a young boy who had left the institution before an additional blood sample was obtained to confirm our findings.

Unidentified arthropod-borne viruses present in Suffolk County:

In the summer of 1961, a survey was conducted in cooperation with the New York State Science Service to determine whether arthropod-borne (arbo) viruses are present in arthropods and birds in Suffolk County on Long Island. From a total of 452 preparations, 6 infectious agents which seem to be strains of the same agent were isolated. These strains have not as yet been identified. Their relationship to human disease is to be investigated.

Dr. Paul Connors collected and identified 139 birds (35 species) and Dr. Edward Berg and Dr. Hugo Jamnback did the same for 2996 arthropods (30 species) from June through October. The birds were mist netted in 10 areas and the arthropods were collected from 20 areas. The single or pooled arthropods and individual bird spleens were immediately frozen in tightly-capped polyethylene tubes after identification was completed and shipped to the Division of Laboratories and Research for testing.

The period of observation (for both mice and tissue culture) was 21 days. Six infectious agents were isolated in the mice but none in the hamster kidney cells. Five of the strains were recovered from mosquitoes: 4 pools of Culiseta melanura, in which the number of mosquitoes were 25, 9, 7, or 3; and 1 pool of Culex pipiens, in which there were 13 mosquitoes. The sixth agent was from the spleen of an oven bird. Reisolation of five strains from original frozen suspensions was accomplished. The mosquitoes were collected at 3 different sites and the bird was caught August 29 within 2 miles of 2 of these sites. Three of the positive pools consisted of mosquitoes trapped on August 2, and the other 2, of mosquitoes caught at 2 of the same sites but on different dates--July 18 and August 8. Incubation periods for the 6 infectious agents were similar--from 4 to 7 days, depending on the strain. The agent passed through Seitz EK filters. The LD_{50} titers were in the same range for all strains, 10^5 to 10^6 per 0.03 ml. These agents caused encephalitis in suckling mice, involving cerebrum and cerebellum; two strains also caused myocarditis. The pathology is not similar to that of the Coxsackie viruses. No inclusion bodies and no bacteria were seen. Four-week-old mice and young guinea pigs did not become ill when inoculated intracerebrally with a 10-per cent suckling mouse brain suspension in 5 serial passages. The strains were not adapted to primary cultures of hamster kidney cells, human amnion FL cells or Hep_2 cells. There was some indication of propagation of one of the strains in the amniotic sac of embryonated hens' eggs. On treatment with ether or sodium desoxycholate, there was a decrease in titer of all the isolates of one or two logs.

By neutralization and complement-fixation tests, all 6 strains so far as studied have been shown to be similar if not identical. The agents were not neutralized with immune serum prepared with the following viruses: psittacosis, lymphocytic choriomeningitis, herpes simplex, Theiler's mouse encephalomyelitis TO, MM, Newcastle disease, Colorado tick fever, Eastern or Western encephalomyelitis, St. Louis encephalitis, or Powassan. Hyperimmune mouse sera for 3 of the agents were sent to Dr. Casals. He examined them in a hemagglutination-inhibition test with a number of antigens; no reaction in 1/10 dilution of the sera was found with the following antigens:

Group A

Chikungunya
Sindbis
Semliki
Mayaro
Eastern encephalomyelitis
Western encephalomyelitis
Venezuelan encephalomyelitis
Aura
Una

Group B

Yellow fever
West Nile
Zika
Wesselsbron
Uganda S
St. Louis
Ilheus
Bussuquara
Powassan
Dengue type 2

Group C

Oriboca
Caraparu

Bunyamwera group

Bunyamwera
Cache Valley
Ilesha
Germiston
Guaroa

California encephalitis virus (Hammon-Reeves)

Ticks from Schuyler County:

We failed in our attempt to isolate infectious agents from 45 pools of frozen Ixodes cookei which had been collected during June-August 1961 from foxes and raccoons, whose sera we had examined, and from several woodchucks which had not been bled. In addition, ticks of the same species collected from a skunk trapped in Genesee County were tested. No blind passages were made.

REPORT FROM JOAN B. DANIELS, VIRUS SECTION
DIAGNOSTIC LABORATORIES, INSTITUTE OF LABORATORIES
MASSACHUSETTS DEPARTMENT OF PUBLIC HEALTH
BOSTON 15, MASSACHUSETTS

A grant for the study of arthropod-borne encephalitis in Massachusetts has been awarded to Robert A. MacCready, M.D., and Joan B. Daniels by the NIH with future support approved for four years.

The specificity of serological methods discussed in the last Information Exchange is still a major concern. Neutralization by plaque reduction being both specific in Group A and very sensitive (Science 133 No. 34533 '61, see also Porterfield in March '62 Information Exchange) is used as the reference test.

HI is not always demonstrable in sera containing neutralizing antibody for EE or WE. Conversely, sera which do not neutralize either virus may have high titers for either or both viruses. A total of 10/50 turkeys (1959) and 28/100 sentinel chickens (1961) (chicken HI tests in Greeley, CDC lab) showed the latter reaction.

It is possible that an unrecognized Group A virus is infecting domestic fowl in Massachusetts. In cooperation with the Taunton Field Station, a flock of sentinel chickens at Site I in Pine Swamp is being tested thrice weekly for arbovirus viremia during the month of August. Five out of about 500 sentinel chicken bloods studied to date have been cytopathogenic for chick embryo tissue cultures. Some difficulty is being encountered in getting transmission in tissue cultures and in chick embryos. An agent has also been isolated from a catbird bled in July.

An alternative hypothesis to explain the HI reaction is presence of non-specific inhibitors. It is entirely possible that as for influenza viruses there are several non-specific inhibitors for arbovirus HA. If so, they may not all be removed by standard methods.

If 1962 sentinel chickens show this phenomenon, it is hoped to obtain large amounts of blood from such birds at the end of the season so that some careful studies can be made here and in a reference laboratory.

A word of warning is offered to laboratories using tissue cultures. One lot of the antimycotic, mycostatin (Nystatin) has been found to contain a factor toxic for chick embryo tissue cultures. One per cent horse serum detoxifies for a time. Often the complete experiment is lost on

the third day after planting. In the absence of serum, cells fail to attach to glass and despite highly buffered media pH drops below 6.8. Controls without mycostatin or containing only 10 units/ml. form a good monolayer and maintain neutral pH. Another lot of mycostatin was nontoxic for chick embryo tissue cultures even in 100 units/ml. Monkey kidney cultures, HeLa and WS amnion cell lines were not affected adversely by either lot. Chick embryo and duck embryo tissue cultures are appparently particularly sensitive.

Cleaning residues on glassware may also be toxic for these cells. Certain media (199) detoxify and permit good growth. Plastic plates (Falcon TCPD 6015) are preferred. The plastic tubes have rather poor optical properties but are useful if complete cell destruction is the expected criterion of viral activity.

REPORT FROM DR. R.C. WALLIS
THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION
NEW HAVEN, CONNECTICUT

Study of the ecology of EEE has continued in 1962 on the bionomics of potential mosquito vectors, mosquito biology, and the relation of mosquitoes to the wild and domestic bird population in Connecticut. Extremely dry weather in the spring, and dry, cool weather throughout the summer has resulted in very low mosquito population. The springtime pest, Aedes stimulans, was the principal mosquito collected in study areas throughout the springtime and summer season and has persisted in local accumulations in swampy woodland valleys through mid-August. Aedes vexans, Aedes triseriatus, and Culex restuans began developing three weeks earlier than expected, but have not occurred in large numbers. As of mid-August, there has been no indication of EEE virus activity detected in the state except for the presumptive clinical diagnosis of encephalitis in one horse in Avon, Connecticut, July 8th. The horse, since that time, has exhibited a clinical picture of remittent fever inconsistent with that of EEE. Acute and convalescent blood samples were taken for serological study. Mosquito collections have been taken from three permanent study areas throughout the spring and summer, and pooled for virus isolation study, and from the vicinity of the sick horse in Avon since mid-July, but results are not yet available from these studies.

REPORT FROM DR. J. R. HENDERSON
DEPARTMENT OF EPIDEMIOLOGY AND PUBLIC HEALTH
YALE UNIVERSITY SCHOOL OF MEDICINE

South Carolina Field Study:

As a continuation of investigations of the ecology of arboviruses in South Carolina started in 1961, collections of mosquitoes and bird and rodent bloods were extended through a three-week period during June and July of this year. Collections were concentrated in Beaufort County as was the case last year. New Jersey type light traps were utilized for collection of mosquitoes. About 24,000 mosquitoes, divided into some 600 pools and representing 15 species, are being examined for virus by the plaque technique using chick and duck embryo cell cultures.

Birds collected in mist nets were bled, banded, and released. Virus isolation studies on 177 bird bloods were negative. Preliminary testing of these bloods for HI antibodies against 4-8 HA units gave the following results.

SLE positive--10 of 160 including 6 immatures
EEE positive--11 of 160 including 7 immatures
WEE positive--0 of 160

Connecticut Field Study (R. C. Wallis):

During the past five years, bird collections have been concentrated in the springtime nesting season and in the fall EEE transmission season. Because few virus isolations have resulted, the emphasis on collections in 1962 was shifted to the period following the beginning of the southward migration of the Purple Grackle (July 10 to August 10), with the purpose of sampling newly arrived juveniles and the domestic starlings and English sparrows associating with the migrants in the vicinity of two pheasant farms where EEE has occurred in the past.

A portable 8 x 8 x 5 foot house trap was built for operation at the Albert Soli pheasant farm in Plainville, Connecticut, and with the collaboration of Mr. Leslie Williamson of the State Board of Fisheries and Game, two 10 x 10 x 5 foot house traps were constructed for operation at the Osborn State Prison Farm in Enfield, Connecticut.

To date, the birds listed in the following table have been collected and blood samples taken for virus and serological study.

Birds Sampled in Connecticut Field Studies During The Mid-summer North-South Migration Season

Bird	No. adults	No. juv.	Place
Purple Grackle	47	21	Enfield
Starling	70	139	Enfield
Blue Jay	29	1	Enfield
Red-winged Blackbird	2		Enfield
Cowbird	2		Enfield
English Sparrow	63	11	Plainville
Totals	213	172	

Hemolytic Activity by EEE and WEE (Dr. Karabatsos):

In a continuation of studying certain aspects of hemolysis by Group A arthropod-borne viruses, experiments were performed to determine whether the hemolysin was closely associated with the hemagglutinin or whether it represented a soluble product elaborated during virus infection. Ultracentrifugation of tissue culture preparations of EEE and WEE revealed that both the hemolysin and hemagglutinin were recoverable in the sediment. EEE and WEE viruses were assayed for HA and hemolytic activity in the presence of an extract of normal chick embryo tissue culture, and it was found that the HA and hemolytic activity of EEE was significantly reduced by non-specific inhibitory tissue components present in the extract, whereas the HA activity of WEE was not appreciably affected. Thus, observation lends support to the previously advanced hypothesis that EEE virus is more readily bound by non-specific inhibitor, and it also may explain the necessity of subjecting these virus preparations to cycles of freeze and thaw before hemolytic activity is revealed. Presumably, the hemolytic activity is masked by non-specific inhibitor bound to the virus, and some inhibitor is dissociated from the virus during the process of freeze-thaw.

REPORT FROM DR. LARS KARSTAD, HEAD DIVISION OF ZOONOSES AND DISEASES OF WILDLIFE ONTARIO VETERINARY COLLEGE, GUELPH, ONT.

A surveillance type study is in progress in Ontario to detect arbovirus activity in wild birds sampled during spring and fall migrations.

Birds are examined and bled at Long Point, Ontario, in cooperation with personnel of the Ontario Bird Banding Association. Several hundred birds were bled in each of the spring and fall migrations in 1961 and this program is being continued in 1962. Blood is obtained by jugular puncture, diluted immediately in saline and held on wet ice for one to two days pending separation of plasma and cellular elements. Diluted plasma is then used in serological tests and the cells are used in attempts to isolate viral agents. Most of these latter specimens have been processed by inoculation of infant mice but embryonated eggs and newly-hatched chicks have also been used. The only confirmed viral isolate to date was made in suckling mice inoculated with blood from a slate-colored junco trapped at Long Point on October 7, 1961. With the assistance of Dr. P.H. Coleman at the WHO Regional Arbovirus Reference Laboratoryat the USPHS Communicable Disease Center (CDC) in Atlanta, this agent has been identified as eastern encephalitis virus.

It may be interesting to note that this is, as far as we can determine, the first isolation of eastern encephalitis virus made in Canada since the equine epizootics of 1938. This may be no more than a reflection of lack of study in this area.

Virus neutralization tests in cell cultures have been performed on the 1961 plasma samples by Dr. L. Lauerman of the University of Wisconsin and HI tests on the 1962 specimens will be performed at CDC. Mention will be made at a later date of the results of this serology.

Just prior to departure from the Virus Research Laboratory at Waycross, Georgia, in 1961, a small study was carried out to measure the duration of survival of eastern encephalitis virus in dried whole blood and plasma adsorbed on small paper discs (as used for the collection of blood and sera in the field; Jour. Inf. Dis. 101: 295-299, and Jour. Inf. Dis. 106: 53-59). The virus was found to be surprisingly stable when dried in whole blood and stored under conditions of normal room temperature and lowered humidity. Titrations performed in newly-hatched chicks showed that infectivity persisted for at least 347 days in paper disc-adsorbed whole blood stored in a desiccator over $CaCl_2$. This information may have some value to anyone contemplating use of paper disc-adsorbed blood specimens.

REPORT FROM DR. C.E. GORDON SMITH
LONDON SCHOOL OF TROPICAL MEDICINE AND HYGIENE
LONDON, ENGLAND

Studies of the ecology of louping ill:

In the spring of 1960, several severe outbreaks of louping ill occurred in Scotland involving the death of up to 50% of the lambs in certain flocks.

Since then, a study has been undertaken with Mr. A. L. Wilson, Veterinary Investigation Officer for the West of Scotland, and Mr. K. J. O'Reilly of the Wellcome Research Laboratories on three farms near Dalmellington in Ayrshire, Scotland. The sheep have been numbered and are bled at intervals for serology. Lamb deaths are investigated and studies have started of ticks (Ixodes ricinus) and of possible wild vertebrate hosts.

In sheep the haemagglutinin-inhibiting antibody is less persistent than the neutralizing antibody. Serological studies have shown that the incidence of infection varies markedly between 1 natural area of hill grazing (hirsel) and another. Comparing 2 adjacent hirsels in 1961, the infection rate appeared to be about 5 times higher on 1 than 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 the 3 farms were found to be 100%, 39%, and nil although tick infestation was heavy on all of them. 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.

The onset of spring was exceptionally late in 1962 and, judging by tick infestation, over a month later than in 1961. An experiment in trapping vertebrates and dragging for ticks in collaboration with Dr. M. G. R. Varma in the study areas was, therefore, timed before the spring increase in tick population instead of during it as intended. The weather was very cold throughout the experiment (17 March to 17 April) and the activity of mammals was minimal. The mean number of female Ixodes ricinus per infested sheep increased from 1.2 in the last week of March to 15.7 by the second week in April. Ticks were collected from rough pasture by dragging a lint "blanket" over the ground. Appreciable numbers were not collected till the second week in April when the grass temperature rose to about 8° C. Most of the ticks collected by dragging were larvae and nymphs, with a preponderance of larvae. With the rise in grass temperature to 13-16° C at the end of April and in early May, the number of active ticks had increased considerably. Louping ill virus was isolated and reisolated from a pool of 42 unfed nymphs of I. ricinus collected by drag on 29 April. These must have been infected as larvae in the previous year and as a considerable proportion of larvae feed on small wild vertebrates, the infection may have been acquired from such hosts.

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

Reference Serum against louping ill virus:

As part of the WHO program on arthropod-borne viruses, a pool of louping ill serum prepared in sheep at the Wellcome Research Laboratories has been dried in 2 ml volumes and is available on request to other reference laboratories and to investigators participating in the Information Exchange. The serum is not intended to be a monospecific serum reacting only with louping ill virus; it is known to cross-react with other tick-borne viruses in neutralization tests and with other Group B antigens in HAI tests. In spite of these disadvantages, the serum may be of value to laboratories which either do not have or are unwilling to handle viruses of the tick-borne encephalitis complex.

Collaborative assay on tick-borne virus antisera:

As part of another WHO project, a set of standard antisera are being tested in several different centers. The methods in use in London include HAI tests and neutralization tests carried out by plaque techniques in chick embryo cells using louping ill, Central European, Kyasanur Forest, and Langat viruses.

Plaque titration of Guama and Oriboca viruses:

A visiting worker, Miss M. V. T. de Figueiredo, working with Dr. H. G. Pereira, has developed a satisfactory plaque method for titrating Guama virus and antisera in HeLa cell cultures. Successful results have also been achieved with Oriboca virus in the same cell system.

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

Work on arboviruses was started at the end of 1961 and has been chiefly devoted to the application of tissue culture techniques. After a preliminary training of personnel:

1. A few strains of the B group were studied in stationary and roller tubes of HeLa cell cultures in Hanks lactalbumin medium in order to find out if increased oxygenation would induce a cytopathic effect. No such effect was observed with RSSE, Jap B, and WN viruses.

2. No significant cytopathogenesis was observed in monolayers of trypsinized calf kidney tissue inoculated with WEE, WN, Jap B, and RSSE.

3. A cytopathic effect was observed in hamster kidney monolayers with WEE, SF, Chikungunya, West Nile, Yellow Fever, Dengue 2, or Jap B. No CPE was observed, however, with a mouse adapted Graz strain of RSSE.

4. Plasma clot tissue cultures of gills and spleens of fresh water fish (goldfish and roaches), Caratius caratius and leuciscus rutilus, incubated in a roller drum at room temperature were inoculated with Chik, SF, WEE, Jap B, RSSE, Bunyamwera. No cytopathogenesis was observed during a 14-day observation period. Moreover, at the end of this period the supernatant fluid phase was shown to be virus free by mouse inoculation. No multiplication of the virus took place.

5. Plaque assay on chick embryo tissue cultures as described by Porterfield were successful. This technique gives very good results with the group A arboviruses tested: WEE, SF, and Chikungunya. More difficulties are encountered with virus strains of the B group. Efforts are now made to increase the quality of the cell sheet so as to obtain plaques with this very important group of viruses.

Using the Porterfield technique, antibodies against SF virus were demonstrated by the serum dipped filter paper discs (6 mm diameter) method proposed by De Somer for the detection of antibodies to poliomyelitis antibodies. Care was taken to incubate all neutral red containing agar overlaid petri dishes in the dark in view of minimizing eventual photo inhibition as described for enteroviruses.

6. Using the plaque vibration method, as described by Porterfield, a study was made of the kinetics of virus multiplication of SF virus in hamster kidney monolayers (HKM) and HeLa cells. It was found that in HK TC, virus multiplication starts about 3 hours after virus adsorption, and reaches its maximum after about 6 hours. The titer of the extracellular virus follows closely that of the cell-associated virus, which means a very short release time. Maximal virus titer is reached before the appearance of cytopathogenesis.

In HeLa cells, however, extra- and intracellular virus titer remain at a constant level during the first 6 hours after virus adsorption. At the 32nd hour, there is a marked increase of 60th intra- and extracellular virus, the titer of the latter being higher. They remain constant thereafter. The titer in HeLa cells is lower than that obtained in the same number of HKT cells.

Incomplete cytopathic effect is observed in HeLa cells and this starts again after the maximum virus titer is obtained.

7. The hypothesis that small rodents might act as virus reservoirs for many arboviruses (C.N. Johnson in Viral and Rickettsial Diseases of Man) was tested by searching viremia in white laboratory rats inoculated subcutaneously with the following viruses: SF, Chikungunya, WEE, WN, Jap B, Yellow Fever (neurotropic strain), RSSE, and Bunyamwera. On the third day after infection, serum of the inoculated animals and/or an aqueous suspension of their liver was inoculated intracerebrally into white mice. No evidence of viremia was found. In one instance, a pregnant rat was inoculated subcutaneously with RSSE virus. In the offspring born 7 days later, no virus could be demonstrated in the brains of the young rats at day 4, 7, and 12 after birth.

REPORT FROM DR. GODSKE NIELSEN
DEPARTMENT OF VIRUS RESEARCH, TROPENINSTITUT
HAMBURG, GERMANY

The Isolation of Infectious RNA from Brains of Mice Infected with Yellow Fever Virus:

From brains of mice infected with yellow fever virus, a ribonucleic acid was isolated by phenol-extraction; its infectivity reached 0.03% of that of the original virus. The preparation revealed a UV-adsorption typical for nucleic acids. No protein impurities could be demonstrated by the biuret-reaction. The infectivity of the RNA could be inhibited by ribonuclease, trypsin, or serum-globulin, whereas desoyxribonuclease, chymotrypsin, and ethanol had no inhibitory effect. It is suggested that the inhibition by trypsin or globulin is not specific.

REPORT FROM DR. ARNE SVEDMYR, CHIEF, VIRUS DEPARTMENT CENTRAL BACTERIOLOGICAL LABORATORY OF STOCKHOLM CITY SWEDEN

Our laboratory has been involved in studies of tickborne encephalitis in Sweden since 1955, partly in collaboration with epidemiologists, entomologists, and vertebrate zoologists. The results may be summarized as follows:

1. Detroit 6 cells are suitable for propagation of Central European tickborne virus in high titer as well as for neutralization tests based on cytopathogenicity of the virus. However, the degree of cytopathic changes may vary considerably with different sublines of the cell strain. The tissue culture fluid, after treatment with B-propiolactone for inactivation of the virus, has proven highly satisfactory as CF-antigen for routine diagnostic tests on human sera. References: G. von Zeipel and A. Svedmyr, Arch. Virusforsch, 1958, 8:370; A. Svedmyr, G. von Zeipel, B. Holmgren, and J. Lindahl, Arch. Virusforsch, 1958, 8:565.

2. A systematic study in 1958 of cow sera collected from all over Sweden revealed that infections with a virus belonging to the RSSE-Louping ill group are prevalent particularly in the southeastern part of the country, a geographic distribution that apparently fits with the occurrence of Ixodes ricinus ticks (no specimens of Ixodes persulcatus have been found in Sweden as yet). A serological investigation of human cases of meningoencephalomyelitis indicated a closely similar distribution of the human RSSE infections. Reference: G. von Zeipel, A. Svedmyr, and B. Zetterberg, Arch. Virusforsch, 1959, 9:449.

3. The tickborne infection rate among patients with CNS disease hospitalized in Stockholm has kept at a moderately high level since the first year completely studied, 1956, when the maximum so far, 68 out of 168 cases, was found. The clinical picture has been the same as that observed elsewhere for cases of "Central European tickborne encephalitis"; a paralytic rate of 15-20 per cent is noteworthy, however. References: B. Holmgren, J. Lindahl, G. von Zeipel, and A. Svedmyr, Acta. Med. Scand., 1959, 164:507; B. Holmgren, Nord. Med., 1961, 66:1705.

4. Virus strains recovered from Ixodes ricinus ticks and from human cases of meningoencephalitis during the viremic phase could not be antigenically differentiated from a Czechoslovac strain of Central European

encephalitis virus. The sensitivity of various isolation techniques was studied. Reference: G. von Zeipel, Arch. Virusforsch, 1959, 9:460.

This summer (1962) a strain of tickborne virus has been recovered from the brain of a moose calf which was shot because it kept walking in circles. Professor H. Hansen, who provided the brain material, had found that it showed signs of severe encephalitis.

5. During the summers of 1960, 61, and 62, small mammal surveys have been performed at three localities, two within the endemic southeastern region and one in an almost free area in central Sweden. Although most of the mammals in the endemic localities carried one or often several Ixodes ricinus ticks, only about 3 per cent of them showed serological evidence (NT) of a previous virus infection. Laboratory experiments with field mice indicate that the low frequency of immune animals is not due to a fatal outcome of the tickborne virus infection nor to lack of detectable antibody response. These results seem to indicate that even in endemic areas only a minority of the ticks spread the virus. This conclusion was further confirmed by a study of the age distribution of antibodies among cows and conforms well with von Zeipel's sporadic recoveries of virus from ticks.

6. A survey of migratory birds for ectoparasites, viremia, and antibodies to RSSE-like viruses was initiated in 1961 and is again taken up in 1962. No results are available as yet.

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

Study of the Relation of the Birds to Natural Foci of Tickborne Encephalitis:

The findings of specific antibodies against tick-borne (TE) virus in pheasants of the area of Central Europe have stimulated the question whether the pheasants could be regarded as natural reservoirs of the TE virus.

Thirteen pheasants were infected with TC virus.

Number of Pheasants	Modus of Infection	Amount of Inoculum	LD_{50} ic for mice in 0.1 ml
5	sc	0.5 ml	10^7
2	im	0.5 ml	10^9
2	ic	0.1 ml	10^9
4	3 x sc	0.5 ml	10^7

The last pheasants, four in number, were reinfected in the intervals of seven days. The blood was taken every 24 hours (from the first to the fourteenth day) from vena alaena in heparin. The blood was injected in white mice sucklings. The blood samples for examination of virus neutralizing antibodies were taken on every 7th day to the 28th day during the experiment. The brain, liver, spleen, and pancreas were taken at the same intervals for virological and histological testing.

The experiments were carried out with TE virus passed on HeLa cells and mice (Hypr M_{42} H_{52} M_3) and once through transovarial passage on Ixodes ricinus. The titre of the virus in 10% brain suspension was 10^9 LD_{50} ic for mice in 0.1 ml.

Table 2

The study of virus neutralizing antibodies in pheasants infected with Tick-Borne Encephalitis Virus

Pheasant No.	Modus of Infection	Weeks after Infection 1	2	3	4	Killed after:
1	sc	-				1 week
2	sc	-	-			2 weeks
3	sc	-	-	-		3 weeks
4	sc	-	-	-	-	4 weeks
5	sc	-	-	-	-	4 weeks
6	im	-				1 week
7	im	-	1:16			2 weeks
8	ic	-				1 week
9	ic	-	1:8			2 weeks
10	3x sc	-	-	-	-	5 weeks
11	3x sc	-	-	1:8	1:8	5 weeks
12	3x sc	-	-	1:8	1:32	5 weeks
13	3x sc	-	-	-	1:8	5 weeks

The virus neutralizing test on HeLa cells with 100 CPD_{50} of virus.

- = negative result

Both after sc and im inoculation, no virus was present either in blood or in brain or in inner organs. The two animals infected sc gave positive histological findings for meningoencephalitis of the viral type. The virus neutralizing antibodies were present only in sera of pheasants infected im and ic and after repeated subcutaneous application.

We therefore assume that the pheasants could play no decisive role in the circulation of tick-borne encephalitis virus because no viremia occurred after the infection of birds even with large doses of virus.

References:

Nosek, J., Gresikova, M., Rehacek, J., Kozuch, O., Albrecht, P. (1962): The Role of Birds in Natural Focus of Tick-borne Encephalitis: Experimental Infection of Pheasants (Phasianus colchicus) with Tick-borne encephalitis virus. J. Hyg. Epid. Immunol. (in press).

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

The Tahyna Virus:

Two lines of Tahyna virus, the "neuroadapted" and the "extraneural" (isolated from the blood of Syrian hamsters after their im inoculation with the original mosquito suspension) of the strain 236 were compared in hamster kidney cell cultures (HK) (Dr. Sefcovicova, L.). The "neuroadapted" in its 7th ic mouse brain passage was causing a regular cytopathic effect (CPE) already in the first HK passage in 2-3 days and the titer of the virus according to the CPE paralleled those determined ic in young white mice. The "extraneural" line also multiplied in HK but the CPE was in the first HK irregular and appeared only after 10-14 days.

Young hares of 0.4 kg of weight were inoculated sc with 0.05-0.5 ic mouse (7.0 gr) LD_{50} of Tahyna virus per 1.0 kg of body weight (Dr. Simkova, A.). A viremia of 5 days of duration was observed. The titer of the virus in the blood was from the second to the fourth day 2-3 log units (LD_{50}).

Viremia in sc infected pigs of 30.0 kg body weight with 7.1 log units of LD_{50} could be ascertained from the first to the third day only after ic inoculation of suckling mice (Dr. Bardos, V. and Dr. Jakubik, J.). In pigs of 3.40 to 5.80 kg of body weight after inoculation of 3.6 log units of Tahyna virus was viremia ascertained from the second to the fourth day also after ic inoculation of young white mice. Virus was present in both experiments only in the undiluted blood of pigs.

The pathogenesis of the Tahyna virus infections was studied in white mice (Dr. Bardos, V.). Mice of 8-9 gr weight were inoculated sc with 4.5 log units of the "extraneural" line of virus, strain 236. The virus was present in their blood, spleen, kidney, and lungs up to the 6th day after their infection. On the 7th day, the virus was still present but only in the lungs, spleen, and kidneys. The virus was present in the brain of killed mice till the 9th day, when the experiment was closed. The titer of the virus in the blood of mice was on the third day 2.5 log units and in the brain of mice on the 7th day 2.0 log units. In mice of 20.0 gr of body weight inoculated alike with the same line of virus, we did not detect any viremia. The virus could be isolated in spite of this from the lungs and spleen on the 7th day post infection. No virus was isolated from the brain of killed mice.

In Syrian hamsters of 100.0 gr of weight infected alike, viremia of 3-6 days of duration was found (Dr. Simkova, A.). The virus could be isolated from their lungs and spleen still in these days when no virus was isolated already from their blood.

The Calovo Virus:

The Calovo virus, the second mosquito-borne virus in Czechoslovakia, was isolated from a pool of 300 Anopheles maculipennis in 1960 (References: Bardos, V., Cupkova, E., J. Hyg. Epid. Microb. 6, 186-192, 1962). J. Casals found that the Calovo virus is very closely related to, or almost identical with Chittoor viruses isolated in Malaya and India, which belong to the Bunyamwera group.

Trying to find the potential reservoir animals of this virus in nature, we have infected sc chickens of 24 hours of age, rabbits of 1.15 kg of body weight, and Syrian hamsters of 150.0 gr of weight with approximately 2.0 log units of virus (Dr. Bardos, V.). Virus isolation experiments were undertaken from their blood during 6 days by ic inoculation in young white mice. No virus was isolated. White rats (Wistar) of 70.0 gr weight were sc infected with 3.8 log units of virus. No virus was isolated from their blood during six days.

Two pigs of 30.0 kg body weight were inoculated sc with approximately 1.5 log units of virus (Drs. V. Bardos and J. Jakubik). Virus isolation experiments from their blood were undertaken for 7 days by ic inoculation of young white mice. Traces of virus were found in the undiluted blood of one pig on the fifth day after infection. Pigs of 5.5 to 7.20 kg body weight were inoculated sc with 3.7 log units of virus. No virus was isolated from their blood during 7 days.

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

Ecological and Virological Investigations on Tick-borne Encephalitis in East-Austria:

Ecological and virological investigations concerning a tick population contaminated with tick-borne encephalitis virus were carried out in a natural focus in the environments of Pottschach (district Neunkirchen, Austria) during the year 1961. In the course of 14 excursions from April to September in two selected areas with different biotops in size of 7200 m^2 on 32 marked fields (each field 20-30 m^2) ticks were regularly collected and their virus content tested. Altogether 21,439 ticks (16,131 larvas, 4,609 nymphs, and 231 imagoes) were collected.

The seasonal course of the fluctuation of the ticks was a biphasic one and showed two distinctly separated summits, one in June and the second in September. The relation between the climatic conditions registrated at the same time and the fluctuation of the ticks was existent but not precisely marked. The statistical results raise the idea that the ticks of the species Ixodes ricinus have a life cycle of 1.5 - 2.5 years. The stages of development during the year were not regularly distributed both to the region and to the season.

The observation that during the time of the highest accumulation of the ticks in this natural focus most of the virus strains could be isolated is conspicuous indeed; the incubation period of the clinical cases in the total district agrees with the summits of accumulation of the ticks. On the other hand, the number of ticks collected in each field was not decisive for a successful isolation of virus.

As in all primary materials, there were nymphs and the isolation of virus out of nymphs was proved in one case only, this stage may be regarded as a vector first of all. Thirty-five experiments to isolate the virus out of a greater number of larvas were not successful. We may suggest that the transovarial transmission of this virus under natural conditions is minor.

Date	Ticks collected in the areas			Virus isolations out of a pool			No. of virus strains
	La	Ny	Im	La	Ny	Im	
April 16-20	50	150	4	8	-	-	-
May 5-7	601	340	17	-	-	-	-
May 19-21	933	436	12	1	37	4	1
				41	14	0	1
				0	13	0	1
				12	29	1	1
				8	10	0	1
				6	15	0	1
				15	9 (engorged on Clethrionomys)		1
June 2-4	638	319	16	-	-	-	-
June 16-18	902	294	34	26	7	3	1
June 30-July 2	697	202	15	-	-	-	-
July 13-15	374	255	35	-	-	-	-
July 28-30	452	71	16	-	-	-	-
August 12-15	2104	92	17	-	-	-	-
August 25-27	2405	300	8	-	-	-	-
Sep. 8-10	2628	362	12	14	5	0	1
Sep. 22-24	1593	501	12	21	34	6	1
Oct. 6-8	1616	699	17	17	39	8	1
Oct. 27-29	904	394	12	-	-	-	-
Nov. 10-12	224	194	4	12	4	2	1

Tick-borne virus infections of the Central Nervous System in the district Neunkirchen during the year 1961

Month	Tick-borne encephalitis	Presumed tick-borne encephalitis	Other Infections	Total
March	1			1
April	1		2	3
May	6		1	7
June	5	1	2	8
July	11	4	4	19
August	2	3	1	6
September	1			1
October	5	2	2	9
November				
December			1	1
	32	10	13	55

REPORT FROM THE DEPARTMENT OF MEDICAL ZOOLOGY U.S. NAVAL MEDICAL RESEARCH UNIT NUMBER THREE CAIRO, EGYPT, U.A.R.

Data from studies of migrating birds during the past three fall seasons have been collated and the report submitted. The following is an abstract:

The need for imaginative thinking and research in the epidemiology of diseases transmitted by arthropods is pointed up by new concepts of longevity and host ranges of arthropod-borne viruses, as well as by other biological and medical phenomena. Among these is the intercontinental transport of ticks by migrating birds. During the fall migration periods of 1959, 1960, and 1961, 32,086 birds comprising 73 forms were examined for ticks in Egypt while enroute from Asia and eastern Europe to tropical Africa. Of these, 40 forms represented by 31,435 birds were tick-infested. Hosts, numbering 1040 birds, 3.31% of the tick-infested bird forms examined, bore 1761 ticks, or 1.69 ticks per host. Common ticks taken were Hyalomma m. marginatum, Haemaphysalis punctata, and Ixodes ricinus. Ixodes frontalis and Hyalomma aegyptium were less common and Haemaphysalis sulcata, H. otophila, and H. pavlovskyi were rare. The common tick species are known to be reservoirs and vectors of pathogens causing a number of human and animal diseases in Europe and Asia. Several of the bird hosts have also been incriminated as reservoirs in their summer ranges. Over 20 strains of pathogenic viruses were isolated from these birds and their ticks in Egypt in the 1961 fall migration period. The most difficult problems in investigations such as this in many parts of the world are taxonomic ones, the correct identification of bird hosts, of immature stages of ticks, and of viruses.

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

Since one of the first yellow fever strains in the world was isolated by Mathic, Sellards, and Laigret in 1927, Dakar Pasteur Institute has devoted much attention to the study of this disease and to the production of a vaccine with the object of eradicating yellow fever in the French-speaking area of West Africa.

It was rational that the yellow fever research laboratory should enter upon the study of the other arboviruses, but this purpose could only be achieved in January of this year when a new building and the requisite

equipment became available. The arbovirus laboratory is now supplied with a low temperature apparatus (-60° C), a refrigerated centrifuge, a freeze dryer, everything required for tissue culture and with an important stock-farming of mice (14, 000) and hamsters.

At the beginning of this year, the arbovirus laboratory studied the yellow fever strains isolated by the Addis Ababa Pasteur Institute and contributed to the serological survey and to the vaccination campaign undertaken as a consequence of the Ethiopian outbreak.

Relating specially to arboviruses in the Republic of Senegal, preliminary work had first to establish whether it is possible to perform serological surveys in the countries where systematic vaccinations against yellow fever have been effected without break since 1939. It has been demonstrated by testing a few specimens of sera that, after vaccination with the Dakar strain, complement fixation tests performed with neurotropic amarillic antigen is almost always positive and can remain so very long after a vaccinal reaction. Likewise, inhibition haemagglutination tests reach high levels. These results contrast with the lesser serological modifications observed after vaccination with the 17D strain (Bres, P., Bull. Soc. Path. Exot. 1961, 54, 995-1001). Survey just completed with 500 sera corroborate these data; it will permit the determination of the limits of overlapping inside the group B and the consequent difficulties regarding the interpretation of results in serological surveys.

Other laboratory studies are in progress. Potent antigens and sera are being produced from the principal arthropod-borne viruses following receipt of strains kindly supplied by the East African Virus Research Institute at Entebbe (Uganda). Isolation attempts are in progress from mouse sentinels, from mosquitoes, and from febrile patients by means of newborn mice and tissue culture (plaque technique) inoculation.

REPORT FROM DR. J. DOUCET, MEDICAL ENTOMOLOGIST INSTITUT D'ENSEIGNEMENT ET DE RECHERCHES TROPICALES ABIDJAN, COTE D'IVOIRE

For the last four years, the laboratory has been devoted to studies on the incidence of arthropod-borne viruses in the Republic of Ivory Coast.

Entomological Studies:

a) General survey.

A study on the distribution of mosquitoes (with emphasis on those known as vectors of virus diseases in other parts of Africa) was conducted in several towns and forest areas in connection with the "Service d'Hygiene" of the Republic of Ivory Coast. Seventy towns and ten forest areas were prospected during two years.

The results are briefly summarized in the table on the following page.

			Southern coastal Forest-area		North-eastern and North-Western Forest-area		Coastal Savannah area		Northern Savannah area
			F	T	F	T	M^t Oroum-boboka	T	T
Anopheles		funestus		+		+	+	+	+
		gambiae	+	+	+	+	+	+	+
Mansonia	(Coquillettidia)	metallica	+						
	(Mansonioïdes)	africana	+		+				
		uniformis			+				
Eretmapodites		chrysogaster gr.	+	+	+				+
		grahami	+						
Aedes	(Finlaya)	ingrami	+						
		longipalpis	+						
	(Stegomyia)	aegypti	+	+		+	+	+	+
		africanus	+	+	+		+		
		apicoargenteus	+				+		
		metallicus					+		
		simpsoni		+					
		vittatus	+			+	+	+	+
	(Aedimorphus)	argenteopunctatus	+			+	+		
		cumminsi					+		
		minutus						+	+
		nigricephalus						+	
		stokesi	+	+					+
		tarsalis gr.			+		+		
	(Neomelaniconion)	circumluteolus	+			+		+	
		punctocostalis	+						
Culex	(Neoculex)	rubinotus	+		+				
	(Culex)	antennatus	+	+					
		univittatus	+						

F = Forests T = Towns

b) Studies on a steel tower (45m high) amidst the trees (the canopy of which reaches 42m) of the Banco Forest near Abidjan. This tower was built for ecological studies by the UNESCO-Wet Tropical Area Commission. For two years, the ecology of the mosquitoes living in the forest was studied by means of:

1) Bamboo pots placed on the tower at different levels from ground to 45m. The bamboo were observed at intervals of a fortnight.

2) Night catches with Mercury Vapor light at 7.50m and 20m high.

3) Twenty-four hour catches on human bait. The man-biting species most prevalent in the canopy seems to be Aedes africanus.

An acrodendrophilic tendency of Eretmapodites chrysogaster gr. is to be noted (it breeds from ground level up to 42m). Aedes apicoargenteus is one of the most prevalent species in the breeding places at all levels. Aedes aegypti (ssp. formosus and typical form) bred in small numbers in the bamboo up to the canopy. Aedes longipalpis bred from ground level up to 23m. Surprisingly, more females than males were caught with the Mercury Vapor light in the following groups: Anopheles, Uranotaenia, Ficalbia and Mansonia.

Virology:

This study is just at its beginning. One-day old mice are systematically inoculated with ground mosquitoes. Periodically, baby mice are used as sentinels in the vicinity of Adiopodoume. One pure strain of Aedes aegypti ssp. formosus (F type of McClelland) is maintained for further transmission trials. The virus strains, after isolation, will be sent to the Dakar Pasteur Institut (Director: Dr. Chambon; Virologist: Dr. Bres) for grouping until Abidjan Pasteur Institut will be opened.

WHO Fellowship:

From October 15, 1961, to January 9, 1962, Dr. Doucet visited laboratories working on the transmission of the arthropod-borne virus diseases in Africa: Yaba, Lagos (W.A.C.M.R.), Entebbe (E.A.V.R.I.), and Johannesburg (S.A.I.M.R.).

Expected activity for the next year:

As Dr. Doucet is going on leave in Europe from September 1962 to April 1963, only the routine mosquito collections and mice inoculations will be pursued. In addition, systematic mosquito catches will be started very soon by means of Lumsden traps baited with rodents and birds.

REPORT FROM DR. G.R. SCOTT, ACTING DIRECTOR EAST AFRICAN VETERINARY RESEARCH ORGANIZATION MUGUGA, KIKUYU, KENYA

Rift Valley Fever in Camels:

Abnormally intense rain fell in the Northern Frontier Province of Kenya between October 1961 and February 1962. Early in 1962, abortions in camels (Camelus dromedarius) were reported. Sera from 11 camels which had aborted neutralized Rift Valley fever virus and a subsequent limited survey revealed the presence of antibodies in 27 out of 60 healthy camels. Differences attributable to age were not significant. A brief report will be published by Scott, G.R., Coackley, W., Roach, R.W., and Cowdy, N.R.

The proven and independently confirmed natural infections of vertebrate hosts by Rift Valley fever virus are limited to man, cattle, and sheep. Proven but unconfirmed reports implicate four white-nosed monkeys, two moustached monkeys, two mona monkeys, one drill, one chimpanzee, one mangabey, one talapoin monkey (Pellissier and Rousselot, 1954), one African polecat (Gear et al., 1955), one arvicanthis rat (Mims, 1956) and two goats (MacOwan, 1960). In addition, springbok and blesbok were suspected as having been infected during the 1951 epidemic in South Africa (Joubert, Ferguson, and Gear, 1951).

Rift Valley fever virus has been propagated in several types of tissue culture (see Murphy and Easterday, 1962) and this information should be included under experimental host range.

In regard to Nairobi sheep disease virus, I suggest that its apparent inability to infect cattle is significant and is worth recording. (Montgomery, 1917; Daubney and Hudson, 1931).

References:

Daubney, R., and Hudson, J.R. (1931): Rep. Kenya Agric. Dept. 1930, p. 127.

Gear, J., De Meillon, B., Le Roux, A.F., Kojsky, R., Innes, R.R., Stegn, J.J., Oliff, W.D., and Schulz, K.H. (1955). S. Afr. Med. J., 29:514-518.

Joubert, J.D.S., Ferguson, A.L., and Gear, J. (1951): S. Afr. Med. J., 25:890-891.

MacOwan, K.D.S. (1960): Rep. Kenya Vet. Dept., 1959, p. 23.

Mims, C.A. (1956): Rep. E. Afr. Virus Res. Inst., 1955-1956. p. 25.

Montgomery, E. (1917): J. comp. Path. 30:28-57.

Murphy, L. C. and Easterday, B.C. (1962): Proc. 65th Meet. U.S. Livestock San. Assoc., Minneapolis, 1961. pp 397-412.

Pellissier, A. and Rousselot, R. (1954): Bull. Soc. Path. Exot. 47, 228-231.

REPORT FROM ARBOR VIRUS RESEARCH UNIT SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH JOHANNESBURG, REPUBLIC OF SOUTH AFRICA

Outbreak of Chikungunya in Southern Rhodesia:

During March-May, 1962, there was a fairly extensive epidemic of chikungunya in the area along the Sabi and Lundi Rivers in the southeastern part of Southern Rhodesia, adjacent to the Mozambique border. The area was visited in early May, just when the outbreak was subsiding, for the collection of mosquitoes and blood samples from patients. The southward movement of o'nyong-nyong, which has now been diagnosed at the southern end of Lake Nyasa, added additional interest to the identification of the causal agent of the Sabi-Lundi outbreak. A virus was isolated from a human being, but no isolations resulted from the 190 mosquitoes collected. Because of its high pathogenicity for infant mice as originally isolated, the ease with which it was transmitted by Aedes aegypti (in contract to a failure of transmission with Anopheles gambiae), and by immunological tests, this virus was identified as chikungunya. HI and N tests on the bloods collected from acute and convalescent patients confirmed the aetiological role of the isolate.

It was evident soon after our arrival in the affected a
would be little chance of isolating virus from mosquitoes b
small size of the populations due to the dry conditions pre
late stage in the season. Taking habits and prevalence at the time
visit into consideration, the following species may have been implicated in domestic and/or wild cycles: Culex p. fatigans, Mansonia uniformis, M. africana, Aedes aegypti formosus, A. cumminsi, A. lineatopennis, A. fulgens, and Culex poicilipes.

Monkeys as possible hosts of chikungunya virus:

Little information is available on the wild cycle of chikungunya virus. Antibody tests on fair numbers of sera from wild birds and rodents collected in South Africa and Mozambique have resulted in entirely negative results. Inoculation of the virus into cattle egrets and four species of wild duck produced neither viremia nor N antibody. Inoculation of the virus into five species of wild African rodents indicated that only one of them, Mystromys, possessed the necessary susceptibility to be involved in transmission cycles. However, of six sera collected from vervet monkeys, Cercopithecus aethiops, trapped at Lumbo in Mozambique, four were positive in the N test to chikungunya virus. The isolation of chikungunya from Aedes africanus and from mosquito catchers on a platform in the forest canopy by the East African Virus Research Institute, Entebbe, Uganda, also suggested that monkeys may be involved.

To test the susceptibility of monkeys, mouse passage 2 of the Rhodesian strain of chikungunya was inoculated into four vervet monkeys. All four monkeys circulated virus for the first three days with viremia levels varying from 2.8 to 5.3 (day 1), 4.2 to 4.7 (day 2), 1.8 to 2.8 (day 3). Two monkeys showed febrile reactions, but no other symptoms were observed. One monkey died suddenly on day 7. At autopsy, small areas of pneumonia in both lungs were present.

Aedes aegypti and Anopheles gambiae engorged upon one monkey with a viremia of 5.3. Fifteen days later, a successful transmission resulted when four of these A. aegypti fed upon a susceptible vervet monkey. On the same day, another group of four A. aegypti and twenty A. gambiae failed to transmit to vervets. Some of these A. gambiae were shown to harbor virus on this day by inoculation into mice.

Antibody response of domestic fowls following inoculation with Sindbis and West Nile viruses:

As it is intended to use fowls as sentinels at various study sites on the highveld where Sindbis and West Nile viruses appear to be prevalent,

these viruses were inoculated into eight fowls each to observe the reliability of the HI and N tests in detecting infection. A low grade (+ 1.0) viremia occurred in both groups of fowls. Except in one fowl inoculated with Sindbis which remained negative, the fowls responded with high titre HI antibody when tested at 1, 3, and 6 months after inoculation. In N tests in infant mice inoculated IP, the same sera (diluted 1/5) gave mostly completely negative or inconclusive results. However, the addition of fresh monkey serum to the serum/virus mixtures and a lower challenge dose of virus resulted in a significant increase in the number of positive sera. The superiority of the HI test over the N test was clearly evident.

Virus isolations and antibody studies at Olifantsvlei.

Olifantsvlei is an area seven miles south of Johannesburg and is fairly representative of the South African highveld habitat. Mosquito activity is practically nil during winter. During last summer, 7,667 mosquitoes were collected there and inoculated into mice. Two isolations of Sindbis from Culex pipiens and Culex univittatus, and one isolation of West Nile from Culex theileri resulted. The mosquitoes were collected alongside a marsh. Culex pipiens was the predominating species in the catches.

Recently, 300 sera were collected from fowls owned by small plot owners living at Olifantsvlei. These sera are being tested by HI against six viruses. The results on 120 sera from five flocks are tabulated below. All five flocks produced positive results with Sindbis and West Nile antigens.

HI tests on fowl sera collected at Olifantsvlei

No. sera tested	No. sera positive to virus.					
	Sindbis	Middel-burg	H 336	Wessels-bron	West Nile	Germiston
120	22	1	6	7	25	1

Each serum positive to either H 336 or Wesselsbron was also positive to West Nile to a higher titre. Three of the flocks were located some two miles from the mosquito-trapping sites. Two of these flocks were young birds which had only been exposed for one summer at Olifantsvlei.

REPORT FROM DR. FRED R. MCCRUMB, JR.
DIVISION OF INFECTIOUS DISEASES
DEPARTMENT OF MEDICINE, UNIVERSITY OF MARYLAND SCHOOL
OF MEDICINE, BALTIMORE, MARYLAND

During the past year, members of the Division of Infectious Diseases have engaged in a study of viral isolates obtained from wild-caught phlebotomine flies collected in Pakistan and Iran in collaboration with members of the Department of Entomology, Walter Reed Army Institute of Research. In addition, attempts have been made to isolate viruses from plasma of human beings with short-term febrile illnesses in these two countries. Summary of virus isolations from Phlebotomus and Sergentomyia species derived from data accumulated by the Department of Entomology, Walter Reed Army Institute of Research (see report from Dr. Herbert Barnett, Arthropod-Borne Virus Information Exchange, April 1961) is presented in Table 1.

Table 1. Summary of Viral Isolations from Phlebotomine Flies in Pakistan and Iran*

Locale	Species	Sex	Specimens	Lots	Viruses
Pakistan	Phlebotomus	Female	3,691	37	7
		Male	1,728	10	3
	Sergentomyia	Female	1,590	15	3
		Male	34	1	1
	Subtotals		7,043	63	14
Iran	Phelbotomus	Female	4,123	40	22
		Male	1,490	11	3
	Subtotals		5,613	51	25
GRAND TOTALS			12,656	114	39

* Arthropod-Borne Virus Information Exchange - April, 1961.

Similarly, the experience with isolation of viral agents from acute-phase human plasma is presented in Table 2.

Table 2. Summary of Viral Isolations from Human Plasma in Pakistan and Iran

Locale	Specimens Tested	No. Viral Isolates
Pakistan	47	4
Iran	6	4
TOTALS	53	8

Characterization of isolates from sandflies has been impeded by failure of the majority to propagate readily in laboratory animals. In most instances, serial intracerebral passage of infected suckling mouse brain has not resulted in rapid adaptation of these viruses to growth in suckling brain, the usual intracerebral titer not exceeding $1 \times 10^{2.0}$ infective units per 0.01 ml. In general, attempts to induce immunity in mice or produce serologically active antigens with agents of low infectivity have been unrewarding. Of additional interest is the observation that even the few well adapted viruses have proven to be weakly antigenic in rabbits and guinea pigs. To date, five viruses isolated from sandflies have been studied with sufficient confidence to justify this preliminary report of their relationships among the group as well as to other arthropod-borne viruses. The most encouraging results have been obtained with complement fixing and hemagglutinating antigens derived from suckling mouse brain and antisera prepared in adult mice. Viruses isolated from human plasma have not presented the technical difficulties observed with arthropod-derived agents; however, it should be noted that serial blind passage of human material received less attention. Furthermore, as will be noted, viruses isolated from human sources appear to fall into one of two previously known serologic groups. Of the eight viruses isolated from human plasma, six have been partially characterized.

Table 3. Serologic Relationships Among Viruses from Pakistan and Iran (Complement Fixation)

Antiserum	Antigens												
	SSF	I-17	Kali	Ahas	Asdul.	Jaafar	NSF	Igbal	Saita	I-67	I-47	I-58	I-81
SSF	20	20	10	10	10	20	0	0	0	0	0	0	0
I-17	160	320	160	>80	320	>80	0	0	0	0	0	0	0
Kali	160	160	320	160	160	160	0	0	0	0	0	0	0
Ahas	160	160	80	160	80	80	0	0	0	0	0	0	0
Asdul.	80	80	80	160	160	160	0	0	0	0	0	0	0
Jaafar	160	160	160	160	160	160	0	0	0	0	0	0	0
NSF	0	0	0	0	0	0	40	80	40	80	20	0	0
Igbal	0	0	0	0	0	0	160	320	160	160	40	0	0
Saita	0	0	0	0	0	0	160	160	160	160	40	0	0
I-67	0	0	0	0	0	0	40	40	40	80	20	0	0
I-47	0	0	0	0	0	0	10	20	10	10	160	0	0
I-58	0	0	0	0	0	0	0	0	0	0	10	320	0
I-81	0	0	0	0	0	0	0	0	0	0	0	0	320

Antigenic Characterization. Results of complement fixation tests presented in Table 3 suggest that agents studied during the course of this investigation represent five serologically distinct groups. Numbered viruses were isolated from sandflies, those with names from human beings. I-17, Kali, Igbal and Saita Khan were derived from material collected in West Pakistan; the remainder originated in Iran. Sicilian and Naples sandfly fever viruses are descendents of the strains isolated by Sabin.

The data presented in Table 3 seem to justify the following major groupings:

Group I	Group II	Group III	Group IV	Group V
Sicilian	Naples	I-47	I-58	I-81
I-17	I-67			
Kali	Saita Khan			
Ahas	Igbal			
Asdullah				
Jaafar				

Minor antigenic relationships exist between Groups II and III as evidenced by partial bi-lateral cross complement fixation reactions.

Hemagglutination inhibition tests have revealed similar antigenic inter-relationships as well as minor cross reactions between Groups IV and V which were not measurable by complement fixation. For the sake of brevity, detailed results of cross hemagglutination inhibition tests are not presented.

All but one of the viruses have acquired, on serial passage in suckling mice, pathogenicity for adult mice following intracerebral inoculation. In spite of this degree of adaptation, neutralization tests are performed with difficulty. In general, the problem is one of poorly neutralizing antisera strongly reactive in other serologic procedures. Additional studies on the dynamics of neutralization with these agents are now in progress.

Prevalence of Viruses in Human Disease. Absence of convalescent serum from patients yielding viruses has precluded establishment of their causative role in human febrile disease. However, reisolation of four agents would seem to validate the original isolation. Furthermore, the appearance of Sicilian or Naples-type sandfly fever viruses in the blood of human beings with benign febrile illnesses in Pakistan and Iran is not surprising. However, the occurrence of what appear to be new viruses among human populations is of somewhat greater interest and attempts to establish their importance in human disease are being made. Results of sero-survey studies to date are presented in Tables 4 and 5.

Table 4. Incidence of Neutralizing Antibodies to Several Arthropod-Borne Viruses in Human Populations of Pakistan and Iran*

Virus	Pakistan	Iran
Sicilian Sandfly Fever	12**	7
Naples Sandfly Fever	8	32
West Nile	34.8	17
Japanese Encephalitis	12.6	3.4
Dengue-2	16.4	ND
Chikungunya	5.8	ND
Semliki Forest	0	3.0
Sindbis	ND	4.5

* Single dilution screen neutralization tests using 50-500 ID_{50} of virus and neat uninactivated serum were performed on 130 and 170 serum specimens from human beings residing in Pakistan and Iran respectively.

** Percentage of serum specimens protecting majority of 5 or 6 mice inoculated with serum virus mixture.

Table 5. Incidence of Hemagglutination-Inhibiting Antibodies to Sandfly Isolates in Human Populations of Pakistan and Iran

Virus	Pakistan	Iran
Sicilian Sandfly Fever	39	ND
Naples Sandfly Fever	15.5	18
I-47	5.3	ND
I-58	3.0	6

It would appear that the viruses of Sicilian and Naples sandfly fever and a Group-B agent closely related to West Nile virus have infected a significant part of the populations sampled. The low incidence of antibodies to the Group A representatives tested is of interest. On the basis of the data presently available, it would appear that viruses of Groups III, IV, and V infect man in this area but at a lower rate than the Sicilian and Naples strains of sandfly fever virus.

Variation in the incidence of specific antibodies was observed in several serum collections from Pakistan. It is noteworthy that Pakistani military personnel tested demonstrated a higher incidence of hemagglutinating-inhibiting antibodies to both a Sicilian-type isolate (Jaafar) and a Naples-type isolate (Saita Khan), there being 86 and 28% respectively positive by hemagglutination-inhibition. This would suggest that military operations may have exposed troops to hyperendemic foci of these agents.

A recent collection of serum specimens more likely to be representative of populations in and around Lahore is being surveyed for the presence of antibodies to a wide variety of infectious agents including 25 arthropod-borne viruses representing the recent isolates from sandflies and other major groups of arboviruses. In addition, inauguration of the University of Maryland International Center for Medical Research and Training program in Pakistan within the past several months will permit detailed investigation of arthropods and arthropod-borne human disease in the future.

REPORT FROM DR. N.F. STANLEY
PROFESSOR OF MICROBIOLOGY
UNIVERSITY OF WESTERN AUSTRALIA, PERTH, W.A.

Hemagglutination inhibition and neutralization tests have now been completed on the human sera collected in Western Australia. The percentage of sera with demonstrable antibody at a dilution of 1/10 or greater is given in the table (see next page).

It is interesting to note the absence of dengue antibody in the sera of the native population. It appears that several group B viruses are endemic in the northwest area, but none in Central Australia. With the exception of MRM39, the group A strains tested seem to be widely distributed.

Tissue culture studies have been attempted using Porterfield's techniques. With chick embryo fibroblasts, plaques could not be demonstrated with the group A strains under test. Of the group B viruses, MVE, MRM16, and Dengue 1 produced plaques but Dengue 2 and MRM32 did not. Three different techniques for the estimation of antibody in tissue culture systems have proved to be successful. The responses of monkey kidney cells, mouse L fibroblasts, and HeLa cells to infection are being studied.

TABLE

PERCENTAGE OF HUMAN SERA WITH HI AND NT ANTIBODY

Sera	Locality	GROUP B						GROUP A			
		HI	NEUT. TESTS					HI	NEUT. TESTS		
		MVE	MVE	MRM16	MRM32	DEN.1	DEN.2	AMM2354	AMM2354	MRM39	AMM2021
White (207)	South West 1958	34	3	0	8	4	12	28	10	0	5
" (50)	Kimberley 1960	60	18	40	25	5	38	25	4	0	18
Aboriginal (285)	Kimberley 1960	92	(50)	78	66	0	0	50	(68)	19	8
" (86)	Leonora	4	0	7	7	0	0	22	28	0	12
" (25)	Carnarvon	7	11	50	36	0	0	8	8	0	0

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

Virus Isolation:

Attempts at virus isolation and reisolation have been completed on 25,901 mosquitoes collected at three centres in North Queensland in 1960-61. Sixty strains were isolated and found to belong to 11 virus types; five in group A, two in group B, two constituting the new "Koongol" group, and two ungrouped. Only one of these types has not been mentioned in previous Information Exchanges--Stratford virus (C338), a group B virus related to Kokobera but considered here to be sufficiently distinct to warrant its own name. It has been sent to the Rockefeller Foundation Virus Laboratories for further study.

Antibody Surveys:

Sera from young domestic fowls collected in April 1961 gave evidence of recent group B infection at Mitchell River (confirmed by virus isolation) and of recent group A infections at Mitchell River, Cairns and Townsville.

Groups of human sera collected from the Torres Straits islands in 1962 had HI antibodies to MVE, dengue 1, dengue 2, Edge Hill, Sindbis, and AMM2021. The results suggested that several group B viruses had been active in the area, and neutralization tests are in progress to test this.

Eggs were obtained in July 1960 and August 1961 from fowls sent to various centres in North Queensland the previous October-November. Yolks were tested for neutralizing antibodies with results that suggested widespread activity of MVE and Kunjin in each summer.

Epidemic polyarthritis:

Cases of this syndrome occurred in the Brisbane area and in western New South Wales and Queensland from Broken Hill to Blackall in the period January-May, 1962. CF tests with group A viruses showed antibody response, especially to AMM2021, in a minority of cases only.

Properties of Viruses:

Filtration experiments showed that Mapputta virus (MRM186) passed a 200 mμ APD membrane filter but not 100 mμ; Koongol (MRM31) and MRM1 passed 100 mμ but not 50 mμ; MVE passed a 50 mμ filter.

REPORT FROM DR. J.A.R. MILES
DEPARTMENT OF MIBROBIOLOGY
UNIVERSITY OF OTAGO, NEW ZEALAND

During the course of a field trip to the West Coast of the South Island of New Zealand during February and March 1962, some 20,000 mosquitoes were caught for virus isolation attempts. There were 17,000 Theobaldinella tonnoiri and 3,000 Culex pervigilans. Three virus isolations were made, 2 from T. tonnoiri pools and 1 from a pool of C. pervigilans. In all cases, primary isolation was made by plaque production on chick embryo fibroblast monolayers, and confirmed by reisolation from the mosquito pools by ic inoculation of suckling mice. Cross neutralization tests using immune fowl sera indicated that the 3 isolates are identical. All further work was carried out on the isolate M78.

The virus produces large plaques in 48 hours on monolayers of chicken or duck embryo cells. It multiplies in suckling and adult mice, chicken embryos, young chickens, and adult fowls. Gradocol filtration indicates that the size of the infectious particle is 45-75 mμ. The infectivity is decreased by DCA and by ether. A haemagglutinin for goose cells has been prepared by sucrose-acetone extraction of infected suckling-mouse brain. It shows optimal activity at pH 6.0 and 37° C. H.I. tests have shown slight inhibition by WEE and Sindbis antisera. The sample of WEE antiserum neutralizes to low titre when titrated against 100 pfu of M78 virus. There is also some neutralization by an antiserum against the MRM39 strain of Sindbis virus. Of 98 human adult sera from South Australia, 1 contained a low level of neutralizing antibodies. A limited number of avian and mammalian sera have also been tested, and antibodies have also been found in 1 harrier (Circus approximans), 1 horse, and 2 fowls, one of which was exposed to mosquito bites in a stable trap.

Plaque production by arboviruses:

We have been investigating the effects of the addition of DEAE dextran or protamine to agar growth media both used as an overlay and in suspended cell cultures.

With the limited number of strains we have available, we have not yet found any whose plaque production deteriorates with these additives. The four shown in the table are all greatly improved. In suspended cell

cultures DEAE dextran has a very clear optimal dose of 100 µgm/ml. This optimum is not so clearly marked in monolayers and with this type of culture 200 µgm/ml is equally good.

Different samples of protamine sulphate vary in their efficacy, but, among the viruses we have available, only with Chittoor is there a very much poorer response to appropriate doses of protamine than to doses of DEAE dextran.

Dengue viruses have not produced plaques on avian cells but, when Dr. Stoker's diploid line of hamster embryo fibroblasts is used, dengue 2 virus will produce plaques in the presence of DEAE dextran. There is some difficulty in keeping the hamster cell monolayers in good condition for the 6-7 days required. However, the addition of DEAE dextran appears to be of some assistance in preserving these cells. We are now adding 100 µgm/ml of DEAE dextran to our agar overlays as a routine.

Table 1

FORMATION OF PLAQUES IN DUCK EMBRYO CELLS SUSPENDED IN AGAR WITH VARIOUS ADDITIONS BY CERTAIN ARBOVIRUSES.

Virus	Virus dilution	Additive and dose: DEAE DEXTRAN (µgm/ml) 0	50	100	200	PROTAMINE SULPHATE (µgm/ml) 100	200
AMM 2354	10^{-4}	0	38*	200+	a	37.5	200+
	10^{-5}	0	9.5	21	0	6	25
St. Louis	10^{-4}	50[a]	80.5[b]	120.5[b]	53[a]	80.5[b]	124.5[b]
	10^{-5}	4[a]	9[b]	6.5[b]	3[a]	7[b]	14[b]
Cache Valley	10^{-4}	0	200+	200+	a	150+	150+
	10^{-5}	0	29	88	a	21.5	25.5
Chittoor	10^{-5}	0	1.5[a]	200+	150+	0	?[c]
	10^{-6}	0	0	80	23.5	0	2

* Mean of two plates

a faint plaques present

b fuzzy plaques, not easy to count

c plaques present, but not countable

This line of cells has proved very susceptible to a range of arboviruses. Comparative titres of BHK 21 and duck embryo T.C.s together with weaned mouse I.C. titres is shown for various viruses in Table 2. M78, which produces excellent plaques to high titre on avian embryo cells, only produces plaques slowly and to a very low titre in BHK 21.

Table 2
Titres of Virus in BHK 21, Duck Embryo, and I.C. in Mice

Virus	BHK 21 monolayers pfu/ml	Duck embryo (suspended) pfu/ml	Mouse I.C. LD_{50}/ml
AMM 2354	1.4×10^9	2.5×10^7	3.0×10^7
SFV	2.4×10^9		
MVE	3.0×10^8		1.0×10^9
SLE	1.0×10^8	1.3×10^7	6.3×10^7
CV	1.2×10^8	8.8×10^7	1.3×10^7
Chittoor		8.0×10^8	1.0×10^8

REPORT FROM DR. K.A. LIM
DEPARTMENT OF BACTERIOLOGY, UNIVERSITY OF SINGAPORE
SINGAPORE 3, MALAYA

An outbreak of a dengue-like illness occurred in Singapore in 1960 (over 200 cases) with sporadic cases continuing into 1961 and 1962. Although the disease was mild and no fatalities were observed, it differed somewhat from classical dengue fever because of a marked tendency to petechial skin haemorrhages.

A number of dengue-type viruses were isolated from the sera of patients in the acute phase of illness, i.e., within the first three days of onset. Isolation was by intracerebral inoculation of suckling mice and in some instances successful adaptation to adult mice. The serial numbers given to these isolates are S-601 and S-843 isolated in 1960 and S-132, S-212, S-378, S-430, S-554 isolated in 1961. All patients had rise of antibody for both JEV and dengue virus antigens of which both dengue-1 and dengue-2 have been used without much difference in the result.

S-601-60 has been identified as most closely related to dengue type 1 (Hawaii) and S-843 to dengue type 2 (New Guinea). These strains were kindly examined in Pittsburgh by Dr. Hammon who found them to be even more closely related to the Thailand isolates, TH-Sman and TH-36, respectively, than to the prototypes dengues 1 and 2 we used. This observation, if confirmed, is of some importance because the Thai strains were never imported into Singapore and raises the question why closely

related viruses isolated in neighboring regions and times should cause different classical syndromes, e.g., the Thailand disease compared with the Singapore disease.

Some difficulties were experienced in the typing of the other isolates as the results obtained did not give a clear indication of their relationship. S-554/61, however, could be identified as a dengue type 4 virus by cross complement fixation tests. Block titrations were carried out with six 2-fold dilutions each of immune sera from 1/4 to 1/128 and dilutions of antigen from undiluted to 1/32.

Results of this nature are conveniently represented as shaded squares, each square representing one serum/antigen mixture which fixed complement strongly. In the table below, for ease in reproduction, the results are represented as Units of fixation, each unit corresponding to a shaded square in a conventional diagram.

Identification of S-554 by block CFT (drop method)
Units of Fixation

Viruses	D-1	D-2	D-3	D-4	S-554
D-1	28	7	13	5	0
D-2	18	27	11	0	0
D-3	6	3	25	9	0
D-4	4	0	10	23	10
S-554/61	0	0	3	19	15

D-1 = Dengue type 1, etc.

All the viruses isolated in 1960 and 1961 were from cases presenting very similar clinical features. Relatively few cases in children were observed and these were mild in character. In 1962, however, a number of cases of a more severe character, more like the Thailand disease have been observed. These cases in 1962 present with petechial haemorrhages, hematemesis, melaena, collapse, and thrombocytopaenia. Cortisone was given and there were no fatalities. Attempts to isolate a virus have been unsuccessful but antibody rises were demonstrated for dengue virus antigens. Japanese encephalitis virus antibodies were present only in some cases. We are unable at present to assess the significance of these cases.

Vector Studies:

Since 1961 a study of mosquitoes caught in houses and their environs has been carried out in the urban area. It is hoped that these data collected over two years may give a clearer picture of the vector variation throughout the year.

After identification and enumeration, batches of mosquitoes were passed in infant mice, particular attention being paid to Aedes mosquitoes. So far, attempts at the primary isolations have been uniformly negative--not a surprising result with dengue-type viruses. It is intended to do serial passage when our mouse colony is enlarged in the near future. Dr. Rudnick, who initiated these studies in 1960, made a number of isolates by serial passage from similar catches.

Encephalitis Study:

Hitherto our studies on encephalitis have been mostly confined to cases admitted to the civilian General Hospital, including a pediatric unit. To widen the scope of our studies and to get a closer estimate of the incidence of encephalitis of all kinds, an Encephalitis Study Group has been organized. This group comprises all medical units in the State of Singapore--civilian and military, government and private, and their associated laboratories where these are available.

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

Singapore Hemorrhagic Fever:

Processing of all the arthropods collected during the 1960-61 outbreak has been completed. Seven agents have been isolated and adapted to suckling mice. SM-1, isolated from a pool of Aedes aegypti, has been identified as a dengue II, or most closely related to dengue II. It is essentially identical to S-843 isolated from the blood of a patient by Professor K. A. Lim. The remaining five mosquito isolates, four from A. aegypti and one from A. albopictus, are dengues or dengue-related on the basis of dengue challenge results. Their identification, however, has not been completed yet. The seventh isolate is from a pool of

Boophilus microplus ticks collected from native cattle in Singapore. It kills suckling mice in 3 days but does not produce illness in weanling mice by the intracerebral and intraperitoneal routes. It is filtrable and is desoxycholate sensitive. Immunological identification has not yet been attempted for this agent.

Bangkok Hemorrhagic Fever:

Over half of the 1961 mosquito collection has been processed for virus isolation with at least two definite agents recovered, both from Aedes aegypti pools. The portion of this project being done in San Francisco has been transferred to Dr. Hammon's laboratory in Pittsburgh for completion, due to Dr. Rudnick's transfer to Kuala Lumpur.

Ecology of Dengue Viruses:

Field and laboratory studies of the ecology of the dengue viruses have been initiated in the Virus Division of the Institute for Medical Research, Kuala Lumpur, Malaya. This is expected to be a two-year project.

REPORT FROM DR. AMBHAN DASANEYAVAJA, DEPARTMENT OF PATHOLOGY, CHULALONGKORN HOSPITAL MEDICAL SCHOOL BANGKOK, THAILAND

The occurrence of Thai hemorrhagic fever has reached epidemic proportions this year. The increase in the number of patients became marked in April. A steady increase has occurred since late May. The disease is, at this writing, (August, 1962), being reported not only from Bangkok, but from many other parts of Thailand.

Our method of approach was the same as in the past: serologic studies using especially the CF technic on paired sera of patients; clinical and general laboratory studies including liver function tests and hematologic examinations of the hospitalized patients; family and environmental studies; examination of patients' sera, autopsy materials, and mosquitoes collected in the homes of the sick for arboviruses; classification of the isolated virus strains and animal experiments to establish their relationship and pathogenicity. The methods applied have not been changed since early 1961. In late 1961, the use of fluorocarbon was introduced as an aid to isolate virus from the blood of patients already showing antibodies.

The patients studied since the beginning of 1962 may be divided into four groups:

1. With shock, vascular collapse, and related phenomena which develop usually 4 to 5 days after the fever started. Patients in this group have rare petechial hemorrhages. Liver and lymphatic gland enlargement may be absent. Some have hematemesis and melena. Both coagulation and prothrombin times are prolonged and liver function tests show marked liver dysfunction. The hematologic picture is that usually found and previously described in this disease, with numerous atypical lymphocytes. The mortality rate is between 50 and 60 per cent. Only dengue but no group A virus was isolated.

2. Philippine type, with the characteristic mulberry rash with white areas between the patches on the extremities, plams, and soles, with the rash only rarely appearing on the trunk and abdomen. This rash appears on the 6th or 7th day after the onset of the fever. No mortality was observed in this group. The viruses isolated belong to the dengue group only. The paired sera in CF tests show no increase of chikungunya titers.

3. Fine petechial type of rash, mostly over the extremities but often also over the body. It appears usually on the 4th to 5th day after the onset of the fever. Lymphatic glands are enlarged, especially the axillary group, then the supraclavicular, postcervical, epitrocheal, and inguinal. Both dengue and chikungunya viruses were isolated from this group. It is noteworthy that those infected with chikungunya virus show a greater enlargement of the lymphatic glands than in those afflicted with dengue infections.

4. Mild non-hemorrhagic form, with fever, liver and/or lymphatic enlargement, diagnosed only by virus isolation and/or antibody production. Only chikungunya group infections were diagnosed in these patients.

Out of 74 acute sera collected on the 1st to 7th day of disease, 12 chikungunya and 10 dengue viruses were isolated in 1962. As stated before, from shock and fatal cases only dengue viruses were isolated.

CF antibody studies showed more dengue than chikungunya infections. The results are being tabulated and evaluated. In one group of the case — a definite increase in the dengue titers (tested against 6 strains according to the classification of Hammon) and no reaction with chikungunya antigens.

In the second group, antibodies (CF) against certain types of dengue viruses are present in the acute serum, and the convalescent serum showed increase against other dengue types. An infection with a different dengue virus can be postulated in these patients. Finally, in the third group, all patients have had high CF antibody levels against all dengue viruses at the onset of the disease. Nevertheless, they became ill with hemorrhagic fever. Neutralizing antibody tests have not been carried out as yet on these sera. Since the observed cases belonged in the first group according to our clinical classification, i.e., went into shock, new avenues of investigation have to be opened which may lead to a better insight into the possible role of sensitization in hemorrhagic fever. We have been setting out to study this problem but publishable data are not available as yet.

Irregular bleeding into the peritoneal cavity and intestines was observed when experimenting with the TH SMAN virus while adapting dengue viruses to adult mice.

Scientific papers on our present observations are being prepared for publication.

REPORT FROM DRS. SCOTT B. HALSTEAD AND JOHN E. SCANLON OF THE SEATO MEDICAL RESEARCH LABORATORY AND DR. CHARAS YAMARAT OF THE SCHOOL OF PUBLIC HEALTH BANGKOK, THAILAND

Hemorrhagic Fever Studies in Thailand:

The Kingdom of Thailand is suffering its largest recorded outbreak of hemorrhagic fever this rainy season. Beginning in April and up until the end of July, there were 1800 THF patients hospitalized in Bangkok and an estimated 500 patients hospitalized outside of Bangkok. A total of 121 deaths have been recorded. Cases have been reported from 23 of Thailand's 71 Changwad (Provinces). House-to-house surveys in Bangkok by this laboratory suggest that there are more than two times as many patients who receive the medical diagnosis of THF as the in-patient data show.

Previous outbreaks of THF have reached maximal proportions in August or September and it would be surprising if cases dwindled significantly until October when the rains cease.

Age distribution resembles that reported in previous years. Nearly all severe cases occur before the age of puberty and all deaths (with one very atypical exception) have occurred in children below the age of 13. The modal age is 5. Perhaps, because of closer observation, the complex of symptoms and signs appears to be broadening this year; lymphadenopathy is now seen quite frequently and it is recognized that on occasion maculo-papular rashes and mild myalgia may be seen in Thai children accompanying dengue or chikungunya virus infection. In general, the cardinal features of hemorrhagic fever remain as previously described (1, 2): history of fever for 3-4 days, followed by sudden collapse; on admission physical the child has a hot trunk, coolness, and occasionally cyanosis of the extremities, low blood pressure (occasionally), narrow pulse pressure, tachycardia, restlessness, positive tourniquet test, liver enlargement and manifestations of hemorrhage such as petchiae, purpura, easy bruisability, and gastro-intestinal hemorrhage. The latter sign and severe shock are of grave prognosis and death may occur suddenly within 24 hours of admission. Intra-cranial hemorrhage occurs rarely and has lead to the diagnosis of encephalitis.

Beginning in January, the Virus and Entomology Departments of the SEATO Medical Research Laboratory and the Virus Department of the Thai School of Public Health have conducted joint epidemiologic and ecologic studies of THF. Twenty basic area study units were selected randomly; each consists of 360-400 households and as a group they form a racial, economic, and geographical cross section of Bangkok. Of 20, 5 representative areas were selected for intensive mosquito collection. Household serologic samples have been obtained sequentially from the same areas. In addition, linear serologic samples have been taken from wild and domestic animals and nearly 50 species of birds netted within the municipality. To supplement the area studies, a collaborative virus investigation was initiated at Children's Hospital late in April to determine the etiology of hospitalized infectious diseases.

The virus etiology of Thai hemorrhagic fever again is mixed. Of 52 carefully studied but randomly selected HF patients, 45 had serologic (HI) or isolation evidence of infection with either dengue or chikungunya (or

closely related) viruses. In over 30% of HF sera inoculated in suckling mice either a dengue-like virus or a chikungunya-like virus has been recovered. Serologically, 17.1% of HF patients at Children's Hospital have been caused by chikungunya; 65% due to dengue-like viruses and 4% have shown 4-fold rises to dengue and chikungunya. In one patient, a chikungunya virus was recovered but no HI antibody was observed in sera taken at an interval of 6 weeks. A series of parallel studies are being made at the Children's Hospital of out-patients and in-patients with a variety of febrile syndromes. Based upon a continuous sample since April (the month of onset of HF in Bangkok) over 40% of all febrile diseases of in- and out-patients has been caused by arbor viruses. In randomly selected out-patients with fever, arbor viruses were isolated in 29% of those studied before the end of June. Among in-patients with diagnoses other than THF, isolation rates for arbor viruses were 23%.

Linear mosquito collections and human serum collections are being made in representative areas of the city. It is expected that specific data concerning overt and inapparent attack rates and pre-infection antibody status will be available since at the end of July, 75 cases of hemorrhagic fever had occurred in sampled study areas and 6 cases had occurred in patients who had been bled before disease onset. Mosquito populations in the study areas are being followed by a number of standard methods, including: light traps, domicile and animal shelter resting collections, human biting collections and animal and human baited mosquito traps. Those females which are collected in a satisfactory condition are retained for virus isolation. The most important species of mosquitoes in all areas thus far have been Culex pipiens quinquefasciatus and Aedes aegypti. Other important species are: Culex tritaeniorhynchus summorosus, Mansonia uniformis and Anopheles vagus. Weekly rates by all methods of collection are being calculated for each area.

No attempt as yet has been made to characterize the over 50 viruses isolated from mosquito pools (Aedes aegypti and Culex pipiens quinquefasciatus) and human blood. Nor has any attempt been made to distinguish dengue virus infections serologically. Virus characterization and further epidemiologic data will be reported in later communications.

References:

1. Nelson, Ethel R. (1960): Hemorrhagic fever in Children in Thailand. Jour. Pediatrics. 56:101-108.

2. Hammon, W. McD., Rudnech, A., Sather, G., Rogers, K. D., and Morse, L. J. (1960): New Hemorrhagic fevers of Children in Philippines and Thailand. Trans. Assoc. Am. Phys. 73:140-155.

REPORT FROM DR. IRVING J. GREEN
DEPARTMENT OF VIROLOGY AND TISSUE CULTURE
NAVAL MEDICAL RESEARCH UNIT NO. 2, TAIPEI, TAIWAN

By the tissue culture neutralization test, two strains of virus isolated by Capt. H.S. Hurlbut from pools of Culex tritaeniorhynchus mosquitoes collected on Okinawa in 1960 have been shown to be closely related, if not identical, to the Sagiyama virus isolated by Scherer et al in Japan in 1956. This is believed to be the first isolation of a Sagiyama-like virus from Okinawa. No cross neutralization with Chikungunya antiserum (rabbit) was noted with the prototype Sagiyama virus (obtained from the 406 Medical General Laboratory in Japan) nor with the two Okinawan isolates (OK-964 and OK-1005). OK-964, OK-1005, and the prototype Sagiyama viruses (30-100 $TCID_{50}$) were neutralized by rabbit anti-Sagiyama serum to a titer of 1:160 in each case. Chikungunya virus was neutralized by only a 1:10 dilution of the same Sagiyama antiserum.

An extensive study of the tissue culture spectrum of selected Group A, B, and Bunyamwera viruses is currently in progress. Scherer et al recently reported the serial induction of CPE by Sagiyama virus in primary embryonic pig kidney and hamster kidney cultures only. We have obtained the serial propagation of the prototype Sagiyama and usually both of the Okinawan isolates, as evidenced by CPE and $TCID_{50}$ determinations, in the following cell cultures: primary cultures - hamster kidney, hamster testis, porcine testis (except OK-964), Guinea pig kidney, guinea pig testis, rat kidney (except prototype Sagiyama), and porcine kidney; continuous cultures - FL, HeLa (obtained from Microbiological Associates), HeLa (obtained from 406 Medical General Laboratory) (except OK-1005).

These preliminary observations indicate that the ability of an arbor virus to propagate in a cell culture system depends not only on the virus type and the cell type, but also on the virus strain and the cell strain.

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

Immunogenic properties of Japanese B encephalitis virus cultivated in tissue culture (Hotta, Ohyama, Takehara, Tokuchi, and Murakami):

Virus tested was the Gl strain of Japanese B encephalitis (JBE) virus cultivated serially in trypsinized hamster kidney cell cultures and harvested in culture fluid from infected tubes.

The active cultured JBE virus was apparently non-pathogenic for mice through intramuscular route, for rabbits through intravenous route, and for monkeys (Macacus rhesus, M. cynomolgus, M. fuscatus) through intracutaneous route.

Mice were injected intramuscularly with the active or formalinized cultured virus and were challenged subsequently by intracerebral injection of mouse-passaged Gl strain virus. The survival ratios were higher among immunized mice than among control non-immunized mice. Little difference was noted between the results concerning the active and formalin-inactivated viruses. Rabbits injected intravenously with the active or formalinized cultured virus produced specific neutralizing (NT) and complement fixing (CF) antibodies against the original mouse passaged JBE virus. Following the second injection given 2 weeks after the first injection, NT indices were 100 to 1, 000 and CF titers were 1:16 to 1:64. Antibody levels increased rapidly after the third injection given 15 weeks later. There was little difference in the pattern of serological response between the animals injected with the active or formalin-inactivated viruses.

In monkeys injected intracutaneously with the active cultured virus, NT and CF antibodies were produced. The increase of antibody titers was somewhat gradual after the first injection, and was more rapid after the second injection.

JBE viruses purified partially by the cellulose column chromatography or fluorocarbon deproteinization stimulated rabbits and monkeys to produce the specific antiviral antibodies as did the crude viruses. Viruses which were purified and inactivated by being treated with trypsin or lipase lost the capacity to stimulate antibody production in rabbits.

Effect of enzymes on partially purified Japanese B encephalitis and related arboviruses (Takehara and Hotta):

JBE and related arboviruses (WEE, dengue, yellow fever) were partially purified by cellulose column chromatography or by fluorocarbon deproteinization and tested for sensitivity to enzymes. Infectivity of viruses decreased markedly or was lost completely on exposure to trypsin or pancreatic lipase. Bacterial proteinase or lipase also reduced the infectivity of purified JBE virus, whereas wheat-germ lipase, a-amylase, ribonuclease and lysozyme had little effect. Poliovirus was resistant to pancreatic lipase, although it was apparently affected by trypsin to a certain extent. (Science, 134, 1878-1880, 1961.)

Some physico-chemical properties of tissue culture-adapted Japanese B encephalitis virus (Takehara and Hotta):

JBE virus investigated was the G1 strain which was cultivated serially in trypsinized hamster kidney tissue cultures for about 150 generations and harvested in infected culture fluid. The mouse-passaged G1 strain, of about 250 generations, obtained in infected brain homogenate, was used for comparison. The former was designated as TC-strain and the latter as M-strain.

1) Chromatographic fractionation of viruses on cellulose ion-exchanger:

The virus suspensions, diluted with 0.07 M phosphate buffer solution (PBS, pH:7.2), was loaded onto DEAE-cellulose column and eluted with either 0.07 M-PBS (pH:7.2) or 0.005 M-Tris buffer (pH:7.2), each containing 0.15 M-NaCl. The eluates were collected separately in every 3 ml, and each fraction was measured for its viral contents by being inoculated into hamster kidney cell cultures. Examples of results obtained are as follows:

(see next page)

Fraction No.	Viral activity of eluates			
	Series I*		Series II**	
	M-strain	TC-strain	M-strain	TC-strain
1	-#	+	+	+
2	-	+	+	+
3	-	+	+	+
4	-	+	-	+
5	-	+	-	+
6	+	+	-	-
7	+	+	-	-
8	+	+	-	+
9	+	+	+	+
10	+	-	+	+
11	+	-	+	+
12	+	-	+	+
13	+	-	+	+
14	+	-	+	+
15	+	-	+	+
16	+	-	+	-
17	+	-	+	-
18	+	+	+	-
19	+	-	+	-
20	+	-	+	-
21	+	-	+	-
22	+	-	+	-
23	+	-	+	-
24	+	-	+	-
25	+	-	+	-
26	+	-	+	-
27	+	-	-	-
28	+	-	-	-
29	+	-	+	-
30	+	-	-	-

* Eluted by 0.07 M-PBS with 0.15 M-NaCl.

** Eluted by 0.005 M-Tris with 0.15 M-NaCl.

(-): No active virus was eluted; (+) Active virus was eluted.

There was a difference in the elution pattern between two virus strains.

2) Interaction between virus and tissue homogenate:

Homogenates of tissues (brain, liver, and kidney) from normal white mice were prepared by being added with Hanks' balanced salt solution. The virus suspensions were mixed with the tissue homogenates, incubated at 37° C for 2 hours, centrifuged at 13,000 rpm for 1 hour. The supernatant fluids were titrated for active virus contents by being inoculated into either mice or hamster kidney cell cultures. Examples of results obtained are as follows:

Tissue homogenate	Viral infectivity of supernatant fluid	
	M-strain (mouse-LD_{50})	TC-strain ($TCID_{50}$)
Brain	0*	$10^{1.00}$
Liver	$10^{2.25}$	$10^{0.75}$
Kidney	$10^{2.50}$	$10^{1.50}$
Control (culture medium)	$10^{2.25}$	$10^{2.25}$

* No active virus was detected.

It is apparent that infectivity of M-strain virus in the supernatant fluid was lost after being mixed with brain homogenate, but not by liver or kidney homogenate, whereas infectivity of TC-strain virus was affected to similar extents by being mixed with any of the brain, liver, or kidney homogenates. Mechanism of the decrease of viral infectivity is being investigated.

3) Thermal inactivation of virus:

TC-strain virus was demonstrated to be more 'thermostable' than was M-strain virus. For instance, M-strain virus was destroyed completely by heating at 55° C for 30 minutes, while about 0.01% of TC-strain virus remained active under the same condition. Comparative thermal inactivation curves of both virus strains are being studied.

Experimental infection of Japanese B encephalitis virus in dogs (Hotta, Ohyama, Funasaka, Ohno, and Yasui, in collaboration with Kuromaru and Mizoguti):

JBE virus, Gl strain, which had been passed through white mice intracerebrally for about 240 generations, was mainly used. One-tenth ml of a 10^{-3} dilution from infected mouse brain homogenates in Hanks' balanced salt solution was injected into the left cerebral hemisphere of dogs, 30 to 180 day old. The inoculated virus dosis was estimated to be about 10^4 times mouse-intracerebral LD_{50}.

Some of the inoculated dogs showed neurological signs as ataxia or tremor after incubation periods of 6 to 13 days. Some of the affected animals ended by dying, and others showed a tendency to recover. In the remaining few, no apparent symptoms were noticed. From the animals which died or were killed after exhibiting the specific signs, organs were removed and subjected to measurement of active virus contents as well as to histological examinations.

Active JBE virus was detected in brains; the viral titers obtained in the infected cerebrums or cerebellums were, in the majority of cases, 10^3 to 10^4/0.02 ml in mouse-intracerebral LD_{50}. Small amounts of virus were recovered from the lung, spleen, kidney, as well as blood. Active virus was demonstrated also in the visceral organs from a dog which showed no apparent neurological signs and was killed 4 weeks after the virus inoculation.

Histological changes noted in the central nervous system were as follows: 1) There were circumscribed or diffuse cell infiltrations of glial or mononuclear elements which were often accompanied by thickening of capillary walls and of arterioles. The infiltrative foci were disseminated in both the gray and white matters throughout the brain and spinal cord. 2) The ganglion cells showed degenerative alterations including chromatolysis, pyknosis, shrinkage of the cell body, fragmentation of the dendrites and neurites, etc. These changes were observed solitarily or with cell infiltration and were variable in locations or in animals. 3) Focal swelling of the meninges or small necrotic foci of spongy appearance in the white matter were also noted.

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

At this time our small unit is not engaged in any active studies of the arboviruses. We are, however, preparing preliminary studies for a proposed project to investigate the arbovirus situation in Alaska. The combined efforts of the virologist, the entomologist, and the ornithologist will be utilized in this program. In addition, a very limited pilot study is underway to check for the presence of specific CF antibodies to Eastern and Western equine encephalitis in the serum of Eskimos and Indians from our Alaskan Native Serum Reference Bank. Our virology laboratory is being structurally modified to afford us a stronger security for the study of arboviruses. The arrival in October of Dr. Jacob Brody, our new Chief of Epidemiology, will coincide with the completion of these preliminary studies and laboratory preparations.

The arbovirus story in Alaska is presently a closed book and we are not only anxious, but quite enthused about the potential of these studies. It is conceivable that Alaska, with its millions of migratory birds, clouds of mosquitoes, and numerous animal species for reservoirs, may play an important role in the continental picture. Clinically, there is very little evidence, thus far, to encourage such a study, but the sparse population and scanty intelligence reports may be masking the true picture.

Presently we are faced with the age-old problem of recruiting a virology technician at the GS-7 position. Our population does not support any individuals with this specialty, and recruitment on two year contracts from the "South 48" is the general procedure. However, we do have the 25% (Federal tax-free) bonus to add to the base pay, and of course the "Great Land" of Alaska to offer to any interested party. In addition to our unlimited scientific potential, I could talk for hours about the many wonderful aspects of life in Alaska. This GS-7 position would require a lady or gentleman of fairly rounded experience in virus techniques, including serology. There are in progress several virus projects and the future will undoubtedly add to this list. We have a well equipped laboratory, an excellent library, and our needs will be honored as our mission demands. Therefore, if you have any knowledge of a qualified technician who may be interested in such a scientific, and Alaskan, adventure, please pass the word along. Incidentially, we cover the general costs of transportation, moving, etc.

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)

Western equine encephalitis (WEE) was first recognized in Saskatchewan in 1935; since then, a number of outbreaks have occurred in man and horses and the disease is recognized as endemic in at least one area of the province. The present project is a study of the epidemiology of WEE and an attempt to determine what other arboviruses exist in Saskatchewan.

Mosquito Investigations (J. McLintock):

The object of these studies is to measure variations in the relative abundance of mosquito species in Saskatchewan; first in relation to temperature and precipitation under dry land conditions, and later during the introduction of irrigation under the South Saskatchewan River Development Project; to collect and identify mosquitoes for virus isolations and to study biological factors in mosquito life history in relation to virus transmission.

Variations in mosquito abundance will be measured by means of light traps. These traps take the mosquitoes alive. These mosquitoes, as well as mosquitoes collected by other means, will be used for virus isolations. Studies of biological factors are being done in the laboratories of the Entomology Research Institute in Ottawa where mosquito colonies and facilities are available for the studies.

Five mosquito light traps have been placed in different locations in the province with the most northerly at Melfort (approx. lat. 52° 51' N) and the most southerly at Swift Current (approx. lat. 50° 17' N). One of the traps is in an area that will eventually be irrigated and efforts are being made to establish another survey trap in another locality where irrigation will be introduced. The traps run nightly and each night's catch is sent to the laboratory in Saskatoon for identification and counting. This year, the first trap did not begin operation until June 28th but in future years trap operations will begin on May 1st and continue until September 15th. On arrival in the laboratory, the surviving mosquitoes are lightly anaesthetized with chloroform, identified and sorted, and the different species distributed

in ampoules and frozen on dry ice until they can be examined for virus. In the past 2 months over 10,000 female mosquitoes - 4 genera, 12 species - have been taken by the 5 traps. The most abundant species in the light traps has been Culiseta inornata, followed closely by Culex tarsalis and Aedes dorsalis. Survival of the mosquitoes in some of the catches has been reduced by the presence of large numbers of moths, first of the beet webworm, and later of cutworms.

For virus isolation, more reliance is placed on mosquitoes collected in their resting places with aspirators and by the use of the survey light trap supplemented by dry ice. Mosquitoes taken by these means are not anaesthetized but identified through the glass wall of the aspirator tube. Collections by these means are made in localities where virus is suspected to be active, either from the epidemiological history, or from the reports of suspect cases in horses or man. The carbon dioxide baited trap of Dow (J. Econ. Ent. 52:496, 1959) has been tried but the results with this trap have been disappointing.

Mosquitoes collected by aspirator or by the attraction of carbon dioxide are either frozen in ampoules or examined immediately for virus. Blood-engorged specimens are kept separate and examined for virus either individually or in small pools.

To date, over 8,000 female mosquitoes distributed in 351 pools plus 40 individual blood-engorged specimens have been frozen and/or examined for virus. Each pool contains 2-45 mosquitoes. The majority of pools consist of Culiseta inornata, Culex tarsalis, or Aedes dorsalis.

In the laboratory in Ottawa, experiments are being conducted to determine the effect of rearing temperature on the ability of adult Culex tarsalis (colony established with wild stock from Alberta) to survive at different temperatures. Two temperature ranges (70°-72° F and 80°-83° F) are being used. The larvae are reared in a yeast and rabbit chow medium and the adults are held in a saturated atmosphere without access to food, but with access to water. The studies are not yet complete but, as expected, they show that the length of the larval stage and adult survival times are shorter at the high than at the low temperature. At both temperature ranges there is a considerable difference in the length of the larval stage of individual mosquitoes and a mosquito with a long larval stage is likely to live longer in the adult stage, i.e., longevity is a characteristic of both the larval and adult stages.

Virus Studies (R. Connell, J. Spalatin, and A. N. Burton):

In 1960 and 1961, it was found that high percentages of wild ducks, migrating from the south to breeding grounds in Saskatchewan, carried WEE neutralizing antibodies. Attempts to recover WEE virus from wild ducks in the Province, however, failed.

In 1962, attention was turned to species other than wild ducks. To date, 1,771 specimens, representing 24 different species, have been collected. Of these, 827 have been examined serologically for WEE antibody. There were 36 positives as shown in the following tables:

Birds:

Chicken	4/407
Crane	2/59
Duck	6/46
Blackbird	1/23
Crow	0/14
Owl	4/12
Gull	0/3
Hawk	2/2
Willet	0/2
Pelican	0/1
Dove	0/1
	19/570 (3.3% positive)

Mammals:

Muskrats	0/28
Ground Squirrel	1/18
Bats	0/10
Horse	0/3
Rabbit	0/7
	1/66 (1.5% positive)

Reptiles:

Snakes	15/113 (13.3% positive)

Amphibia:

Frogs	0/60

Human:

Human	1/16
	Total 36/827

Attempts to isolate virus from the above specimens were not successful.

Garter snakes, Thamnophis radix and Thamnophis sirtalis, experimentally infected with WEE virus developed viremia within 24 hours. The viremia had a maximum duration of 30 days and reached a maximum titre of $10^{-5.2}$. After the viremia disappeared, neutralizing antibody appeared in the blood and reached a peak of more than 4 logs ($10^{-4.2}$). By the 250th day, neutralizing antibody had declined to 2 logs or less.

WEE virus has been found to propagate in frogs (Rana pipiens), reaching a maximum titre of 10^{-2}. Further work with frogs is in progress.

Gravid garter snakes have been infected to determine whether the virus would transmit to the offspring, but results are not yet available. Work is in progress to develop snake cell tissue cultures. Optimum temperature for growth of tissue cultures from garter snakes is in the vicinity of 22° C.

In endemicity studies, 5 sentinel chicken flocks have been exposed in different areas. So far, positive results have been obtained only in the mid-central part of the province. In 1 sentinel flock of 12 birds, negative serologically at the commencement of exposure on July 13th, 1962, 2 birds were positive and 1 suspicious for WEE antibody on August 17th.

REPORT FROM DR. CARL M. EKLUND
ROCKY MOUNTAIN LABORATORY OF THE
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
HAMILTON, MONTANA

I. Tick Transmitted Viruses.

A. Colorado Tick Fever Virus.

Field Studies: The objectives of field study are to:

1) Discover the types of clinical disease and their incidence .
2) Determine the geographical distribution of disease and of virus infected ticks.
3) Obtain quantitative data regarding the virus cycle between larval and nymphal ticks and small mammals.

Data Obtained Since Last Newsletter:

Table 1. 1962 CTF Patient Isolations

	April	May	June	July	Unknown	Totals
Colorado		1	1			2
Idaho		3	1	1		5
Montana		3	2	1		6
Nevada	1	1	1			3
Oregon	1	2	4*			7
South Dakota		1		1*		2
Wyoming		4	4	2	1	11

* One isolate not yet identified

No history of encephalitis or hemorrhage has been reported among these patients. As far as known, all had a benign febrile disease. Exposure was in known endemic areas. Data accumulated since 1952 regarding the geographical distribution of patients from whom virus has been isolated and the location of infected ticks indicate that virus has been limited to the Rocky Mountains and adjacent high plateau regions. Low average annual rainfall and high altitude appear to characterize regions favorable for maintenance of CTF virus. This area is roughly bounded by drawing parallel east-west lines through the northern half of Nevada and the southern half of Montana and parallel north-south lines through eastern Oregon and eastern Colorado.

Four hundred nineteen D. variabilis collected in northeastern North Dakota were examined with no isolation of CTF virus. Up to the present time, 14,546 D. variabilis from the eastern and midwestern U.S. have been examined without any isolation of CTF virus.

Laboratory Studies:

Present studies are concerned with growth of virus in larval, nymphal, and adult D. andersoni under various temperature conditions and holding times. Kennedy has infected larvae by having them feed on hamsters

infected by subcutaneous inoculation of 1000 suckling mouse LD_{50} of a recently isolated CTF virus. During the quiescent period after feeding, no increase in amount of virus could be detected. The average titer of virus in a pool of 10 was $10^{-2.2}$ to $10^{-2.4}$ and the amount of virus 15-40 LD_{50}. Pools of 10 flat nymphs derived from these larvae were examined daily through the 21st day and at less frequent intervals thereafter. The titers immediately after molting from larvae were $10^{-2.9}$ to $10^{-3.6}$, and 21 days later $10^{-5.2}$ to $10^{-5.9}$. Two months later titers as high as $10^{-6.2}$ were found. During the next month, titers dropped and were in the range $10^{-2.4}$ to $10^{-3.9}$. The corresponding amounts of virus were 75 to 400 LD_{50}, 10,000-46,000 LD_{50}, 100,000 LD_{50} and 5-400 LD_{50}. An aliquot of nymphs was allowed to engorge on hamsters 21 days after emerging from the larval stage. At this time titers of virus were as given above. The titers found in nymphs immediately after engorgement were $10^{-4.0}$ and the amount of virus 30,000 LD_{50}. After the 1st week titers were generally in the range $10^{-2.7}$ to $10^{-3.6}$ and amounts of virus 1600 to 6000 LD_{50}. In flat adults derived from these nymphs, titers were usually in the range $10^{-1.9}$ to $10^{-2.5}$ and the amounts of virus 25 to 1000 LD_{50}. These results correspond to those found in naturally infected adults.

Rush has dissected flat adults and consistently found virus in the salivary glands and less frequently in the gut. He has also carried out preliminary work on the histology of flat adult ticks. Portions of the exoskeleton were dissected off without disturbing the internal anatomy and rapid penetration of fixatives was then obtained. Good cellular detail is obtained with conventional stains. The work is preparatory to a study of distribution of virus in D. andersoni and its effect on the tick.

B. Powassan Virus

Field Studies: The objectives of field study are to:

1) Determine its importance as a cause of human disease.
2) Study its ecology.

Data Obtained:

The initial strain isolated here from D. andersoni in 1952 differs from the Powassan virus by being neutralized to a slight extent by St. Louis immune serum and by some human sera containing SLE antibodies. In areas where there is a high incidence of SLE antibodies, there is an increase in sera which neutralize our strain (791A-52) of Powassan virus.

Table 2. Incidence of SLE & 791A-52 Antibodies

	791A-52	%	SLE	%
Minnesota	1/434	.2%		.7%
Wyoming	3/566	.5%		*
North Dakota	1/58	2%		1%
Nebraska	9/144	6%		?
Oregon	8/68	12%	60/119	50%

* A SLE antibody study has not been carried out in Wyoming - neighboring Montana has 3%

Our original strain of Powassan virus cannot, therefore, be used in antibody surveys with any assurance that Powassan antibodies are being detected and our results to date must be revaluated.

Work has continued in the Black Hills of South Dakota. This June virus was isolated from a blood clot obtained from a pool of 2 peromyscus, 1 of 49 examined, and once from 175 adult D. andersoni. Since there is a relatively high incidence of CTF virus infection of D. andersoni in this area, it is possible that this virus may interfere with isolation of Powassan virus. Small mammals in this area are infested with Ixodes sp. of larvae and nymphs as well as with those of D. andersoni. Data obtained to date are too scanty to allow more than a guess as to the ecology of the virus. It is suspected that small mammals and some tick parasites maintain the virus.

II. Mosquito Transmitted Viruses.

A. Western Equine Encephalitis Virus.

Objectives of Study: By a systematic study of vertebrate species and their ectoparasites obtain clues as to the overwintering mechanism.

Field Data:

During September and October 1961, in the Oregon study area, vertebrate species, mammalian and avian, were bled to get comparative antibody rates. Swine and jackrabbit sera had an appreciable incidence of WEE antibodies making it necessary to consider mammals in the study of an overwintering mechanism.

Table 3. Antibody Survey - Oregon and North Dakota 1961

	WEE	SLE
OREGON		
Swine - 9-28-61	27/49	18/45
Chicken (not sentinel) 10-2-61	4/71	1/71
Rabbit (domestic) 10-3-61	0/6	0/6
Horse - October 1961	4/4	2/3
Sentinel chickens 10-4-61	2/12	0/12
Packrats	0/6	2/6
Peromyscus	0/10	0/4
Cottontail	0/2	0/2
Jack rabbit	3/16	0/15
Magpie	1/1	0/1
Bullsnake	0/1	0/2
Mus sp.	0/2	
Mule deer	0/1	0/1
NORTH DAKOTA		
Sentinel chickens -Sept. 1961	1/12	0/11
Swine - September 1961	11/50	0/49
Turkeys - September 1961	0/53	1/53

In September and October 1961, after the mosquito season and in May and early June, 1962, prior to the appearance of an appreciable number of the 1962 generation of mosquitoes, blood was collected from vertebrate species, primarily mammals, to determine whether virus

could be isolated. No virus was found although there had been a high incidence of WEE virus infection in C. tarsalis the summer of 1961. The animals bled are listed in Table 4.

Table 4. Vertebrate Bloods - Vale, Oregon - 1961 and 1962 - Isolation Attempts

	Sept. - Oct. 1961	May - June 1962
Ground squirrels		135
Peromyscus	18	54
Owl		1
House mouse		1
Jack rabbit	16	2
Cottontail	3	9
Marmot		16
Bullsnake	2	6
Kangaroo rat		3
Packrat	6	3
Magpie	2	19
Racer		1
Lizard		1
Hawk		1
Pocket mouse		5
Mus. sp.	2	
Totals	49	257

To date, examination of C. tarsalis collected in Oregon during 1962 shows a very low incidence of WEE virus and a moderate incidence of SLE virus infection.

Table 5. Isolation of Virus from C. tarsalis - Oregon 1962

	No. Mosq. Examined	No. Pools	+ WEE	+ SLE
May	45	20	0	0
June	24	15	0	0
July	2308	49	1	5

This contrasts with the summers of 1960 and 1961 when WEE isolations predominated and the incidence of infection in C. tarsalis was high. The nearly complete disappearance of WEE virus following summers with a high incidence of infection is difficult to explain on the hypothesis that either hibernating C. tarsalis or birds carry virus through winter.

North Dakota Study Area:

Examination of animal sera (Table 3) at the end of the 1961 mosquito season indicated that, as in Oregon, swine are better indicators of virus presence than chickens or turkeys. Examination of sera as well as previously reported mosquito examinations indicated a low level of WEE virus activity - a situation that has prevailed for 3 years. The incidence of WEE virus infection of C. tarsalis has been so low and the population of C. tarsalis at such a low level that it is difficult to account for the minimal presence of WEE virus by a C. tarsalis-bird cycle and makes any overwintering of virus by either birds or C. tarsalis unlikely. The 1962 mosquitoes have not been examined yet.

Alaska Field Studies:

Alaska appeared to be a favorable area to test the hypothesis that latent infection in birds may be an overwintering mechanism since many water birds spend the winter in endemic areas and the summers in Alaska where a summer virus cycle appears unlikely. Therefore, any virus isolated could be presumed to represent a latent infection acquired in a southern area. The Arctic Health Research Center, Dr. Hopla, Dr. Sladen of Johns Hopkins have all supplied material. Although an occasional bird has serum neutralizing SLE or WEE virus (Table 6), it has been impossible to isolate virus from 117 bird tissues and 52 bird bloods received in August 1961 from the Arctic Health Research Center or from 159 bird bloods examined from the Aero Medical Laboratory (Dr. Hopla).

Table 6. Presence of SLE and WEE Antibodies in Arctic Bird Sera

	Sentinel Chickens	Wild Birds	
	WEE	SLE	WEE
Aero Med. Laboratory (Dr. Hopla)			1/34*
Arctic Health Research Center	0/20	1/100	0/101
Pribiloff's (Dr. Sladen)		1/28*	0/31

* An additional one was positive on screen but there was insufficient quantity of serum to repeat.

Laboratory Studies:

To get information regarding the effect of springtime temperatures on the extrinsic incubation period in C. tarsalis, Rush infected C. tarsalis by feeding on a chick. These were divided into 2 groups. Those kept at room temperature had virus in the salivary gland within 4 days. Those kept outside during April had no virus in the salivary gland at the end of 21 days and 1 mosquito had not completely digested its blood meal within this period. It is considered to be further evidence in support of the belief that a C. tarsalis-bird virus cycle does not take place during spring in the northwestern U.S.

Since there is some evidence that adapting C. tarsalis to the usual insectary conditions selects a population which differs in its capacity to support virus growth and because it is desired to get more data on ability of infected C. tarsalis to pass the winter successfully under conditions found in the northern U.S., Rush has attempted to maintain C. tarsalis throughout the year in an outside insectary by building an insectary adjacent to an artificial hibernating quarter (rocks covered by earth). Several thousand mosquitoes were placed in this insectary during the summer of 1961 and exposed to infected chickens. During the spring of 1962, only 2 mosquitoes emerged.

B. St. Louis Encephalitis Virus.

Objectives of Study: More adequate data on summer and winter maintenance are required. Evidence obtained here and elsewhere indicates that WEE and SLE virus have a different ecology and that it cannot be assumed that birds play the important role in the maintenance of SLE virus that they do with the WEE virus.

Field Data:

As shown in Table 5, SLE virus has been isolated from C. tarsalis more frequently during 1962 than WEE virus. Similar observations indicating a lack of correspondence between the occurrence of WEE and SLE virus have been obtained in the past. Correlation between SLE and WEE infection rates in C. tarsalis would be expected if the ecology of SLE and WEE viruses were similar.

Antibody Surveys:

Results of antibody surveys during late summer and fall of 1961 are shown in Table 3. In Oregon, swine appear to be better indicators of the presence of SLE virus than chickens. Birds had a low incidence of antibodies. The high antibody incidence in swine sera is remarkable in view of the absence of virus in C. tarsalis during 1961.

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

Investigations have been continued on the wintertime presistence of WEE and SLE viruses in an endemic area, Kern County, California. SLE virus was isolated from Culex tarsalis collected in February, 1961. These were deplete mosquitoes that had probably fed on a virus source in January. No virus isolations were made in tests of 4,900 Culiseta inornata. WEE virus was isolated from blood samples from a white-crowned sparrow collected January 24, 1961, a Citellus nelsoni bled February 24, 1961, and a Mus musculus bled March 3, 1961. Three strains of a virus were isolated from the blood of jack rabbits and cottontail rabbits but are unidentified. No virus was isolated from pools of ticks or fleas. Serologic evidence continues to accumulate of a Group B virus infection other than SLE in rodents. No virus has been isolated from C. tarsalis that took xenodiagnostic feedings on over 1,000 birds collected in winter months. Examinations of ovarioles from samples of a C. tarsalis population allow an accurate separation into parous and nulliparous categories. In the fall and early winter, a high proportion of the feeding population was parous and the shelter population was predominately nulliparous. In mid-winter, all female C. tarsalis were nulliparous. These observations indicate the mid-winter population had not taken a blood meal and would not favor the overwintering of viruses in this population.

A second year of intensive C. tarsalis control was completed. WEE and SLE virus transmission remained at a significant level in the controlled and in comparison areas. Infiltration of female C. tarsalis into the controlled area was a major factor in persistence of viruses. A study on the flight dispersal of female C. tarsalis documented the infiltration pattern. Specimens marked with fluorescent particles were released outside of the

29 square mile controlled area and infiltrated from all directions into the center of the area. Numerous marked specimens were recovered at distances up to 5 miles and the maximum dispersal was to 9.8 miles. The experimental control project has been shifted to another area of 50 square miles for the summer of 1962.

The development of a series of screening and specific precipitin antisera allowed accurate studies of the feeding habits of C. tarsalis. It was found that most C. tarsalis fed on birds, particularly passerines. In some habitats, the dove was a favored host. The most available animal species in an area was most likely to be fed on. In some habitats up to 50 per cent of C. tarsalis feedings were on mammals. Ninety-eight per cent of Culiseta inornata feedings were on mammals. Studies have continued on the development of more specific precipitin antisera for the identification of blood from various vertebrate species. Chickens develop high titers of specific antibodies to the serum antigens of some mammalian species. Immunoelectrophoresis and Ouchterlony techniques are being applied to a further understanding of the various immunologic problems.

In 1961, there was one WEE case in man, and two in horses in Kern County. The vector population was small in most areas, and except in special study areas, there was little WEE and SLE virus transmission to chickens. There was shortage of water which limited mosquito sources and favored effective vector control. Temperatures were above normal and favorable for rapid virus transmission. In 1962, there were no human or horse cases as of August 1. WEE virus was first detected in C. tarsalis in mid July, but in most of the area the vector population remained at a low level.

CF antibodies to WEE and SLE viruses were undetectable in 50 per cent of proven clinical cases after three years and in almost all cases after six years. Antibodies persisted longest in those individuals with maximum initial titers. An equation was developed which may allow epidemiologic interpretation from a single CF serologic survey with respect to infection rates in previous years. In clinical cases, HAI and neutralizing antibodies lasted longer than CF antibodies.

Surveys for HAI antibodies in samples of normal exposed populations indicated that a number of persons had inapparent infections with WEE and SLE viruses over an 18-month period. A number of individuals with WEE neutralizing antibodies had no HAI antibodies. The reverse was true for SLE antibodies, a possible indication of infection with another group B virus.

Fluorescent antibody studies with WEE virus have progressed to the point of planning their application to virus detection and identification problems.

REPORT FROM DR. HUGH H. SMITH
ARBOVIRUS UNIT, UNIVERSITY OF ARIZONA
TUCSON, ARIZONA

Material collected during the hot season of 1961 in the Tucson area has been tested for evidence of activity of Western and St. Louis encephalitis viruses.

Using the HAI technique, the following results were obtained:

	No. Sera	+ SLE	+ WEE
Human sera (Adults-mostly from Blood Bank donors)	293	12	0
Wild caught birds	535	14	1
Sentinel chickens	136	2	2
Wild caught mammals (mostly rodents)	375	6	0
Horses	8	0	2
	1347	34	5

Attempts to isolate virus by inoculation of specimens into less than 4-hour-old chicks and by inoculation into hamster kidney cell cultures, to date, has been negative. Specimens tested have consisted of mosquito pools and blood and tissue samples from several species of wild birds and rodents. So far, some 191 specimens have been tested in chicks and 165 have been given two passages in hamster kidney cell culture. Work along these lines is continuing.

It is expected that an adequate supply of suckling mice will soon be available for use in virus isolations.

Collections of field specimens are proceeding along the same lines during the current hot season.

The use of other viral antigens for testing local specimens is planned, especially Eastern EE.

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

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

Efforts to isolate viral agents in the Colorado study areas have yielded a California virus strain from a pool of Culiseta inornata, collected between 9 June and 29 August, 1961, at the 6,500 foot elevation in our mountain study area. Modoc virus was isolated from the heart tissue of a juvenile male Peromyscus maniculatus, collected on 12 May, 1961, in the St. Vrain river bottomland near Greeley. The isolate was identified by Dr. Leo Thomas, Rocky Mountain Laboratory. This is the first isolation of Modoc virus from Colorado and is particularly interesting since it came from a predominantly juvenile Peromyscus population in the early spring.

Search for the overwintering reservoir of WE resulted in the isolation of this virus from the brain of an apparently health Microtus sp. collected 30 March, 1960, in the St. Vrain study area. This isolation was made in 8-day embryonated eggs. A blood clot was negative and a 1:20 dilution of the sera only partially inhibited WE antigen in the HAI test. This isolation was made at the time when small mammals exhibited an HA inhibitor to WE antigen.

II. Report from Taunton, Massachusetts, Dr. Richard O. Hayes.

EE virus was found to overwinter in experimentally infected spotted turtles and a garter snake. Specimens were inoculated on November 2, 1961, and held for one week at room temperature. Snakes were then placed in an outdoor overwintering cage and turtles were held in water in a refrigerator at 40 $\pm$ 5 F. Pre-hibernation bloods taken November 9, 1961, contained EE virus as did blood from the turtles taken February 2, and April 20, 1962. Blood from one garter snake taken on April 20 was positive also. Negative results were obtained with five painted turtles, two box turtles, and one wood turtle.

Studies of the diel activity cycles of adult Culiseta melanura indicate that peak activity occurred during the 2-hour period following sunset. Such activity in its swamp habitat would facilitate feeding on wild birds and reduce the opportunity for feeding on man. This may contribute toward its importance as an enzootic vector of EE virus and its apparent unimportance as an epidemic vector.

III. Report from Plainview, Texas, Bruce Francy.

Residual larvicides applied to all potential mosquito breeding areas within a three-mile radius of Hale Center, Texas, failed to reduce substantially the adult Culex tarsalis population. The anticipated residual effects of the larvicides were not obtained, so that frequent retreatment of many areas was necessary. Larval densities were controlled in this manner, but adults apparently infiltrated the three-mile area in such numbers that light trap and shed trap collections were only slightly lower than would have been expected without treatment. There was some evidence that adult populations in the center of the treated area were suppressed.

Evaluation of the efforts to control vectors on virus transmission rates awaits tests on sera from sentinel chickens.

IV. Report from Wenatchee, Washington, Louis J. Ogden.

Larvicidal control of mosquitoes in an area of approximately 17 square miles in the Columbia Basin effectively reduced mosquito production. Continuous inspections of breeding areas showed .15 total larvae and .07 C. tarsalis larvae per dip in the treated area compared with 1.1 total larvae and 0.4 C. tarsalis larvae per dip in the untreated areas. However, because of rapid percolation through breeding sites into the coarse soils prevalent in the area, the effectiveness of dieldrin residual larvicide was of short duration. Frequent retreatments were required on areas supplied by seepage or by prolonged irrigation of individual fields.

In spite of the reasonably effective control of mosquito production, C. tarsalis female populations at the center of the area were only slightly reduced until late July and exceeded the populations in comparison areas the first two weeks of August. Light traps in the treated area averaged 9.0 C. tarsalis per trap night throughout the summer and 13.9 per trap night in the untreated area. There was circumstantial evidence based on the ratio of males to females that specimens were infiltrating from breeding sites beyond the treated area. The controlled area was roughly circular with average radius of approximately two miles. Flight range studies carried out in Kern County, California, during 1961 indicate that C. tarsalis would be expected to infiltrate much farther than two miles.

Data from sentinel chicken flocks in the treated and untreated comparison areas indicated that the mosquito control operations did not produce a reduction in WE transmission rates. WE infection rates in the comparison area were 60% and 46% for 1960 and 1961, respectively; the rates in the treated area for the same years were 41% and 55%.

These results confirm observations in other areas which indicate that control of encephalitis virus activity by larviciding mosquito breeding areas is not effective within a small area. The effectiveness of this technique on a larger area remains to be demonstrated.

V. Report from Bakersfield, California, Dr. R.E. Bellamy.

(This information is included in the report from the School of Public Health, University of California, Berkeley)

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

In working with one of our virus isolates from Culex tarsalis mosquitoes, collected in 1961 from the Lubbock area, a hemagglutinin has been demonstrated with the sucrose-acetone extraction of suckling mouse brain. The optimum conditions for hemagglutination of goose cells is pH 6.0 at 37C. Adult mice are not killed by this virus, but it is highly pathogenic for chick embryos inoculated either by amniotic sac or yolk sac routes. A viremia is produced by this virus when inoculated into newly hatched chicks. Hemagglutination-inhibition tests with known antisera have shown that this virus is not Western, Eastern, St. Louis, California, Aedes trivitattus, or Cache Valley virus. It is hoped that in the near future antisera will be obtained and tested to determine the relationship of this virus to other known viruses.

Hemagglutination-inhibition tests have been performed with the above virus with human, horse, small mammal, and avian sera from the Lubbock area. There is no evidence of antibodies in the human or mammal sera, but HI antibodies have been demonstrated in chicken sera and one wild bird. As yet no neutralization tests have been done on the HI positive sera, but the HI would seem to be specific in that these sera are negative in HI tests with other antigens.

Antigens and antisera are being prepared with a number of our unidentified virus isolates from mosquitoes which do not seem to be related to the above virus so that we may obtain help from CDC in identifying these viruses. We have about twenty isolates which are pathogenic for suckling mice but not adult mice or chick, and perhaps are the same virus.

So far this summer, this laboratory has not confirmed any cases of arthropod-borne encephalitis. This seems unusual especially since there have been none from the Texas Panhandle area where there has been ample rain in addition to the irrigation which is done. This summer, this laboratory has only confirmed one case of Western encephalitis--in a horse from the El Paso area. However, an isolated case of Western encephalitis was confirmed in a child in the coastal area in March.

Testing of mosquitoes for presence of virus this year has not been very extensive and as yet no virus recoveries have been made. Regular shipments are now being received, however.

REPORT FROM DR. WILLIAM D. HILLIS, CHIEF OF EPIDEMIOLOGY USAF, LACKLAND AIR FORCE BASE, TEXAS

Western Encephalitis Study in Air Force Personnel in California:

Heavy early spring rainfall and unseasonably large mountain snow melt are expected to generate flooding conditions in the northern central valley of California during the late spring and early summer of this year. The resulting anticipated increase in mosquito populations, including Culex tarsalis, together with the known migration of large numbers of wild birds into the area and the existence of a significant susceptible animal population, constitutes reason for concern over the possibility of an outbreak of western encephalitis during the approaching summer.

A moderately large U.S. Air Force population is based in this general area. Previous exposure to WE virus in this group is essentially unknown, but it is expected that many, if not the great majority of this highly mobile group, are susceptible to WE infection. A prospective study is presently underway by the 6570th U.S. Air Force Epidemiological Laboratory, Aerospace Medical Division (AFSC), at Lackland AFB, Texas, to determine the rates of serological conversion in USAF personnel in this area to both WE and SLE viruses, during a season in which mosquito populations and environmental conditions will be studied.

Some 2300 military personnel, randomly selected, will be followed serologically before, during, and after the anticipated height of the mosquito season. Complement fixation tests will be used to determine any recent infections that may have occurred during the period, and neutralization tests in a representative sampling of the preseasonal sera will be utilized to identify rates of previous infection. Simultaneously, an entomologic team, headed by Captain Cyril Hodapp, Chief of the Department of Entomology, will conduct mosquito surveys to determine prevalent species and total estimated population and to ascertain the efficacy of various insecticidal efforts programmed for each of the participating bases. Attempts will be made to isolate virus from mosquito pools.

USAF hospitals in the area have been alerted to the possibility of human disease, so that early acute material from clinical cases will also be available for study. In the event of an actual disease outbreak, bird, rodent, and mammal populations in the area will be studied for evidence of recent viral infection. Viral studies will all be conducted in the Virology Branch of this Laboratory, headed by Major Robert Crandell.

Participating bases include Travis AFB (near Fairfield), McClellan and Mather AFB's (near Sacramento), Beale AFB (near Marysville), and Castle AFB (near Merced). Hospital commanders, together with base preventive medicine officers, veterinary officers, and engineers, are actively participating in the study. The group will be in close contact with Dr. William Reeves and his associates at the University of California School of Public Health, who will be conducting WE studies at Bakersfield and among civilian groups in Kern County, California.

REPORT FROM DR. S.S. KALTER, CHIEF, VIROLOGY UNIT USAF SCHOOL OF AEROSPACE MEDICINE BROOKS AIR FORCE BASE, TEXAS

Forty-one baboon serums were tested in the CF and NT tests for antibodies to various human virus and rickettsial diseases. Table 1 indicates the results showing evidence of infection with EE, LCM, Herpes Simplex, Q fever, Psittacosis, Rocky Mountain Spotted Fever, and ECHO 18. The presence of antibodies to the polioviruses was a result of experimental infection. Of interest was the high incidence of antibodies to Coxiella burnetii. This was substantiated by a report from Dr. G.S. Nelson (Senior Parasitologist, Div. of Insect Borne Diseases, Nairobi, Kenya) who has informed me of studies done by Dr. R.B. Heisch who found 7 of 10 baboons with antibodies for Q fever. "One of the baboons with a titre of 1/100 had a batch of ticks, Riphicephalus simus simus, attached to the chest wall." Also of interest was the finding of two baboons with antibodies to eastern encephalitis virus.

Table 1. Antibodies in normal baboons to human viruses and rickettsia*.

Virus or Rickettsia	No. Baboons with Antibodies	No. Baboons without Antibodies	Serol. Test
EE	2	39	CF
WE	0	41	CF
SLE	0	41	CF
LCM	1	40	CF
Mumps (V)	1	40	CF
Herpes Simplex	3	38	CF
Adenovirus	0	41	CF
Inf. A	0	41	CF
Inf. B	0	41	CF
Q Fever	9	32	CF
Psitt.	1	40	CF
RMSF	1	40	CF
Poliovirus (1-3)	2**	39	CF, Neut.
Coxsackie (B1-6, A9)	0	41	"
ECHO 1-28	1 (ECHO 18)	40	"

* HA-I Tests for arborviruses not completed at this time.

** Experimentally induced.

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

In a previous report from this laboratory (Information Exchange No. 5), reference was made to the fact that transplacental transmission of JBE virus in experimentally infected Mexican free-tailed bats had been demonstrated. This brief account presents observations with both JBE and SLE viruses. Bats netted in progressive stages of the reproductive cycle during the spring and early summer received subcutaneously 150 mouse intracerebral LD_{50} of either the OCT-541 strain of JBE or a strain of SLE virus isolated from a naturally infected flicker. Previous studies had shown the Mexican free-tailed bat to be highly susceptible to these viruses. At intervals after inoculation, the pregnant bats were bled and the single fetus carefully removed from the mother in such a way that placental connections were severed without contamination of the fetus with blood or body fluids from the mother, and, in addition, the fetus was washed in several changes of sterile saline. Both the mother's blood and whole ground fetus were assayed for presence of virus by intracerebral inoculation of weanling mice. Since we are especially interested in tissue tropisms of arboviruses in experimentally infected bats, in several instances brown adipose tissue, brain and kidney were removed from the mother bats and also from their fetuses and assayed individually for virus. The approximate stage of pregnancy of the groups of experimentally infected bats was determined by weighing the fetuses obtained at each harvest period. An earlier study concerned with fetal weight-linear measurement correlation had shown that bats netted at any one time are in approximately the same stage of pregnancy and that the stage could be approximated as early, mid, or late by simply determining fetal weights.

JBE virus was found to cross the placenta and invade fetuses of mother bats infected during all three stages of pregnancy. Virus was demonstrated in the fetuses of approximately 40% of the bats shown to be viremic at the time of sacrifice. In one instance, virus was found in the fetus of a bat whose blood was negative. Virus titers ranged from <1.0 to >3.5 in maternal tissues, and from <1.0 to 1.5 in fetal tissues. In the group of bats inoculated in late pregnancy from which multiple tissues of both mothers and fetuses were assayed, JBE virus was widely distributed in infected mothers, being found in brown fat, brain, and kidneys, as well as in the blood of the individual bats. Infection was found to be similarly disseminated among the fetal tissues tested, although titers were lower and often fewer tissues were involved. In two instances, brown fat was the

only fetal tissue positive by intracerebral mouse inoculation, and in one instance virus was found only in fetal brain tissue. Although the stage of pregnancy in which bats received JBE virus did not appear to influence their susceptibility, transplacental transmission of this agent occurred with greater frequency in the late group. The results of this study would suggest that JBE virus could be perpetuated in nature in bat populations by passage from infected mothers to their offspring.

In striking contrast to the results obtained with JBE virus, transplacental transmission of the flicker strain of SLE virus in bats could not be demonstrated. Although concentrations of SLE virus in the blood of infected mother bats ranged as high as 4.0 log units, evidence of viral multiplication in their fetuses could not be obtained. Whether failure to demonstrate SLE virus in these fetuses was due to the inability of this virus strain to cross placental barriers or to an inability to establish infection in fetal tissues remains to be determined.

REPORT FROM DR. R. WALTER SCHLESINGER
DEPARTMENT OF MICROBIOLOGY
SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE
SAINT LOUIS, MISSOURI

Work on the purification and characterization of dengue-2 virus has continued. Virus is harvested from KB cell monolayers, subjected to extraction with fluorcarbon (Genetron), and to preparative centrifugation at 30,000 rpm for 90 minutes. Banding in cesium chloride or rubidium chloride density gradients usually gives two hemagglutinating peaks, one at $\rho = 1.19$, the other at 1.24. All of the plaque-forming capacity is associated with the latter fraction. It has not yet been determined whether or not the noninfectious hemagglutinating component results from partial degradation by the salts.

Studies have also been initiated on plaque formation by Hammon's strains provisionally designated as dengue-3, 4, 5, and 6. Cross-neutralization tests with these strains and with dengue-1 and 2 are currently being carried out. Results will be communicated in the next issue of this bulletin.

During a summer's work at the Mount Desert Island Biological Laboratory, Salisbury Cove, Maine, we ran out of goose cells and had to improvise with what our marine environment provided. One by-product of this situation was the finding that sea gull red cells can substitute for goose red cells for hemagglutination by dengue viruses.

Characterization of the inhibitory factor present in agar extract has been completed. As reported previously, agar contains a water soluble component which inhibits plaque formation by dengue-2 virus, as well as infectivity for mice and hemagglutination. Partial purification of this material has been achieved by co-precipitation with bovine albumin at acid pH, repeated washing of the precipitate, and dissociation of the inhibitor-protein complex by alkalinization. The protein can be removed by extraction with phenol which, in turn, can be removed by extraction with ether. The resulting product has very high inhibitory activity which is destroyed by acid hydrolysis. Upon hydrolysis, it yields free sulfates and reducing sugar. Thus it is probably identical with the plaque inhibitor described by Liebhaber and Takemoto. Direct evidence has been obtained which indicates that the sulfated poly-saccharide does not combine with red cells, but does combine with virus particles.

Further work is in progress on the kinetics of the interaction between the purified inhibitor and partially purified viruses.

REPORT FROM DR. EDWIN M. ELLIS U.S. DEPARTMENT OF AGRICULTURE, NATIONAL ANIMAL DISEASE LABORATORY, AMES, IOWA

A study was initiated during the summer of 1962 to attempt to isolate the virus of vesicular stomatitis from arthropods collected in vesicular stomatitis endemic areas of the United States. This work was stimulated by the isolation of vesicular stomatitis virus from Phlebotomus sandflies and one lot of Culex mosquitoes in Panama. These isolations by the Gorgas Memorial Laboratory and the Middle America Research Units were summarized in the Arthropod-borne Virus Information Exchange (No. 4 , October 1961, pp. 17-19).

Vesicular stomatitis has been diagnosed annually in livestock of the Southeastern United States (Georgia, North Carolina, South Carolina, and less frequently in Florida, Alabama, Louisiana, and Texas).

Because of its clinical similarity to foot-and-mouth disease, outbreaks are carefully followed by virus identification and serology. The seasonal pattern with annual recurrence of this disease is suggestive of arthropod-involvement.

As an initial probe, an entomologist, Dr. Derl Brooks, was hired for the summer by the Animal Disease Eradication Division to collect Phlebotomus sandflies and other blood sucking arthropods, and to make

professional observations which could bear on the possibility of arthropod involvement.

Intracerebral inoculation of 1-7 day suckling mice, swine kidney tissue cultures, and the chorioallantoic sac inoculation of 7-8 day embryonated hen eggs were chosen as the methods for virus isolation. The tissue culture isolation efforts were carried through three blind passages.

Insect collections started in Livingston Parish, Louisiana, in June, 1962. Thirteen small pools of mosquitoes, hornflies, and Tabanids were examined. No virus isolations were made. No Phlebotomus sandflies were found.

Dr. Brooks transferred his activities to the area of a large outbreak of New Jersey type vesicular stomatitis at Carrolton, Carroll County, Georgia, on July 12. In Georgia he experienced difficulty in making insect collections and has found Phlebotomus extremely rare.

Three small arthropod pools collected from animals in Alabama during VS outbreaks in the herds were negative to virus isolation attempts.

Dr. Brooks' findings and observations will be reported in the next Information Exchange.

REPORT FROM DR. CARLOS CAMPILLO-SAINZ DIRECTOR, INSTITUTO NACIONAL DE VIROLOGIA DE LA S.S.A. MEXICO, D.F., MEXICO

Studies on Arboviruses in Mexico, Preliminary Report:

The staff doing field research is made up of virologists, entomologists, and ornithologists affiliated as well with the University of Minnesota.

Serological inquiries have been undertaken in human beings, cattle, pigs, and fowl. Wild fowl have been caught with the help of nets or shot. Different types of mosquito traps have been set up. They include traps containing human and animal bait, as well as direct capture through aspiration tubes.

Studies were begun in July 1961. Periodically, one of the staff members or the whole group travelled to the different chosen areas.

Chosen areas:

Tlacotalpan, Vera Cruz, and Coatetelco, Morelia, were chosen in order to get a comparative view of two distinct ecological areas. The first is a small town by the Papaloapan River. It has tropical weather as well as flora and fauna typical of the Gulf Coast region of Mexico. Coatetelco, on the other hand, lies on the high Mexican plateau with the lesser rainfall of the high subtropical climate. Its vegetation and animal life are also different from the lower village. Bordering Coatetelco is a lake which was a major point in choosing the place.

Results:

Tlacotalpan, Vera Cruz. Human sera from this town underwent HI and NT tests. The first used the following antigens: EE, WEE, SLE, Ilheus, Dengue 1, and Yellow Fever. The HI test results show there is a marked group B activity, especially SLE and Ilheus, among the human population of Tlacotalpan. Due to serological crossing from several group B members, there was also a response to Dengue 1 and Yellow Fever, although both had low titres. Such crossings are most noticeable in the 30 to 50 age groups. Younger persons only showed IL and SLE antibody activity.

Using the NT test, the aforementioned findings were interpreted with clearness, and thereby exclusive specificity of antibodies against SLE was shown. Supported by the HI and NT test results, we concluded there is a marked SLE virus activity in Tlacotalpan, and that due to the uniform age-group antibody distribution, as well as having found crossings in older groups, we can adduce there is endemic SLE virus activity in the town and that the serological crossing can be interpreted as due to reinfections by the same agent.

Concerning group A, cattle infections with EEE and WEE were considered after having found antibodies against both. Apparently this activity has not included human beings whose sera have come out as negative for both viruses.

In Coatetelco, Morelia, there was a marked contrast with the former data. No evidence was found of any of the above mentioned viruses in Coatetelco. Human and pig sera were studied. We believe it interesting to stress that although this village showed the proper arbovirus ecological conditions, the findings were negative. The nearby lake gives a place of brooding for varied species of birds. Also the mosquito population should run high under this situation. Nevertheless, the mosquito collection at the time of the study was negligible.

REPORT FROM DR. ENID DE RODANICHE
SCHOOL OF MEDICINE, UNIVERSITY OF PANAMA
PANAMA, R. P.

The University of Panama reports that Enid de Rodaniche, Professor of Microbiology in the School of Medicine, was awarded a grant (E-4228) by the National Institutes of Health for the study of arthropod-borne viruses isolated in Panama. The investigation, as planned, includes the identification and evaluation of the epidemiological importance of 50 unclassified viruses recovered in collaboration with the staff of the Gorgas Memorial Laboratory from various sections of the Republic during the period from 1956 to 1961. Isolations have been made principally from mosquitoes, but also from sandflies, birds, and humans. Some 29 additional viruses were previously identified as yellow fever, Ilheus, St. Louis and vesicular stomatitis (Indiana type).

Our major effort during the first year of the grant has been dedicated perforce to equipping the laboratory of microbiology of the University for virological research and to improving facilities for animal care. The most significant findings to date are the identification as Ilheus of a third isolate from the blood of a bird, Ramphocelus passerinii, captured in Bocas del Toro. (Two former isolates were reported in Am. J. Trop. Med. & Hyg., 10:395, 1961, by Galindo and Rodaniche). Other isolates of this virus have been obtained from the mosquitoes, Psorophora lutzii, Psorophora ferox, and Culex nigripalpus obtained in the same Province. Other pools of the Genus Psorophora were found also to harbor Una virus. Epidemiological studies completed include testing for antibodies against Ilheus virus the blood of some 357 birds, 18 other vertebrates, and 645 human beings: 9.6% of the birds and 10.7% of the humans gave strongly positive results.

As isolation of Ilheus virus from the Province of Darien and from Central Panama was reported previously, it is apparent that Ilheus infection occurs at least periodically from one border of the Republic to the other. Birds seem to be the principal hosts and are probably important in its dissemination in South and Central America and the southern part of the United States.

Two different viruses isolated from the blood of 2 male adults with transient febrile disease were identified by Dr. Causey, Director of the Belem Virus Laboratory, as belonging to Group C, one apparently being related to Apeu. No clinical cases of Ilheus infection have as yet been found.

REPORT FROM DR. CARLOS SAN MARTIN-BARBERI AND DR. ROBERT H. KOKERNOT, UNIVERSIDAD DEL VALLE CALI, COLOMBIA

In February of this year, a trip was made along the Pacific coast of Colombia from the border of Panama to Buenaventura. Human bloods were collected in six different localities. In the enclosed table are the results obtained in relation to immunity to VEE virus. For a general description of the methods used, see page 26 of the March 1962 issue of the Arthropod-borne Virus Information Exchange.

In the last two years, the treatment of sera for the H.I. test with kaolin and thereafter adsorbed goose erythrocytes (Clarke and Casals, 1958, American Journal of Tropical Medicine and Hygiene, 7:561-573) has been routinely made in a very simple way which saves a lot of time and work. The method consists of using a single tube for the whole process. After kaolin treatment, the sera are centrifuged for 20 minutes and then the goose erythrocytes are added to the same tube. After adsorption with the erythrocytes, a second centrifugation is carried out for 10 minutes. At the end , three layers are obtained: kaolin in the bottom, goose erythrocytes in the middle, and on top, the serum ready to be tested in the H.I.

DISTRIBUTION OF IMMUNITY TO VEE VIRUS

DATE	LOCALITY		AGE IN YEARS							TOTAL
10 February 1962	Colombia, Chocó, Juradó	Male	0/5	0/14	0/1	1/2	0/5	1/3	2/4	4/34
		Female	0/2	0/ 6	0/4	2/7	0/1	0/3	0/1	2/24
		Total	0/7	0/20	0/5	3/9	0/6	1/6	2/5	6/58
11 February 1962	Colombia, Chocó, Cupica	Male	0/4	1/6	0/3		0/2		1/3	2/18
		Female		0/7	0/2	0/6	0/3		1/4	1/22
		Total	0/4	1/13	0/5	0/6	0/5		2/7	3/40
9 February 1962	Colombia, Chocó, Bahia Solano	Male	0/2	0/1		1/2			0/1	1/6
		Female	0/5	0/2	0/3	1/14	0/5	0/8	1/4	2/41
		Total	0/7	0/3	0/3	2/16	0/5	0/8	1/5	3/47
11 February 1962	Colombia, Chocó, El Valle	Male	0/10	0/8	1/10	0/3	1/3	0/4	2/5	4/43
		Female	0/ 6	0/9	0/10	0/2	0/3	0/1	0/1	0/32
		Total	0/16	0/17	1/20	0/5	1/6	0/5	2/6	4/75
12 February 1962	Colombia, Chocó, Nuqui	Male	1/3	0/5	2/5	3/10	0/2		3/6	9/31
		Female	0/2	0/2	0/2	1/2	0/2	0/1	0/2	1/13
		Total	1/5	0/7	2/7	4/12	0/4	0/1	3/8	10/44
14 February 1962	Colombia, Chocó, Pizarro	Male	0/12	0/6	0/2	0/3			2/3	2/26
		Female	2/14	1/7	0/1	0/5	3/6	1/1	0/1	7/35
		Total	2/26	1/13	0/3	0/8	3/6	1/1	2/4	9/61

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

Serological reactions in rhesus monkeys inoculated with the 17D strain of yellow fever virus:

This report contains some of the results of an investigation into the appearance and pattern of haemagglutination inhibiting (HI) antibodies in the serum of eight rhesus monkeys inoculated intracerebrally with the 17D strain of yellow fever virus during the testing of yellow fever vaccine. Ten days after inoculation all of the eight monkeys showed HI antibodies for yellow fever antigens prepared with 17D; six exhibited evidence of neutralizing (NT) antibodies for the French Neurotropic (FN) of yellow fever virus, but only two gave survival ratios of 6/6; and finally, only one showed complement fixing antibodies against yellow fever antigens prepared with FN virus. However, the sera of all eight monkeys taken on the 20th or 21st day showed complete protection in the neutralization tests and gave positive CF tests with yellow fever antigens.

HI antibodies for 17D antigen, in titers ranging from 6 to 9 on the twentieth to thirtieth days after inoculation, were still demonstrable four years later in titers 1 to 3. (In the HI test, 8 units of antigen were used and non-specific inhibitors in the sera were removed with kaolin. The sera were tested in serial two-fold dilution commencing at 1:10. The titer of a serum was taken as the highest dilution giving complete inhibition of hemagglutination and was recorded as the exponent of the power of 2, which multiplied by 5, gave the denominator of the dilution).

HI antibodies against antigens prepared with the FN and the JSS strains of yellow fever virus developed also in all monkeys and were still demonstrable four years later. The antibody titers for FN antigen and especially for JSS antigen were lower than those for the 17D antigens.

Heterologous HI antibodies against other Casals' Group B viruses developed in six of the monkeys: in five for Dengue 2 and Ilheus and St. Louis; and in one, for Ilheus and St. Louis. Antibodies for Ilheus and St. Louis were still demonstrable four years later in some of the monkeys. The titers of the heterologous antibodies were lower than those for 17D antigens.

CF antibodies for yellow fever developed in all monkeys; however, they were not demonstrated in the sera taken 204 or more days after inoculation. Two of the monkeys showed traces of CF antibody to Ilheus antigen on the twentieth day, but this appeared to be a transient phenomenon.

It is believed that the results were not influenced by any intercurrent arborvirus infection of the monkeys. The animals were kept outside the laboratory and although their cages were not mosquito-proof, there is no evidence of the natural existence in Bogota of any Group B arborvirus.

Neutralizing and haemagglutination-inhibiting antibodies to yellow fever 17 years after vaccination with 17D vaccine.

This report contains some of the results of a study on the duration of immunity confered by yellow fever vaccine. This study was carried out with the cooperation of Dr. Rubem Bahia Ribiero, from the Departamento Nacional de Endemias Rurais, Ministerio de Saude, Brazil.

Persons resident in Pouso Alegre, Brazil, a locality where yellow fever has never been recognized, were vaccinated with 17D yellow fever vaccine between 27 December, 1940, and 5 February, 1941. In the period from 23 to 30 September 1958, specimens of blood were collected from 108 individuals whose names were found in the bound vaccination registration books that were still available from the 1940-1941 studies, during which a total of 5,275 persons were vaccinated. The age and sex of the donors of blood in 1958 were carefully checked against the entries in the books. In addition, bloods were taken from 78 persons--presumably unvaccinated--from 7 to 16 years of age, born after the 1941 vaccinations were finished. No yellow fever vaccinations were done in the Pouso Alegre area between 1941 and the time the 1958 specimens were taken.

Among the 108 vaccinated persons, only 3, or 3 per cent, gave negative results in neutralization tests for yellow fever, while 82, or 76 per cent, gave strongly positive results and 23, or 21 per cent, gave "inconclusive" results which we have interpreted as weak positives. It is the usual practice to consider sera with survival ratios (SR's) of 2/6, 3/6, 4/6, 3/8, 4/8, and 5/8 to be "not positive"; we have interpreted them as weak positives because we consider them to be not negative. As to details: of the 23 weak positives, 6 gave SR's of 2/6-3/8; 2 gave SR's of 4/8; and 15 SR's of 4/6-5/8.

Among the 78 persons born after 1941, only one showed neutralizing antibodies for yellow fever. Inquiry revealed that this person had been vaccinated against some disease, possibly yellow fever, in Itajuba, a few years previously.

In the group of the vaccinated individuals, there is no suggestion of increasing positivity with increasing age. This is additional evidence of the absence from the area not only of yellow fever virus but of any other virus that stimulated the presence of yellow fever neutralizing antibody.

The sera were also submitted to neutralization tests with Dengue-2, Ilheus, St. Louis, and Bussuquara viruses. These tests showed that 6 persons in the vaccinated group and 2 in the unvaccinated group gave positive results with one or more of the four aforementioned agents. Again, no increasing positivity with increasing age was observed.

The majority of the sera were tested also in hemagglutination inhibition (HI) tests. In the group of unvaccinated persons, of 65 sera tested by HI (and with no demonstrable neutralizing antibodies for Group B viruses) only one showed HI antibodies against 17D and St. Louis antigens. This serum, having a titer of only 1:10 with both antigens, gave negative results with Ilheus, Dengue-2, and yellow fever (French Neurotropic) antigens. In the group of vaccinated individuals, of 60 sera tested by HI (and with no demonstrable neutralizing antibodies for Dengue-2, Ilheus, and St. Louis) 30, or 50 per cent, gave positive results with a 17D antigen. In the same group, the proportions of sera giving positive HI results with other yellow fever antigens were lower; 39 per cent with an antigen prepared with French Neurotropic Strain and 38 per cent with an antigen prepared with the JSS Strain. The titers for the 17D antigen were also higher than those for the other two.

REPORT FROM DR. ANDRIES H. JONKERS
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Continued and intensified activities on Bush Bush Island, the field station of this laboratory, have led to the following results:

1) VEE virus and agents of group C and the Guama group were isolated throughout the dry season of 1962 (Jan-July). This has been the first year in which this was achieved.

2) Twenty-four of the 29 isolations were from Culex sp. #9, a not yet further identified Culex which was, however, not the most abundant species around but was the one receiving most attention as a result of observations during the preceding rainy season.

A program of trapping, marking, and releasing of small Bush Bush mammals is underway and gives promise of interesting results.

Some Ornithodoros capensis ticks taken from Soldado Rock, a sea bird nesting place off the southwest point of Trinidad, have yielded a filtrable agent which is presently still unidentified. This isolation is the first from ticks in this laboratory.

The agent isolated from Gigantolaelaps mites combed from Oryzomys rats (TRVL 40233) has been shown to be related to vesicular stomatitis virus (Indiana). Intensive study of this agent's epidemiology is presently underway. Antibody surveys in Bush Bush rodents indicate that antibody rates in these animals increase sharply about two months after the beginning of the rainy season. At that time, antibody rates to VEE have already been going up for two months. Laboratory studies with colonized Oryzomys and Zygodontomys rats indicate that viremia in these species is of very low level and less than 24 hours duration if inoculated subcutaneously with TRVL 40233.

REPORT FROM DR. OTTIS R. CAUSEY
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Virus Isolations, 1962:

Virus isolation from sentinel mice in the IAN forest study area during the first seven months of 1962 indicates that a reduction in transmission rates has occurred, especially for the Group C and group Guama types which were prevalent in 1961. While 104 or 21% of 490 mouse groups exposed January through July 1961 became infected with 13 different arbovirus types, only 24 or 7% of 326 groups became infected with 9 types in an equivalent interval and under the same conditions in 1962. Thus, although only one-fourth as many infections were encountered in sentinel mice in 1962 as in 1961, they represent more than two thirds of the types found in 1961. Of group C, Caraparu, Marituba, and Itaqui are present; in group Guama, both Guama and Catu types have occurred, but a number of the isolations are still untyped. The Capim group is represented by all three of the known types, and the ungrouped virus AN27639, which was first found in sentinel mice in February 1961, appeared again in March 1962. Noteworthy for its absence since November 1961 is VEE. In addition to these 9 viruses isolated from sentinel mice so far this year, eleven other arbovirus types were isolated from other sources. Eight of these eleven

(including two viruses new to the Belem area, AR39377 and AR40578) were from arthropod pools, and 6 of the 8 were picked up only from arthropods during this period. Pixuna, yellow fever, Wyeomyia and AR40578 were from mosquitoes; two isolates of AN28873 were from Phlebotomus, and AR39377, new to Belem, was found from mites. Four of 8 types isolated from wild animals likewise were obtained only from this source during this period, including the two new viruses from lizards (AN40290 and AN42217), and two viruses first isolated from rodents in 1960 and 1961, (AN24262 and AN27326). The isolates from man were Caraparu, obtained from an agricultural worker at the Japanese colony on the Guama River, and Marituba from a visiting mosquito catcher in the IAN forest. Apeu was isolated only from a sentinel Cebus. (Table 1)

Wild Animal Recaptures in Utinga Forest:

In June the long-planned wild animal recapture program was inaugurated in the Utinga Forest about 3 kilometers distant from the Ian study area. The plot selected for study is in forest virtually surrounded by swamps and streams. The 18 traps are set in grid formation, covering a square area 500 x 500 meters. Three parallel rows, 200 meters apart, contain 3 traps each 200 meters distant from the other. Another 3 rows perpendicular to these but offset 100 meters and also at 200 meter intervals, complete the grid. Maximum distance between any two adjacent traps is 200 meters and minimum distance is about 150 meters. The traps are numbered in the order in which visited so that numbers 1 and 18 are neighbors.

During the first 50 days, 61 rodents and 39 marsupials were captured, bled, measured, marked, and released, at the site of capture. During this period nearly two-thirds of the rodents and more than one-half the marsupials were recaptured one or more times for a total of 308 captures. Two Proechimys were captured 11 times and two Oryzomys 10 times each. One Didelphis was captured 9 times. At the first capture sufficient blood is obtained for virus isolation and antibody studies. On recaptures at short intervals, a drop of blood is taken from the tail into bovine albumin diluent for virus isolation in infant mice, and larger quantities are obtained only at about 20-day intervals for serological testing. A group Guama virus was isolated from one Proechimys and Caraparu was isolated from an Oryzomys. Both animals were recaptured at the original site and convalescent serum obtained (Table 2).

The data on recapture of the three rodent species found in this area indicate a greater than anticipated movement. One Oryzomys travelled at least 600 meters between traps and 4 Proechimys travelled 300 to 400

meters between traps. Nectomys was found only in one corner of the plot, indicating that the chosen area was largely outside the home range of the species. In general, the territory seems to be divided between the other two species, roughly 40% with Oryzomys and 60% with Proechimys.

The marsupials appear to wander more widely and show less predilection by species for any particular area in the outlined territory.

Table I

ARBORVIRUS INFECTIONS DURING PERIOD JANUARY THROUGH JULY, 1962

IMMUNOLOGICAL			NUMBER INFECTIONS SOURCE				
GROUP	TYPE	TOTAL	HUMAN	WILD ANIMAL	SENTINEL CEBUS	SENTINEL MOUSE	ARTHROPOD
A	Pixuna	1					1
B	Yellow fever	1					1
C	Apeú	1			1		
	Caraparú	7	1	1		5	
	Itaqui	1				1	
	Marituba	3	1		1	1	
Guama	Guama	1				1	
	Catu	1				1	
	Untyped	13		1	1	8	3
Capim	Capim	7		1		1	5
	Guajará	3				3	
	AN20076	2				2	
Bunyamwera	Wyeomyia	2					2
Ungrouped	AN24262	1		1			
	AN27326	1		1			
	AN27639	1				1	
	AN28873	2					2
	AN39377	1					1
	AN40290	1		1			
	AN40578	1					1
	AN42217	1		1			
Unidentified		8		8			
TOTAL		60	2	15	3	24	16

Table II

Animal	Total No.	NUMBER TIMES CAPTURED 1	2	3	4	5	6	7	8	9	10	11	Total Captures	Number Animals Recaptured	% Recaptured	Virus	Death in Lab
Proechimys guyannensis	45	15	5	5	6	3	3	3	2	1		2	165	30	66	1	10
Oryzomys goeldi	14	7	1	3		1					2		43	7	50	1	1
Nectomys aquaticus amazonicus	2		1	1									5	2	100	0	
Total Rodents	61	22	7	9	6	4	3	3	2	1	2	2	213	39	64	2	11
Didelphis marsupialis	22	10	2	3	3	2		1		1			61	12	54.5	0	0
Marmosa murina murina	6	2	2	1	1								13	4	33	0	1
Metachirus nudicaudatus	5	2	2			1							11	3	75	0	2
Metachirops opossum	3	2			1								6	1	33	0	2
Calluromys	3	2	1										4	1	25	0	1
Total Marsupials	39	18	7	4	5	3		1		1			95	21	54	0	6
Total Animals	100	40	14	13	11	7	3	4	2	2	2	2	308	60	60	2	17

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

The study of arboviruses in Sao Paulo is being done as it was outlined in Information Exchange No. 4. There is a definite virus transmission season here from September through April, when the temperature is high and there is a heavy rainfall. From May till August, there is a drop in temperature and it is dry. So our intensive period for field work is during the hot wet season, slowing down in the cold dry months.

From Station A, Boracea, we received 102 mammals and 111 families of sentinel mice were exposed.

From Bertioga, Station B, we captured 12 mammals and exposed 141 families of sentinel mice.

In Station C, Cotia, 162 mammals were trapped and 137 families of sentinels exposed.

From the 3 field stations, 109 pools of mosquitoes were inoculated in mice. They were captured using human bait. All mammals were bled to death. The blood was used for serological work and a pool of liver, kidney, and spleen was inoculated in mice for virus isolations.

From the trapped mammals, 7 agents were isolated. One of them, An 476, is a group C virus, close to Caraparu. The others are being identified.

Six agents were isolated from the sentinel mice exposed. From these, An 934 and An 936 are being considered as strains of An 232, the virus isolated last season. As it was described in our report for the number 4 issue of the Information Exchange, 40 strains of a virus was isolated from sentinel mice exposed in Cotia from February till April. This season, surprisingly, only two strains were isolated, one in March and the other in April. From the other agents isolated, one, An 741, has shown some slight cross reactions with Marituba and Oriboca.

From the pools of mosquitoes, 6 agents were recovered and they are being identified.

Serology:

Human: Sixty-five sera were obtained from people living around Cotia. Using HI tests, we found no antibodies for the A group (Mayaro,

EE, WE, VE, Aura, Una). With the viruses of group B (SLE, YF, Ilheus, Bussuquara, Powassan), there are about 58% of sera inhibiting them. Some of the reactions were those observed in double infections and others looked like cross reactions. Some people more than 30 years old showed specific reactions with YF. They could have been vaccinated or had the disease in the past. For the C and Bunyamwera groups only two persons reacted with Oriboca, Marituba, and Cache Valley. They have lived there for life.

Animals: From the trapped mammals, 122 were tested for antibodies. For the A group, only one reacted with Una and another with WE. For the B group, few (10/122) were able to inhibit hemagglutination. The reactions of C and Bunyamwera groups with the antigens used above were negative.

For the next season, the field stations of Bertioga and Boracea will be moved to places more easily reached. Boracea will be changed to the "Alto da Serra" in the same chain of mountains but only 25 miles from the laboratory. Bertioga will be moved to "Iporanga", a beach nearer Sao Paulo, served by a good, paved road. These changes will be done to save time for field work.

REPORT FROM DR. MARIANO DUNAYEVICH
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Multiplication of Junin Virus in Adult Mice.

The multiplication of Junin virus in adult mice was studied, in order to clarify some epidemiological aspects of the field mouse population in the affected area, which shows high titer of neutralizing antibodies (Hesperony laucha laucha).

The virus multiplies in the adult mice brain, reaching a titer of 10^4 at the fourth day, titrated in suckling mice, being only pathogenic for about 10% of the inoculated animals. The serial passage of the virus

obtained from sick animals, didn't increase the pathogenicity for adult mice, and continue maintaining the erratic proportion of sick animals.

Adult mice inoculated by intracerebral route with the 25th passage in suckling mice did not circulate the virus, and did not produce antibodies detectable by neutralization.

The titers obtained in cortisone treated animals with 0.3 mg of dexametasone, in three daily injections, did not show any increase, compared with the control, in a seven-day period of study.

The interferon production by the adult mice brain, was not demonstrated in mice challenged with EEE virus.