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

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REPORT OF THE SUBCOMMITTEE ON INFORMATION EXCHANGE

The Arthropod-borne Virus Catalogue:

In the preceding number of the Information Exchange, the general scheme and progress in revision and expansion of the Catalogue to global coverage and the proposed associated current information and abstract service were described. In the latter part of February, the revised Catalogue, containing registration cards of 110 viruses, was distributed. There remains a dozen or more known viruses to be registered, notably of members of the tick-borne encephalitis or RSS complex. When registrations of these missing viruses are received, registration cards will be prepared and distributed for insertion in the Catalogue. Accompanying this issue of the Catalogue are coded abstracts on arthropod-borne viruses with appropriate index cards, selected from Biological Abstracts covering the period January 1, 1959, to December 15, 1961.

In the next issue of the Information Exchange, before which time it is anticipated that all of the known arthropod-borne viruses will have been registered, it is proposed to give a statistical summary of salient information obtainable from the Catalogue.

Since the present number of Catalogues is limited to 100, the Catalogue and accompanying abstract and current information service are to be supplied only to laboratories and institutes actively engaged in research involving isolation, classification, or natural behavioral characteristics of the arboviruses.

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 purpose of general reference in publications is 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.

Subcommittee on Information Exchange

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

REPORT FROM THE AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

An American Committee on Arthropod-borne Viruses (ACAV), which evolved from the original Gould House Group, first convened by The Rockefeller Foundation in October 1959, has continued to support, develop, and enlarge its activities for investigators of arthropod-borne viruses. An executive council for the ACAV was appointed by a special committee of the Gould House Group at its last meeting in Atlanta on April 9, 1961. It consists of William C. Reeves, Chairman, Telford H. Work, Secretary, Wilbur G. Downs, William F. Scherer, Alexis Shelokov, and Richard M. Taylor.

The administrative group of the ACAV has held two additional meetings in the past year. Copies of the minutes of either meeting can be obtained on specific request to Dr. T. H. Work, Secretary to the ACAV, Communicable Disease Center, Atlanta 22, Georgia. The first meeting was held on October 31, 1961, a day prior to the annual general meeting of the ACAV on November 1, to receive reports of the Subcommittee on Serologic Reagents and Information Exchange, and to consider other matters for presentation and hearing at the annual general meeting. The second meeting was held on March 7, 1962. Activities, reports, and discussions can be summarized as follows.

The annual general meeting of the American Committee on Arthropod-borne Viruses was held from 9-12 am, November 1, 1961, at the Willard Hotel in Washington, D. C. as part of the annual meeting of the American Society of Tropical Medicine and Hygiene (ASTMH). The meeting, open to all actively interested or engaged in research on arthropod-borne viruses, was attended by 76 registrants. The idea of loose affiliation with the ASTMH, at least in regard to holding annual general meetings, was approved by the assemblage as were the general principles guiding operation of the ACAV as outlined by Chairman Reeves.

The American Committee on Arthropod-borne Viruses, as a free association of investigators of arthropod-borne viruses, functions not as parent to permanently established responsibilities and activities, but rather to stimulate, initiate, and develop worthwhile and essential scientific activities of pertinence to the arborvirus field. Once operating and of recognized value, the ACAV intends to hand these activities over to the appropriate agency for continued operation.

Recognizing a need for a more coordinated approach in the study of birds in the ecology of arthropod-borne viruses, the ACAV proposed an

exploratory meeting on this subject. Dr. T.H. Work, as an ACAV committee member, was designated as convenor of the meeting which was held February 16-17, 1962, at the Communicable Disease Center in Atlanta, Georgia. It resulted in formation of a new subcommittee whose actual title has not yet been settled. It consists of Donald D. Stamm, Chairman, Joseph J. Hickey (University of Wisconsin), Robert J. Newman (Louisiana State University), David E. Davis (Pennsylvania State University), and Maurice W. Provost (Florida Board of Health Entomological Research Station). A summary of the meeting proceedings is in preparation and can be obtained on request from the Chairman, Dr. D.D. Stamm, Virus Ecology Laboratory, Communicable Disease Center, Atlanta, Georgia.

Dr. R.M. Taylor, Chairman of the Subcommittee for Information Exchange, reported that with distribution of the fourth issue of the Information Exchange in November, international participation had been successfully implemented in accordance with recommendation of the 1960 WHO Arbovirus Study Group Report. The fifth issue of March 1962, of which this report is a part, will go to a somewhat larger distribution as designated in the list of participants and recipients which will accompany this issue. This was suggested from the floor at the last annual general meeting of the ACAV.

The catalogue of Arthropod-borne Viruses was reissued at the end of January 1962. Still incomplete, it now contains 110 entries with another 20 in production, expected to be distributed in a couple of months. It should reach 150 entries within the next year. The cost of the catalogue limits its distribution to one to an institution or geographical locality where it will be accessible for study by interested investigators. In order to keep information currently up to date on viruses registered in the catalogue, an abstract reproduction and distribution service has been devised which will operate in conjunction with the catalogue. These abstracts will be drawn from Biological Abstracts and other published sources and will be issued about every two months.

With revised issue of the arborvirus catalogue and initiation of an abstract service to keep registrations current, it becomes of timely importance to develop a mechanism for catalogue review, particularly in regard to nomenclature and classification. Because of its international importance, this requires a review mechanism developed from an international rather than American origin.

Dr. Jordi Casals, Chairman of the Serologic Reagents Subcommittee, reported on meetings held in November and March, with technical communications exchanged in the interim, considering methods for producing group screening and ungrouped specific arborvirus reference antisera. A meeting of special consultants to the NIAID Reagents Program met February 20 at The Rockefeller Foundation Laboratories in New York to outline protocols and specifications for contractors interested in undertaking production of arborvirus reference reagents. These two groups are essentially the same and are to be drawn from in the formation of an Arborvirus Panel for NIH which is expected to be organized in the immediate future. It is expected that the ACAV Serologic Reagents Subcommittee function will dissolve into activity of the new panel with similar objectives at that time.

The problem of rotation of membership of the executive council of the ACAV was considered at length. An outside nominating committee of the three, under chairmanship of W. M. Hammon, was set up to recommend a formal system of rotation and for nomination of new members at the next annual general meeting of the ACAV. A staggered order of retirement of the present executive council has already been established.

The next annual general meeting of the ACAV will be held 9-12 am, Wednesday, October 31, 1962, at the Biltmore Hotel, Atlanta, Georgia, in association with the annual meeting of the ASTMH. All investigators and others with particular interest in and activity with arthropod-borne viruses are urged to attend. They need not be members of the ASTMH to participate in these meetings.

William C. Reeves, Chairman
Telford H. Work, Secretary
Executive Council, American
Committee on Arthropod-borne Viruses

AFRICAN VIRUS - British Colonial Office, London, reports more than one million Africans in Uganda and Kenya are afflicted by a new virus disease called O'nyong-nyong or "the joint-breaker," characterized by high fever, crippling joint pains, itching rash, and swollen glands. No deaths reported but pathologists fear new and more severe forms of malady may occur. Virus is carried by 2 species of mosquitoes--Anopheles funestus and Anopheles gambiae. Investigators believe disease may be latent among forest animals, possibly monkeys, and has only recently begun to afflict man. (NYT 1-28)

Announcement of Symposium on "Immunization Against Arbor Virus Infections"

A symposium on "Immunization Against Arbor Virus Infections" is being planned for the annual meeting of the American Society of Tropical Medicine in Atlanta, Georgia, on Friday afternoon, November 2, 1962. The symposium will welcome presentations dealing with recent developments regarding attenuated strains of dengue, Japanese, West Nile, Kyasanur and Venezuelan viruses including their efficacy and safety in animals and man. In addition, information bearing on the broad immunologic coverage within a given Casals group which may be obtained by sequential inoculation of selected killed or living viral vaccines, will be considered for inclusion. New and pertinent data on inactivated vaccines against members of the arbor group may be worthy of inclusion. Suggested contributions should be directed to Dr. Joseph Smadel, Division of Biologics Standards, National Institutes of Health, Bethesda 14, Maryland, before June 1, 1962.

Report from American Type Culture Collection, Viral and Rickettsial Registry and Distribution Center, Second Edition 1959, and Supplement 1961

Arthropod-borne Viruses	Strain	ATCC Designation
Group A:		
Chikungunya	S27	D
Eastern equine encephalitis	Massachusetts	D
Mayaro	TR4675	D
Semliki Forest	Original strain	
Sindbis	Ar-334	
Venezuelan equine encephalitis	Donkey No. 1	Spec. Pmt.
Western equine encephalitis	California	D
Group B:		
Dengue Type 1	Hawaii (mouse adapted)	PHS
2	New Guinea C.	PHS
3	H87	PHS
4	H241	PHS

Arthropod-borne Viruses	Strain	ATCC Designation
Group B: (Continued)		
Ilheus	Original strain	
Japanese encephalitis	Nakayama	PHS
Kyasanur Forest	P9605	Spec. Pmt.
Louping ill	1930/1 (England)	Spec. Pmt.
Murray Valley encephalitis	No. 1	PHS
Ntaya	Original strain	
Powassan	Byers	Spec. Pmt.
Russian spring-summer encephalitis	Moscow, B-4	PHS
St. Louis encephalitis	Hubbard	D
Uganda S	Original strain	
West Nile	B956	PHS
Yellow Fever	17D	D
Zika	Original strain (MR 766)	D
Unclassified:		
Anopheles A	Original	
Anopheles B	Original	
Bunyamwera	Original	D
Bwamba Fever	M459	D
California encephalitis	BFS-283	
Colorado tick fever	Florio (N-7180)	D
Sandfly fever - Naples		
Sandfly fever - Sicilian		
Wyeomyia	Original	

Key
D = Dangerous; PHS = Released with approval of United States Public Health Service; Spec. Pmt. = Special Permit, released only after ATCC receives written permit from United States Public Health Service or Department of Agriculture.

REPORT FROM DR. MAX THEILER, DIRECTOR ROCKEFELLER FOUNDATION VIRUS LABORATORIES NEW YORK, N. Y.

The work of the RFVL in New York forms an integral part of the arthropod-borne virus program of The Rockefeller Foundation. The work in New York is primarily concerned with the more theoretical aspects, whereas the field stations are more immediately concerned with the variety, prevalence, and epidemiology of the viruses occurring in their regions. In the 1961 annual report will appear sections dealing with biophysical, biochemical, entomological, tissue culture, and immunological studies. Here it is planned to summarize only certain sections of the report.

One of the main problems of these laboratories is the question of virus classification. This has been summarized in a series of tables which afford an up-to-date summary of our knowledge of the variety and distribution of the various agents proven or suspected of being arthropod-borne.

The method of classification adopted depends entirely on immunological studies. To date, seventeen immunological groups or complexes are recognized. It must be emphasized, however, that there is good evidence that some of these groups may be related to each other. If these findings are substantiated, then several of our groups will become merged, thus reducing the present number. It is quite clear that our knowledge, at present, is quite inadequate to set up a definitive virus classification.

In the tables are listed 143 distinct agents. Of these, 103 are members of groups and 40 are at present still ungrouped. It is quite possible that some of the latter may, on further study, prove not to be arthropod-borne. If to these are added the distinct immunological types belonging to the two groups, African horse-sickness and blue-tongue of sheep, the total number of arbor viruses (proven and suspected) is approximately 160.

The results of studies during the year have added several new arbor viruses. In group A, an agent, AR 35645, from Belem has been shown to be different from all other members of the group. Three agents, all from Australia, are apparently new group B viruses. The Negishi virus from Japan has been shown to be a new member of the Russian tickborne complex. A seventh member has been added to group C. Two new Bunyamwera group agents, one from Africa and the

other from Belem, are described. The Tahyna virus, isolated from mosquitoes in Czechoslovakia, has been shown to belong to the California complex. Several new viruses, as yet ungrouped, have been added to our lists. These originated in Japan, Australia, and Belem.

The geographic distribution of other agents has been extended. The Tshalova virus from Czechoslovakia has been shown to be a strain of Chittoor, previously known only in Malaya and India. Several isolates from Colombia have been shown to be strains of Una. Several strains of Argentinian equine encephalitis have been shown to be strains of EEE. BE AN 32260 from Belem appears to be identical with the Turlock virus of California. BE AN 35112 was found to be identical with the Lukuni virus previously known only from Trinidad. The Chuku virus, isolated from man in Nigeria by Macnamara and described by him as a strain of Zika (Trans. Roy. Soc. Trop. Med. & Hyg., 1954, 48, 139), has been proven to be identical with the Spondweni virus. This immunological identity was established by HI, CF, NT, and antibody absorption studies.

The greatest aid in virus classification is undoubtedly the development of hemagglutinins and hemagglutination inhibition tests. To date, hemagglutinins have been obtained from 96 distinct agents. Of equal importance and of more universal use is the complement fixation test. By the use of these two tests, an agent can usually be identified with speed and accuracy.

An analysis of the distribution of the 143 listed agents reveals that the largest number have been isolated in South America (58), followed by the Ethiopian region (35) and the Oriental region (28). These are largely tropical areas. From the vast Palearctic region, only 20 agents have been described. We have records of 13 agents from North America and 9 from the Australasian region. In this analysis, the remarkable fact emerges that of the 143 distinct agents, only 14 are common between any two or more biogeographical regions. The distribution of some of these, such as yellow fever, which occurs in Africa and South America, and two types of dengue, which have a wide distribution, is probably due to transportation of these infections and their common vector, Aedes aegypti, by man. This marked regional distribution is of prime importance in establishing regional diagnostic centers. Only agents which have been shown to occur in the particular zoogeographical region need to be considered. Group immune sera can be readily prepared in mice. Such sera can be used in HI, CF, and neutralization tests.

In the study of strains of the same virus isolated in different zoogeographical regions, it has been found that there are clearly marked differences depending on the region of isolation. The strains of Chikungunya isolated in Bangkok appear to be identical with each other, but differ from the strains isolated in Africa. Sindbis virus has a very wide distribution, having been isolated in Australia, the Philippine Islands, Malaya, India, and Africa. A study of these revealed that these strains could be divided into three varieties depending on the three regions where they were isolated viz., Australasia, Oriental region, and Africa. In a similar manner, strains of EEE isolated in South America clearly differ from North American strains. These findings afford clear evidence that the exchange of viruses between two distinct zoogeographical regions must be a rare event, unless it be assumed that a very rapid immunological change of the virus occurs when it is introduced into a new region. The arthropod-borne viruses are exceedingly stable immunologically, and it is for this reason that our classification is an immunological one. Strains of the same virus occurring in two distinct zoogeographical regions, must have found suitable ecological conditions in both regions. This implies that in both suitable vertebrate hosts and vectors are present. These are not likely to be the same in two distinct regions. Laboratory passage in different vertebrate hosts or tissue cultures, though leading to marked changes in pathogenicity, produce at most very minor immunological changes. So far we are not aware of any studies in which immunological changes have been induced in a virus by prolonged transmission by two distinct arthropod-vectors.

Some observations made in the study of the tick-borne complex may be pertinent. Extensive studies with numerous strains have shown that this complex could be divided into distinct types. Here we are concerned only with two of these - the Far Eastern RSSE and the Central European virus. The normal vectors of these are Ixodes persulcatus for the Far Eastern RSSE and Ixodes ricinus for the Central European virus. In a recent study of seven isolates from the Leningrad area, five were shown to be Central European strains and two Far Eastern RSSE strains. Both of the latter had been isolated from Ixodes ricinus. In the Leningrad area, the distribution of these two species of ticks overlap. Here we, thus, have an example of an identical virus being transmitted by two distinct species of ticks.

REPORT FROM DR. A. C. SAENZ, MEDICAL OFFICER, VIRUS DISEASES, DIVISION OF COMMUNICABLE DISEASES WORLD HEALTH ORGANIZATION, GENEVA, SWITZERLAND

WHO Arthropod-borne Virus Program

Establishment of a network of Arthropod-borne Virus Regional Reference Laboratories

Following the recommendations of the Study Group on Arthropod-borne Viruses which met in Geneva in September 1960, a network of Arthropod-borne Virus Regional Reference Laboratories was established during 1961 and is now functioning. The seven laboratories listed below have been designated in this capacity:

Africa	Virus Research Institute East African High Commission P. O. Box 49 Entebbe Uganda
The Americas	Virus and Rickettsia Section Communicable Disease Center U. S. Public Health Service Atlanta 22, Georgia
Australasia	Department of Microbiology The John Curtin School of Medical Research Australian National University Canberra, Australia
Central Europe	Institute of Virology Czechoslovak Academy of Sciences Mlynska dolina Bratislava 9, Czechoslovakia
USSR	Virus Encephalitis Section Institute for Poliomyelitis and Virus Encephalitis USSR Academy of Medical Sciences Kievokoce Chaussee Moscow B-27, USSR

Western Europe	Medical Research Council National Institute for Medical Research The Ridgeway, Mill Hill London, N. W. 7 UK
Western Pacific (excluding Australasia)	Department of Virology and Rickettsiology National Institute of Health 284 Kamiosaki-chojamaru Shinagawa-ku Tokyo, Japan

Their main functions may be outlined as follows:

1. Identification and study of virus strains isolated in the areas covered by the laboratories.

2. Maintenance of prototype strains isolated in the areas of coverage and making them available to other laboratories collaborating in the scheme.

3. Preparation and/or control of reference sera from viruses isolated in their areas of coverage, and distribution of small amounts of them to collaborating laboratories.

4. Training of virologists in this field according to facilities available.

5. Consultant advice to national or field laboratories.

6. Assistance or advice when epidemics occur.

7. Collection and transmission of epidemiological information to WHO.

8. Collection and circulation of technical information among collaborating laboratories.

Efforts will be made to insure the appropriate cooperation and coordination in the work of this system of Reference Laboratories through exchange of information, visits between the personnel of the different

laboratories, and the organization of meetings to discuss common problems and reach agreement on the use of laboratory techniques.

Birds as disseminators of arthropod-borne viruses

Assistance has been given to the Bombay Natural History Society to carry out bird migration studies in the Kutch area of the Saurashtra peninsula where a great number of birds from the migration current N.E. to S.E., or vice versa, come to rest. (Principal investigator - Dr. Salim Ali) Large numbers of birds have been caught, identified, and ringed in three successive migrating seasons. Parasite ticks have been collected and samples of organs, tissues, and blood have been obtained for virological studies. The Virus Research Centre in Poona has collaborated in these studies.

Similar investigations were carried out during the spring migration session 1961 in the southern part of Spain by a team of British investigators under the technical direction of Dr. C.E. Gordon Smith of the London School of Hygiene and Tropical Medicine.

Yellow Fever in Ethiopia

An Informal Meeting of Advisers on Yellow Fever Research was held in Geneva from 25-27 October 1961 to discuss the plan for an inquiry into the epidemiology of yellow fever in Ethiopia. Seven experts attended the meeting. Investigations are being carried out at the present moment with the support of WHO.

JOINT REPORT FROM GML-MARU (GORGAS MEMORIAL LABORATORY, PANAMA, AND MIDDLE AMERICA RESEARCH UNIT, CANAL ZONE)

Dr. Pauline Peralta, Dr. Margaret Grayson, and Dr. Pedro Galindo

This first newsletter report to be prepared jointly by these two laboratories relates primarily, but not exclusively, to long-term investigations of the arthropod-borne viruses of Panama. These are being conducted in Almirante, an area of tropical rain forest climate located in the province of Bocas del Toro, in extreme northwestern Panama. The report includes results of the first 27 months of observations from this area, as well as those from Canito and other areas pertinent to our studies on Venezuelan equine encephalitis.

Isolations from Bocas del Toro

A total of 147 viral agents was isolated at Gorgas Memorial Laboratory from the following sources: 13 from human sera; 47 from 178,418 mosquitoes and Phlebotomus sandflies; 17 from sera of rodents; 4 from blood samples of wild birds; 65 from sentinel mice; and 1 from a sentinel chicken.

In addition, 39 isolates were obtained from 180,755 mosquitoes and Phlebotomus sandflies processed at the Middle America Research Unit.

Although many of the isolates await identification, some progress in the characterization of these agents has been made. Those which have been identified are closely related to or identical with the following arthropod-borne viral agents: Venezuelan equine encephalitis (humans, rodents, mosquitoes, and sentinel animals); Ilheus encephalitis (birds and mosquitoes); Apeu (human); vesicular stomatitis (Phlebotomus sandflies and mosquitoes) and Una, Guama, Wyeomyia, and Guaroa (mosquitoes).

Some epidemiological work has been done on vesicular stomatitis, and detailed investigations of the epidemiology of Ilheus encephalitis and Venezuelan equine encephalitis are still in progress.

Ilheus encephalitis

The sequence of Ilheus encephalitis virus isolations from mosquitoes and birds indicates that this virus has been active in the study area during the last two years.

Venezuelan equine encephalitis (VEE)

In April 1961, VEE virus was first isolated in the Isthmus from a human fatal case residing at Canito (located on the west side of Gatun Lake). As a result of this isolation and an outbreak of illness during June 1961 in Almirante confirmed as VEE, investigations were started concerning: 1) Epidemiology of VEE in Canito, 2) Ecology of VEE in Almirante and 3) VEE vaccination with Berge's attenuated virus.

1. Epidemiology of VEE in Canito

Intensive studies in this area were conducted during May. Seven viral agents were isolated from 20,519 insects. While the identity

of these isolates has not been established, one resembles VEE virus as judged by its pathogenicity for mice. From 28 litters of sentinel mice and 200 one-day old chicks exposed in the field for 24-hour periods, no viruses were isolated. Fifteen per cent of 117 human serum specimens collected early in May were found to have HI and NT antibodies to VEE virus. No conversions could be demonstrated between May and July, but a few instances of a drop in CF titer over this period suggested that these were recent infections. Six of eight horses had serum CF antibodies to VEE.

2. Ecology of VEE in Almirante

The epidemiologic investigation of the outbreak in Almirante indicated that during May and June some 60 individuals had been admitted to the Almirante hospital or outpatient clinic with an acute febrile illness of unknown origin. The virus of VEE was recovered from six of 29 serum specimens obtained from febrile cases and from the blood of a mosquito collector who became severely ill on July 5, three days after his arrival in the area. Analysis of the case histories indicated that five of these acquired the infection in Tampico or Patoistown, slum sectors of Almirante bordered on three sides by mangrove and palm swamps. The 720 inhabitants of these two sectors lived under extremely poor sanitary conditions in 160 dwellings with no running water, privy facilities, or screening. The possible VEE morbidity in residents of these two sectors during the months of May and June is presented in Table I. The column of febrile cases represents the number of persons who reported during interview that they had experienced a febrile illness of sudden onset, accompanied by chills and severe headache, during these months. The case of encephalitis was hospitalized five days after onset of illness; blood obtained on the sixth day failed to yield any viral agent. The last column shows the VEE isolation made from residents of these sectors who were hospitalized or who reported sick to the outpatient clinic.

No illnesses or fatalities were reported in equines of the area before or during the period of the human epidemic. The temporal sequence of VEE virus isolations from April to November is shown in Figure 1.

Of 242 sera from wild vertebrates collected in the study area between March and September 1961, seven yielded VEE virus, six from the cotton rat, Sigmodon hispidus, and one from the spiny rat, Proechimys semispinosus.

Mouse inoculations at GML and MARU of all mosquitoes collected between April and November 1961 showed that 13 of 238 pools of three species of Culex yielded the virus, whereas 268 pools of other species of mosquitoes were negative. Seven VEE isolations were made from 2,353 C. taeniopus mosquitoes (51 pools), four from 5,719 C. vomerifer (89 pools), and two from 1,869 C. pipiens quinquefasciatus (98 pools).

The feeding habits of C. taeniopus and C. vomerifer, nocturnal species frequently found attacking humans, rodents and birds, incriminate these two species as the probable vectors of VEE virus in Almirante. The two isolates from C. pipiens quinquefasciatus were obtained from pools of over 100 mosquitoes each collected resting inside the houses of two of the residents of Patoistown who exhibited viremia. The vector efficiency of these species of Culex is being assayed.

Between January and November 1961, three litters of mice were exposed each week for one night at the study area field station two miles outside of Almirante. No VEE isolates were obtained between January and June, but between June and November, nine of the litters and 20 of the suckling mice became positive for VEE virus.. Diurnal exposures of the same weekly number of litters were carried out in the same area between August and November. A single suckling mouse yielded VEE virus in October.

From August to November, 27 groups of four chickens, four to five weeks of age, were exposed nocturnally in the same area. In October, the blood of one chicken yielded VEE virus.

It is not possible at this time to determine whether VEE virus had been active in the Almirante area before the present epidemic. However, results of immunological tests on the large number of human and animal sera collected before and during the epidemic should help to clarify this point. Present accumulated information indicates that VEE virus was absent from Almirante from September 1959 to April 1961.

The coincidence of both outbreaks with the passing of migrating birds through the area from the south and the temporal sequence of viral isolations tempt the speculation that the virus was first acquired, perhaps from viremic migratory birds, by C. vomerifer and C. taeniopus, two wild species of mosquitoes with wide host ranges inhabiting a variety of ecological niches. Infected mosquitoes, in turn, transmitted the virus to the dense and presumably highly susceptible population of cotton rats around Almirante. As the number of viremic wild rodents increased, a correspondingly greater proportion of the

vector populations became infected, resulting in the transmission of the virus to other susceptible vertebrate hosts, such as man, sentinel mice, and sentinel chickens.

3. VEE Vaccination with Berge's Attenuated Virus

Berge's attenuated VEE vaccine was given to 43 employees of the two laboratories between June and August 1961. Many of the vaccinees remained asymptomatic. Others experienced fever, myalgia, headache, and fatigue of mild to moderate severity. The onset of symptoms was from one to ten days after vaccination. Attenuated VEE virus was recovered from serum as early as the second day. It was recovered from the throat and serum of four persons who became ill six to ten days after vaccination and from the throat of one person at 16 days.

Most vaccinees showed at least some serological response at one month; in several, antibodies were no longer demonstrable at three months. The log neutralization index at one month varied from 0 to $\geq$ 2.5.

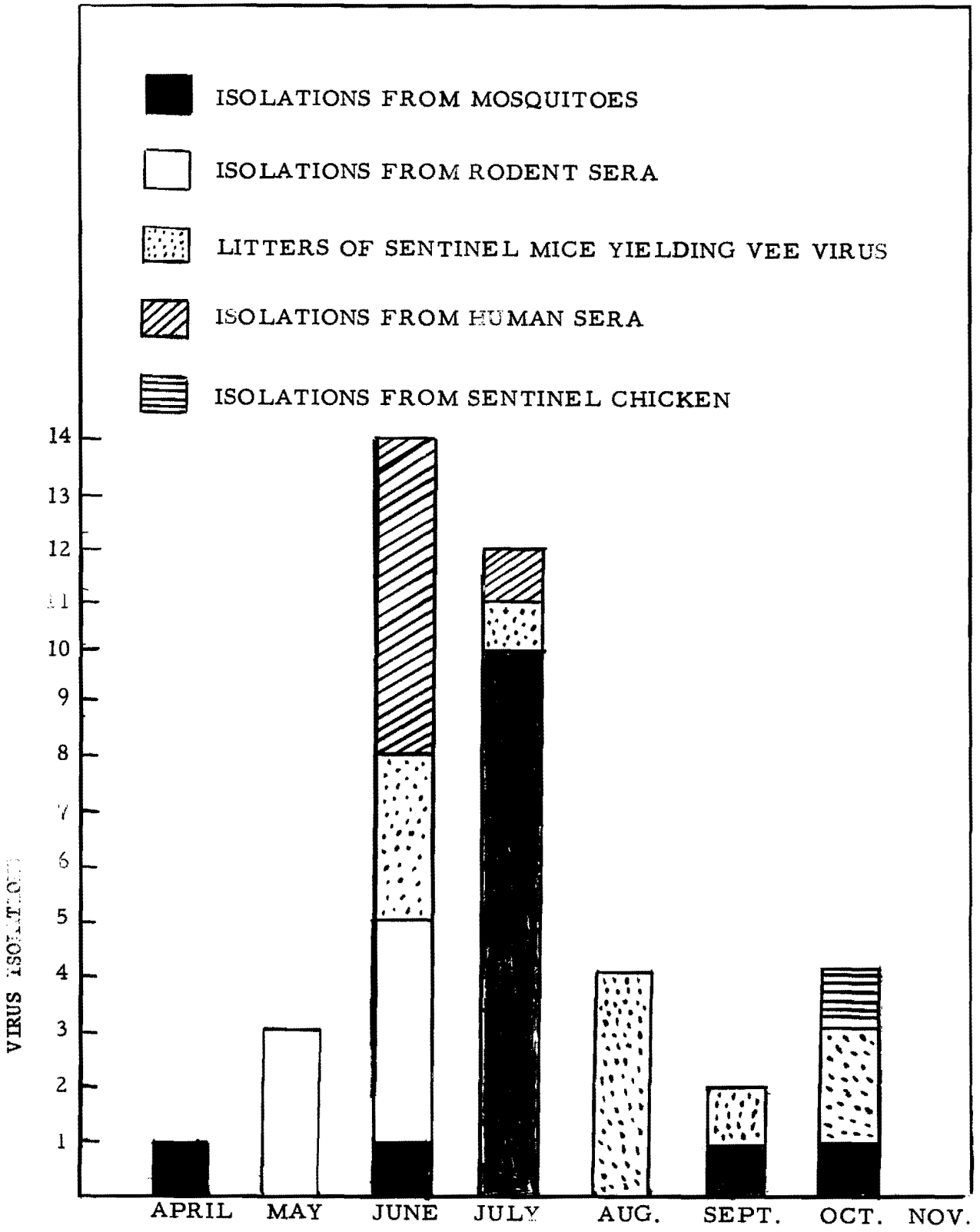
In November one of the June vaccinees developed a febrile illness several days after exposure to VEE virus. Non-attenuated virus was isolated from the throat and a sharp rise in CF, HI, and neutralizing antibodies followed.

Table I. Possible VEE Morbidity in Residents of Patoistown and Tampico (May 1 to July 1, 1961):

Age Groups	No. of Persons	Febrile cases (May-July)	Hospitalized	Encephalitis Cases	VEE Virus Isolations
0-6	185	19	6	0	0
7-12	149	17	5	1	1
13-20	93	1	1	0	1
21-40	142	10	2	0	2
40-on	149	10	2	0	0
Totals	718	57	16	1	4

Figure 1.

MONTHLY PROGRESS OF VEE ISOLATIONS IN ALL HOSTS



REPORT FROM DR. LESLIE SPENCE, TRINIDAD REGIONAL VIRUS LABORATORY, PORT-OF-SPAIN, TRINIDAD

During 1961 the field program was restricted to Bush Bush Forest in the Nariva swamp in eastern Trinidad and was greatly intensified. Table I summarizes the viruses encountered by source. Sentinel mice were exposed both under Causey hoods and in newly developed mosquito traps. These traps did not diminish infection of mice and increased greatly the number of mosquito isolations. Sentinel mice housed in traps became infected only during night hours.

Isolations from nocturnal mosquitoes far exceeded those of diurnal species. Evidence suggests that VEE and a group C virus (the Caraparu-like TRVL 34053-1 prototype) are most likely dependent upon different primary vectors.

Interesting and useful entomological data were collected during studies on bait preference, 24-hour catches at ground and forest canopy (55') levels and the use of another new trap designed for automatic instantaneous mosquito sampling.

Two new Trinidad virus prototypes were isolated, one from Zygodontomys and Oryzomys rats and one from Gigantolaelaps mites collected from Oryzomys rats. Both are ether sentitive. The mite agent has been parenterally inoculated into adult Culex quinquefasciatus mosquitoes and the salivary glands successfully passaged three times at weekly intervals. Infected mosquitoes have also transmitted the virus by engorging on susceptible mice.

Table I. Identification and source of arthropod-borne viruses isolated at TRVL during 1961 (One or more isolations of the same virus from a sentinel mouse family regarded as a single isolation.)

Immunological Group	Virus	No. of isolations	SOURCE			
			sentinel mouse	wild rodent	mosq.	mite
A	VEE	39	13	11	15	
B	Ilheus	7			7	
C	Probably Caraparu	26	13	8	5	
Bunyamwera	Cache Valley	1			1	
	Wyeomyia	1			1	
Guama	TRVL 33579	9	2	5	2	
	Not typed	19	5	12	2	
Ungrouped	TRVL 39316-1-5	2		2		
	TRVL 40233	1				1
Totals		105	33	38	33	1

REPORT FROM DR. A. L. BRICENO ROSSI
VIRUS SECTION, ARTHROPOD-BORNE VIRUS LABORATORY
INSTITUTO NACIONAL DE HIGIENE
CARACAS, VENEZUELA

Our activities during 1961 included the study of viscera, blood, and mosquitoes from wild areas, sent to us by the Yellow Fever Division, by field workers or in response to petitions made to particular regions where small epizootics of equine encephalitis had been reported. Of this latter type, we received mosquitoes in good condition from the State of Apure. Also several specimens of bats and mosquitoes were received in connection with work of the Entomology Section of this institute.

Continuing this work, the material was inoculated into suckling mice, to HeLa cell and chicken embryo cultures.

We have been preparing hyperimmune sera through inoculation of inactivated or attenuated and freeze-dried vaccines from Venezuelan equine encephalitis, eastern equine encephalitis, and western equine encephalitis viruses, followed by inoculations of further dilutions of live viruses.

Between April and July, studies were made of mosquitoes captured in the States of Cojedes and Guarico, and between August and October of new specimens of mosquitoes received from the States of Cojedes, Guarico, and Miranda. Samples of blood from 15 monkeys from wild regions were also studied during this period.

A cabin was built to facilitate these studies, and a room was arranged for the maintenance of inoculated animals under optimal conditions of protection. Special cages for field work were also set up.

In relation to the needs of breeding suckling mice for this work, we can report that the laboratory's animal colony has been expanded so as to make possible the inoculation of 100 families of white mice weekly.

Note: The inoculations were made in mice less than 24 hours old. The inoculated mice were observed each morning and afternoon for 21 days. In most specimens a first blind passage was made, giving the same results as the original specimen under the usual observation.

REPORT FROM DR. G. H. BERGOLD, HEAD, VIRUS DEPARTMENT INSTITUTO VENEZOLANO DE INVESTIGACIONES CIENTIFICAS APARTADO 1827, CARACAS, VENEZUELA

No major changes have occurred in the Virology Department except that Dr. Manfred Mussgay resigned at the end of his contract (January 31, 1962) to take over the Virology Section of the Hygiene Institute in Mainz, Germany.

Laboratory for Domestic Animal Viruses:

A. In cooperation with the Entomology Laboratory, it was found that vesicular stomatitis virus (VSV) multiplies and is transmitted by Aedes aegypti after ingestion or thoracical injection. This means that VSV should now be classified as an arbor virus, in accordance with Casals' definition.

B. It was found, in electron microscopic investigations that intact Newcastle disease virus (NDV) particles can be found in chicken embryo cells 30 minutes after inoculation. This indicates that morphological intact NDV particles are capable of entering cells, which is contrary to conclusions drawn by others. Assuming NDV disintegrates during the infectivity process, this occurs apparently in the cytoplasm, and not at the surface membrane of cells.

C. Electron microscopic investigations of the development of vesicular stomatitis virus (VSV) in KB-cells have revealed the presence of 6 morphologically different structures believed to be virus specific. On the basis of these results, a speculation of the development of VSV is suggested. Accordingly, electron transparent spherical particles with a diameter of about 65 mμ develop either into: 1) similar but slightly denser particles, and further into dense non-infectious spherical particles (70 mμ in diameter) which are probably incomplete virus showing complement fixation activity, or 2) via transparent spherical particles (100 mμ in diameter) with two membranes and further to similar, but dense particles which eventually grow into the typical infectious virus rods.

D. In cooperation with our Servicio Medico, the history of 4 human cases of laboratory infection with VEE was summarized and prepared for publication.

E. VEE virus particles were identified in suspensions by the use of Ferritin-conjugated VEE virus antibodies. Concentration and

purification with protamine sulphate, and separation of VEE virus particles by the use of sucrose density gradient centrifugation revealed that HA and CF activities are activities of the infectious virus particles. A non-infectious hemagglutinin was detected after centrifugation of virus through a gradient column with pH 2.7. This result is interpreted as a disintegration process of VEE virus particles.

Laboratory of Group B Arbor Viruses

A. Apart from the isolation of the IVIC 1 strain of yellow fever from an Alouatta seniculus seniculus, reported previously, another (IVIC 2) isolation has been made from the serum and liver of a fatal human case in Estado Tachira. This strain (IVIC 2) appears to be identical to IVIC 1 since it is completely neutralized by IVIC 1 mouse hyperimmune serum.

B. The electron microscopic investigation of the morphology of yellow fever virus was continued. Spherical particles with a diameter of about 35 mμ, having a well developed membrane, are believed to be the infectious yellow fever virus. This is based on the fact that particles very similar in dimension and structure were found in infected mouse brain, in mouse liver, in KB and HeLa cells, in chicken fibroblasts, in salivary glands of Aedes aegypti, and in suspension of virus particles prepared by ultracentrifugation and sucrose density gradient separation. Different size particles usually occur in only one of the above mentioned preparations. This finding is contrary to reports in the literature which describe particles with a 50-61 mμ and 25-27 mμ diameter, apparently without any internal structure.

C. An apparatus to facilitate the preparation of primary cell cultures, particularly chicken embryo cells, was developed and will be described shortly. This apparatus is time-saving (45 minutes total time of preparation) and decreases considerably the possibility of contamination which is of particular interest in laboratories operating under difficult conditions.

D. Following a suggestion of Lleras and Juliao (II Congreso Latinoamericano y I Nacional de Microbiologia, San Jose, December 10-17, 1961), it was found that some viruses like vesicular stomatitis virus (VSV) produce well defined plaques in chicken fibroblasts when all serum in Porterfield's growth medium and overlay is replaced by freshly prepared egg albumen. The possibility of using only egg albumen in place of serum is investigated with other viruses in respect to plaque formation.

E. Experiments to obtain readily reproducible plaques with yellow fever virus were continued using many different combinations of media and different conditions. However, in spite of the very generous assistance from Dr. J.S. Porterfield, no satisfactory experimental conditions were found so far.

Laboratory of Entomology

A. A colony of Culex (culex) pipiens fatigans was established for future experiments on their probably transmitting ability of viral diseases.

B. Aedes aegypti mosquitoes inoculated with VEE were studied to observe the relative influence of the virus on their biological cycle. This study was done in cooperation with Dr. M. Mussgay.

C. Continuing the study on the Venezuelan fauna and their virus-transmitting capacity, 540 mosquitoes were collected and classified from several regions of the country.

REPORT FROM DR. CARLOS SAN MARTIN
VIRUS SECTION, FACULTAD DE MEDICINA
CALI, COLOMBIA

The Virus Section of the Facultad de Medicina in Cali has different fields of interest, one of them being studies of arthropod-borne viruses.

Up to the present the major emphasis on this particular aspect has been on serological surveys especially in areas along the Pacific coast of both Colombia and Ecuador.

The most important event in the last few months has been the assignment by the Rockefeller Foundation of Dr. Robert H. Kokernot to the Virus Section in Cali

Preliminary studies of arbor viruses on the Pacific coasts of Colombia and Ecuador have included collections of human blood specimens since 1957 in order to get information on antibodies present in those areas.

We were able to obtain the Ecuadorian blood specimens through the help, cooperation, and facilities provided by Dr. Luis Baquerizo, then Director of the Instituto Nacional de Higiene de Guayaquil.

The information reported on this occasion refers only to studies of Venezuelan equine encephalitis virus (VEE). In each instance hemagglutination-inhibition, complement fixation, and neutralization tests will be indicated by HI, CF, and N respectively.

Only persons having spent their entire lives on the Pacific lowlands are included. In relation to a few of the sera from Ecuador, time of residency of the subject in the area is considered equivalent to chronological age, but only when careful questioning proved that the person came directly from the very high Andean mountains to the Pacific coastal areas.

HI was employed as an initial screening test, the N test was performed when indicated.

In each instance the denominator indicates the number of sera tested, and the numerator the number of sera containing antibodies to the VEE virus.

The following table (Table 1) shows the pertinent data from our serological surveys that relate to distribution of the immunity to VEE virus. The localities and dates of the blood samples are indicated.

In a previous publication (San Martin, C. and Duenas, A., 1959. Am. J. Trop. Med. & Hyg. 8:346) the relationship between HI and N tests was presented. After the preparation of that paper, 197 more sera were tested in the same comparative way. The results indicate that, at least for the geographical areas studied (Magdalena Valley, Atlantic coast and Pacific coasts of Colombia and Ecuador), our original statement is still valid. All the work done up to the present day is included in the following table (Table 2).

From results obtained in the Rockefeller Foundation Virus Laboratories (Rockefeller Foundation Virus Laboratories Annual Report for 1956), it has been generally accepted that complement fixing antibodies to VEE virus in human sera disappear within two years after infection or, if persisting, occur only in relatively low titers (1:4, 1:8).

We thought that it would be of interest to see whether the HI test might give some idea about the time of infection as does the CF test. Therefore 196 sera with varying HI titers, all of them positive in N tests, were assayed by the CF test. The table (Table 3) shows that

high titers in the HI test almost certainly indicate recent infection with VEE virus among the samples studied, and vice versa.

In the last two years tissue culture N tests in HeLa cells have been substituted for N tests in mice for VEE virus. Besides obvious advantages such as space, animal care, isolation and so on, the time required for determination of results is certainly shorter when HeLa cells are employed

TABLE 1
DISTRIBUTION OF IMMUNITY TO VEE VIRUS

Date	Locality		Age in Years								Total
			1-4	5-9	10-14	15-19	20-29	30-39	40-49	50 up	
30 May 58	Colombia, Choco (Condoto & Andagoya)	MALE			0/41	0/19	0/7	0/8	0/14	1/8	1/97
		FEMALE					0/2	0/1	0/1		0/4
		TOTAL			0/41	0/19	0/9	0/9	0/15	1/8	1/101
24 Aug 57	Colombia, Bocas del San Juan (Togoroma, Pichima, Charambira, Cuellar, Palestina)	MALE		1/15	5/13	1/5	1/2	0/1	1/1	0/3	9/40
		FEMALE		0/3	1/13	0/3	0/6	0/2	0/1	0/1	1/29
		TOTAL		1/18	6/26	1/8	1/8	0/3	1/2	0/4	10/69
3 Apr 57 & 12 May 58	Colombia, Buenaventura	MALE		0/20	0/9	1/23	4/17	4/19	10/20	3/9	22/117
		FEMALE	0/1	0/4	2/36	0/7	0/1	0/2		0/1	2/52
		TOTAL	0/1	0/24	2/45	1/30	4/18	4/21	10/20	3/10	24/169
2 Jul 58	Colombia, Tumaco	MALE		0/2	2/4	2/15	13/38	7/18	5/10	7/10	36/97
		FEMALE		0/2	1/11	0/4	4/12	3/7	1/4	5/7	14/47
		TOTAL		0/4	3/15	2/19	17/50	10/25	6/14	12/17	50/144
8 May 59	Ecuador, Guayaquil	MALE			0/1	0/3	2/22	6/18	0/10	2/5	10/59
		FEMALE									-
		TOTAL			0/1	0/3	2/22	6/18	0/10	2/5	10/59
8 May 59	Ecuador, Ancon	MALE	0/1	0/4	2/22	3/24	2/11	3/9	2/4	0/2	12/77
		FEMALE	0/1	0/2	2/17	0/19	0/2	0/4	1/1	1/1	4/47
		TOTAL	0/2	0/6	4/39	3/43	2/13	3/13	3/5	1/3	16/124
8 May 59	Ecuador, Milagro	MALE	1/3		0/3	1/2	0/7	1/6	3/9	3/6	9/36
		FEMALE	0/4	0/3	0/1	1/2	0/5	1/3	0/4	1/2	3/24
		TOTAL	1/7	0/3	0/4	2/4	0/12	2/9	3/13	4/8	12/60
7 May 59	Ecuador, Tenguel (Hacienda "Maria Teresa")	MALE	0/2	2/7	1/14	6/18	13/27	4/10	6/9	3/4	35/91
		FEMALE		1/3	1/5	0/3	1/5	1/4	1/1		5/21
		TOTAL	0/2	3/10	2/19	6/21	14/32	5/14	7/10	3/4	40/112

Table 2. Comparative results of tests for antibodies to VEE virus in sera studied by the HI and the N tests.

HI TEST		N TEST	
Sera tested	Titer *	Neg.	Pos.
346	0	346	0
4	<10**	2	2
13	10	7	6
17	20	1	16
34	40	5	29
67	80	0	67
89	160	0	89
114	320+	0	114

*Expressed as the reciprocal of the highest serum dilution inhibiting hemagglutination.

**In all tests the first dilution of serum was 1:10. In these cases the first tube showed only partial inhibition.

Table 3. Relationship between antibodies to VEE virus in sera studied by the HI and the CF tests.

CF/HI	10	20	40	80	160	320	320+	640	640+	1280	1280+	Total
Neg.	2	11	14	14	17	7	1	1	0	0	0	67
4	0	0	0	12	14	3	2	0	0	0	0	31
8	0	0	0	5	7	11	7	5	5	2	0	42
16	0	0	0	1	5	4	4	2	7	2	2	27
32	0	0	0	0	1	1	2	2	4	3	2	15
64	0	0	0	0	1	0	7	0	4	0	1	13
128+	0	0	0	0	0	0	0	0	1	0	0	1
Total	2	11	14	32	45	26	23	10	21	7	5	196

Titers both in HI and CF tests are expressed as reciprocals of dilutions of serum giving positive results.

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

Numbers of samples studied, merely because of volume have little value, but as they delineate trends and mark associations they give substance to an arborvirus picture that was tenuous and vague. This year's 817 isolations (356 infections) bring to 2, 346 the number of strains isolated and identified in the seven years of study at the laboratory. These represent 1, 078 infections, most of them from forests near Belem and on the Belem-Brasilia highway. The infections for 1961 are shown in the accompanying table.

Types new to Belem continue to appear in isolates from nearly all sources. This year there were eight, including a new member of group A, with one isolate each from Anopheles nimbus, Proechimys and man; a new Bunyamwera type in six isolates from Sabethini captured on tree platform; Turlock virus from one sentinel mouse group; Lukuni virus from Anopheles nimbus; and four ungrouped types, from Oryzomys microtis and sentinel mouse. One of the new Oryzomys isolates has been found also in Phlebotomus sp. Two other new hosts of the year are a reptile Mabouya mabouya with Mirim virus, and a group of nestling birds Troglodytes musculus clarus with Guama virus. The field of serological survey has been broadened enormously by improved methods for the production of hemagglutinating antigen from sera of infant mice. This has opened up the Guama group to the HI technique and led to the discovery of relationships between the two big Amazon complexes of viruses classified in groups C and Guama.

The discovery of two human cases of Oropouche early in the year led to an investigation that uncovered 13 other cases with viremia, in an urban epidemic of the virus that probably involved at least 11, 000 persons in the districts on the Guajara bay watershed area of Belem. Because of the simultaneous presence of an influenza outbreak, and numerous cases of alastrim and malaria in the city, there was no general awareness of this arborvirus illness, and without the laboratory search for others, probably only the two original cases would have been diagnosed.

One of the mysteries in past years has been why more viruses were not isolated from mosquitoes. Without any change in technique, one hundred isolations were made from arthropods in 1961. Sixty of these came from the Belem-Brasilia highway. The finding of so many infected mosquitoes is perhaps due to the buildup of number and variety of virus in the study areas, to densities detectable by the methods used.

The exposure of sentinel mouse groups and the capture of mosquitoes associated with them continues to be the best technique for the detection and isolation of arboviruses in the IAN forest near Belem. The method of continuous sampling now used for nearly three years at this place is yielding information about the types, incidence, and cycles of arborviruses that can only be gained over a period of time.

One of the special pleasures in epidemiological research is watching the definitive picture emerge from a mass of accumulated details and appear at last framed in its place among other emerging and finished portraits. The picture of VEE in the Amazon forests is far from complete, but the conception of a rodent-mosquito (Culex) association and of cyclic activity in a given area has become clearer with observations over two complete cycles, including three epizootic years (1957, 1959, 1961), interspersed by two apparently silent years (1958, 1960) in the IAN forest.

The modest beginning in tissue culture techniques begun in March with primary cell cultures by Dr. Henderson and taken up in July with HeLa cells by Dr. Gilda Gomes Bruno-Lobo, paid dividends in the last trimester when poliomyelitis appeared in epidemic form in Belem and was confirmed by laboratory isolation of poliovirus Type I.

Virus Infections by Type and Source 1961

		No. Infections	Human	Wild Animal		Sentinel Animal		Arthropod
				Rodent	Other	Mouse	Monkey	
A:	VEE	22				12	2	8
	Mayaro	29	1					28
	Aura	1						1
	Una	7						7
	AR35645*	3	1	1				1
B:	Bussuquara	4				1		3
	Ilheus	13					1	12
C:	Oriboca	5				5		
	Murutucu	12				12		
	Apeu	2				2		
	Caraparu	61				58	1	2
	Itaqui	26			1	22		3
GUAMA:								
	Guama	33		1	1	27		4
	Catu	15				10		5
	Moju	10		1		7		2
	(not typed)	51		1		45		5
CAPIM:								
	Capim	10		4		4		2
	Guajara	8				7		1
	AN20076	2				2		
MIRIM:								
	Mirim	4			1	3		
BUNYAMWERA:								
	Wyeomyia	3						3
	Cache Valley	2						2
	Kairi	1						1
	AR32149*	6						6
SIMBU:								
	Oropouche	16	16					
TURLOCK:								
	Turlock*	1				1		
ANOPHELES:								
	Lukuni*	1						1
UNGROUPED:								
	Tacaiuma	2						2
	AN27326*	1		1				
	AN27639*	2				2		
	AN28873*	2		1				1
	AN37944*	1		1				
Totals		356	18	11	3	220	4	100

*New to Belem in 1961.

REPORT FROM DR. MARIANO DUNAYEVICH
ARBOR VIRUS SECTION
INSTITUTO NACIONAL DE MICROBIOLOGIA
BUENOS AIRES, ARGENTINA

Multiplication of Junin Virus (Hemorrhagic Fever) in Tissue Culture

Junin virus had been propagated in this and other laboratories in suckling mouse, guinea pig, chick embryo, and HeLa cells. In this last system, it was necessary to maintain the cells in special conditions a long period. We could multiply the virus in primary tissue of different animals as a possible source for the production of an experimental vaccine that is under study. The virus multiplies without CPE in guinea pig, hamster, and mouse kidney and at a lower titer in mouse and chicken fibroblast. The titers of tissue culture fluids showed a maximum at 5 to 7 days of inoculation. We are studying the interferon production by these cultures and these conditions of the virus multiplication as a possible explanation of some biological aspects like absence of CPE, lowering of the titer in serial passage, etc. A hemagglutinin in tissue culture is under study because of the failure of obtaining one from suckling mouse brain.

REPORT FROM DR. JOSE M. VANELLA
DIRECTOR, INSTITUTE OF VIROLOGY
CORDOBA, ARGENTINA

The isolation is reported of two strains of the equine encephalomyelitis virus "western" type, besides one "eastern" type previously reported, from horses in Cordoba, Argentina, during the summer 1957-58. The immunologic study is performed by means of neutralization, complement fixation, and hemagglutination test of these and three other strains received from the province of Buenos Aires and the Republic of Uruguay. These last strains were also isolated during the same period and were classified as "western" type.

Complement fixation tests were performed with 17 blood samples obtained during the same period and in the same areas of the reported isolations. Sera of horses contained CF antibodies to eastern and western types of equine encephalomyelitis virus as well as to St. Louis encephalitis virus.

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

Antibody surveys:

HI and N tests have been done on 980 human sera collected from African residents of 24 localities in the low-lying coastal regions of southern Natal and the eastern Cape Province. The sera were all tested in the HI test against antigens to the following viruses: (1) Group A: Sindbis, Semliki, Chikungunya, Middelburg. (2) Group B: H 336 (Uganda S-like), Wesselsbron, Spondweni, West Nile, Zika. (3) Bunyamwera group: Bunyamwera, Germiston. (4) Ungrouped: Rift Valley fever. The sera positive to these antigens at serum dilutions of 1/20, 1/40, or 1/80 were then tested against the respective virus with the N test utilizing mice. In addition, N tests on most of these sera were done with 4 viruses, viz., Ndumu, Witwatersrand, Lumbo, and Pongola, for which a satisfactory HA antigen is not available. The results showed that Sindbis, H 336, Wesselsbron, West Nile, Bunyamwera, Germiston, Rift Valley fever, and Ndumu viruses have probably infected man in this area. However, the overall infectivity rate was extremely low.

HI and N tests are at present in progress on human, domestic animal (cattle, sheep, goat, horse) and wild rodent sera collected from localities along the Vaal and Orange Rivers. These localities are situated on the highveld (plateau) region which experiences a moderately severe winter. The tests are sufficiently far advanced to indicate that, while the human sera reflect low infectivity rates, the domestic animal and rodent sera show a surprisingly high incidence of arbor virus activity. Although a true assessment of the situation will have to await completion of the N tests, it would appear that Sindbis, Middelburg, H 336, Wesselsbron, West Nile, and Germiston viruses are fairly prevalent in the highveld region.

Isolation of viruses from rodents and mites:

Following the inoculation of tissues from 665 small mammals (mostly rodents), 4 viruses were isolated in mice. Three of these viruses have proved to be encephalomyocarditis virus and the other is still unidentified. A further strain of EMC was isolated from

Laelaps muricola mites collected off the same lot of rodents which produced the other 3 strains. These rodents were collected near Johannesburg.

Entomological field studies and virus isolations from mosquitoes and ticks.

(a) Tongaland.

Collecting trips were made to the Ndumu Field Station during the months of June, September, and October 1961, and during January 1962. Isolations of Pongola virus were made from Aedes (N.) circumluteolus during June and October 1961, at the beginning and end of the dry season (winter). A second, as yet unidentified, agent was isolated from the same mosquito during the October trip. The final results from the January 1962 trip are not yet to hand but, thus far, as yet unidentified agents have been isolated from a Culex (C.) univittatus pool and a pool of Aedes (Aedimorphus) mosquitoes in which A. (A.) dentatus was not distinguished from A. (A.) leesoni. A feature of the January 1962 trip, which followed two dry months, was the unutually large numbers of A. (A.) cumminsi (1, 755) and A. (A.) dentatus/leesoni (479) collected. A specimen of Aedes (Diceromyia) fascipalpis Edw. was collected for the first time at Ndumu and in Natal. Although 4, 740 A. (N.) circumluteolus were collected, they were, on the average, undersized, which was probably merely a reflection of the dry conditions which had prevailed during November and December 1961.

(b) Witwatersrand area.

Since late December 1961, regular trapping of mosquitoes has taken place at Olifantsvlei, near Johannesburg. This is a marshy area near a sewage farm, and it was here that an isolation of H 336 virus was made from Culex (Neoculex) rubinotus in 1958. The traps used were of the Californian Lard-can type baited with solid carbondioxide. So far, 2, 733 mosquitoes have been trapped at an average rate of 48. 8/ trap/night. The figures for the commonest species are as follows: Culex (C.) univittatus 1, 430 (25. 5/trap/night); Culex (C.) theileri 299 (5. 3/trap/night); Culex spp. (mainly members of Culex pipiens s. l.) 1, 000 (17. 9/trap/night). During this period, 3 biting catches were made yielding 646 mosquitoes. Virus isolations have apparently been made from a pool of Culex univittatus from a trap, and from a pool of Culex theileri from a biting catch.

Phlebotomus fever virus has been shown to persist for at least 21 days in laboratory-reared artificially-infected female P. papatasii.

Search for extra-human hosts of phlebotomus fever virus among mammals and birds of the Nile Delta continued. Sera from 22 animal species inhabiting endemic phlebotomus fever areas have been found to be devoid of hemagglutination-inhibiting antibody to the Sicilian type of virus.

Entomologic information on P. papatasii, accumulated during the course of the virus-vector-host studies, is noteworthy. Detailed morphologic study revealed certain differences between P. papatasii from Egypt and from other areas. Quantitative insecticide susceptibility levels have been established for several Egyptian sandfly populations and the influence of colonization on susceptibility to insecticides has been determined. Climatologic factors affecting seasonal and daily fluctuations in Egyptian sandfly populations and activity have been investigated.

Equine encephalomyelitis

Attempts to identify the etiologic agent of this sporadic fatal disease continued. Clinical and epidemiologic data collected in the course of field work have suggested the possible existence of 2 distinct diseases--one affecting horses and the other affecting donkeys. This impression was supported by the isolation of 2 immunogically-distinct viruses, AN-106 and AN-405, from typical cases in horses and donkeys, respectively. Further virus isolation attempts have been discontinued in favor of efforts to determine the relationships of the previous isolates to disease. Following adaptation of the horse virus (AN-106) to adult mice, antibody surveys were conducted among equines from involved and disease-free areas. Neutralizing antibody was found in 11% of horses from involved areas, whereas none was demonstrated in horses from disease-free areas. This finding suggests a possible etiologic relationship between AN-106 virus and the horse disease. Further indication of viral etiology was provided by a histo-pathologic study of central nervous system tissue from typical horse cases. Lesions characteristic of viral encephalitis were demonstrated in two animals yielding AN-106. Similar pathogenic changes were not seen in animals failing to yield the agent. Preliminary attempts to produce the disease experimentally in horses were unsuccessful.

No evidence was obtained for the etiologic role of AN-405 virus in the donkey disease. Donkeys from involved areas were devoid of AN-405 neutralizing antibody, no characteristic brain lesions were seen

in animals yielding the agent, and attempts to reproduce the disease experimentally were unsuccessful.

Studies of the etiologic significance of these two viruses will be continued during the coming year.

Serologic Survey

A serologic survey conducted in Upper Egypt, between Aswan and Wadi Halfa, revealed a high incidence of poliomyelitis (types 1, 2, and 3) and West Nile fever, moderate incidence of Q fever, and the absence of phlebotomus fever and yellow fever. The majority of the subjects were school children under 12 years old who had never travelled outside their native villages.

Migratory Bird-Ectoparasite Study

To assess the importance of migratory birds and their tick parasites as vehicles for the intercontinental transfer of arthropod-borne viruses, a program for studying birds on their seasonal migrations between Europe and Africa has been initiated by the Departments of Virology and Medical Zoology. Over 1100 bird bloods (from 21 species) and nearly 500 ticks (4 species) have been processed for virus isolation. No viruses have as yet been recovered from this material.

REPORT FROM DR. HARRY HOOGSTRAAL
DEPARTMENT OF MEDICAL ZOOLOGY
U.S. NAVAL MEDICAL RESEARCH UNIT NO. 3
CAIRO, EGYPT

In addition to an intensive study on the epidemiology of kala-azar in the Sudan Republic, this department of NAMRU-3 is continuing several lines of research aimed at increasing knowledge concerning the interrelationships of known and potential vectors and their hosts in many parts of the world.

In the area of bird-tick relationships, a summary of African ticks taken in the Cairo area during the spring migration periods of 1956 to 1960, from birds travelling from Africa to central and eastern Europe and Asia, was recently published in Bulletin of the World Health Organization (1961). For comparative data, birds migrating northward over the deserts of Egypt will be studied on a 500-mile coastal front during the 1962 and subsequent spring seasons. A similar review of results from birds migrating southward from Europe and Asia through Egypt during the fall seasons of 1959, 60, and 61, is being prepared

for publication, and this study will continue for several years. Serum and ticks from many of these birds are being examined by the NAMRU-3 Virology Department.

Argas ticks of the subgenus Argas parasitize wild and domestic birds in many temperate and tropical areas of the world, but species criteria have been very poorly established and biological data are practically non-existant. Some of these species are known to harbor and transmit viruses and all are considered potential vectors of some importance. A special effort is therefore being made to obtain living material from around the world for laboratory studies, to review the genus, to provide all available biological data, and to stimulate further research on these ticks and their host and pathogen relationships. Five reports on different species in this subgenus have thus far been published in the Annals of the Entomological Society of America and several others are being prepared. This work has stimulated similar studies in USSR and a preliminary study of the Argas (Argas) fauna of USSR was published in the December 1961 issue of Zool. Zh.

For some years, this department has been amassing data on bird-tick relationships, reviewing literature on the subject, and developing concepts to aid in research on the role of birds and their parasites in dissemination of pathogens; this material is now being assembled into monograph form.

Interest in bats, their ectoparasites, and viruses is increasing in several parts of the world, and a number of bat-infesting ticks have been studied and described during the present program. Several new and poorly known species are presently being studied and a review of bat-tick relationships around the world is being prepared in collaboration with Mr. Glen Kohls of the Rocky Mountain Laboratory.

Mention of other studies relating to ectoparasite-host-pathogen interrelationships was made in the April 1961 issue of the Information Exchange.

The Entomology Division of this department is devoting its attention to biological studies on potential vectors of kala-azar in Sudan and to support of the NAMRU-3 Virology Department in research on sandfly virus. An ecological study of fleas on dogs in the Cairo area is also in progress.

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

Three further viruses have been registered with the catalogue: C281 as "Edge Hill", MRM168 as "Wongal" and MRM186 as "Mapputta."

The identification has almost been completed of viruses isolated and reisolated from mosquitoes collected in North Queensland in April 1961. The most notable features are:

1) The isolation of Edge Hill virus, originally from Aedes vigilax at Cairns, from C. annulirostris at Mitchell River. This increases the number of group B agents recognized at Mitchell River to four.

2) The isolation from C. bitaeniorhynchus and Anopheles amictus amictus collected at Normanton of two strains which appear identical with the Malayan AMM2021. Both were reisolated with ease and appear to be valid isolations. This isolation will make necessary a reappraisal of our earlier epidemic polyarthritis antibody studies.

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

The investigations reported in the No. 4 issue of the Information Exchange suggested the presence of more than one group B arbovirus in the North-West of Australia (Kimberley area). In an attempt to interpret the figures, particularly those native sera which gave 92% positive results by HI and 50% positive by neutralization with MVE, further tests were carried out with the virus strains obtained from Dr. Doherty of Queensland. These were:

Group B - MRM16, MRM32, Dengue 1, and Dengue 2

Group A - MRM39, AMM2021

During 1961, there were 169 cases of Thai haemorrhagic fever admitted to Chulalongkorn Hospital. The highest incidence occurred between June and December. There were no cases reported in February and March. The clinical picture was the same as described in previous reports on this disease. The age of the patients ranged from 4 months to 14 years. There were 7 deaths.

Sixty-five acute sera collected between the 1st to 8th day after onset of the fever were selected for virus isolation. Fourteen viruses were isolated, of which 9 were identified to date. Six are dengue strains and 3 are chikungunya. The other five viruses are now being adapted for complete identification.

Two isolations are of particular interest. One, SH 728, identified as dengue TH 36 (Hammon) was isolated from the blood of a 7-month-old girl with typical symptoms 6 hours before death. The other, SH 674, was also isolated from a patient with classic symptoms and signs of this disease. The patient was in shock but recovered. The virus may be a new type, very close to dengue 4 (Hammon H. 241).

Specimens from three autopsies (tonsils, lungs, livers, heart, stomach, spleen, kidneys, bone marrow, lymph nodes, skin, hypothalamus, adrenal glands, pancreas, and heart blood) were processed but none yielded virus.

Attempts to isolate virus from mosquitoes collected in light traps and from night and day resting collection in this hospital and in some from premises of the patients' residences, was carried out from June to December 1961. The number of mosquitoes processed was: Culex fatigans 10,700; Culex tritaeniorhynchus 1,497; Culex gelidus 1,100; Aedes aegypti 262; Anopheles hyrcanus 117; and Mansonia uniformis 51.

Two viruses (Q 66, Q 69) were isolated from two pools of Culex fatigans collected in this hospital. They have a short incubation period and are now being adapted for complete identification.

This laboratory has been receiving pools of mosquitoes collected by Capt. J.E. Scanlon of U.S. Component SEATO Laboratory in Bangkok for virus isolation. They were: Culex fatigans 1736; Mansonia uniformis 104; Aedes aegypti 73; Culex gelidus 68; Culex tritaeniorhychus 29; Armigeres subalbatus 18; Culex sitiens 15.

One virus (C6) was isolated from a pool of Aedes aegypti. This virus has a much longer incubation period than Q 66 and Q 69. It is being adapted for complete identification.

A serological study of paired sera from patients was performed mainly by the CF method. The result of the testing of 19 paired sera was:

Positive for Group A (chikungunya), 3.
Positive for Group B (dengue), 10.
Positive for both groups, 2.
Negative for both groups including JBE, 4.

During January 1962, there were 9 cases of Thai haemorrhagic fever in this hospital. Seven acute sera were used for virus isolation. Two yielded viruses of short incubation period. Complete identification is now being carried out.

REPORT FROM DR. H.S. HURLBUT, U.S. NAVAL MEDICAL RESEARCH UNIT NO. 2, TAIPEI, TAIWAN

The 1961 epidemic of Japanese encephalitis in Taiwan proved to be one of the worst in recent years. From May to October, 629 cases were reported with about one-half occurring in July. The fatality rate was about 21 per cent.

Mosquitoes were trapped in the Taipei area and tested for virus infection throughout the year. Twenty-three JE virus isolations were made in July and August, all from Culex tritaeniorhynchus. The mosquitoes were collected in New Jersey type light traps set either inside or close to pig shelters.

Experiments with six-months-old pigs, C. tritaeniorhynchus, and JE virus unmodified by mouse passage indicate that mosquitoes are infected at very low levels of viremia.

Growth patterns of arboviruses are being studied in a variety of tissue cultures using both established strains and newly isolated ones.

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

Cultivation of mouse-passaged dengue viruses in human and animal tissue cultures (Hotta, Ohyama, Yamada, and Awai):

Type 1 dengue virus, Mochizuki strain, multiplied in plasma-embedded cultures of human mature lung, lymph node, thyroid, and spleen tissues. The release of virus into the fluid phase continued for 30 to 50 days at 34° C, and the maximum virus titers of infected fluid were approximately $10^3/0.02$ ml in mouse-intracerebral LD_{50}. The active virus persisted for a significantly prolonged period of time (31 days) in the fluid of embryonic cerebrum tissue cultures; the viral multiplication of a low grade was suggested. Apparently no growth of virus was evident in cultures of human adrenal gland tissues.

Trypsinized kidney cell cultures of guinea pig, hamster, and hog supported the growth of dengue virus. Active virus was detected in the fluid phase for more than 30 days at 34° C, and the maximum virus titers were 10^3 - $10^5/0.02$ ml in mouse LD_{50}. A low grade viral multiplication was evident in trypsinized chicken kidney cell cultures. A significant persistence of virus in trypsinized chick embryo skin-muscle cultures was noted. Dengue virus did not grow apparently in trypsinized kidney cell cultures of mouse, rabbit, or ox.

Hamster kidney cell cultures infected with type 1 or type 2 dengue virus exhibited degeneration which was suppressed by addition of specific immune rabbit serum. The general pattern of infection of hamster kidney cells resembled to that noted in monkey kidney cell cultures. The in vitro neutralization using hamster kidney cell cultures was complete when performed with homotypic serum, while a partial neutralization was shown by heterotypic serum. The cellular degeneration practically paralleled the mouse-infectivity in the case of type 1 virus. A discrepancy was noted between the titers in mice and in cell cultures infected with type 2 virus, Trinidad-1751 strain; a tendency was shown that cell culture ID_{50} was higher than mouse LD_{50}.

(Abstracted from Japanese Journal of Microbiology, vol. 5, Hotta, S., Ohyama, A., Yamada, T. and Awai, T. (1961): pp. 61-72. This issue has not yet been distributed.)

Japanese B encephalitis virus infection of puppy cerebellar cells cultivated in vitro:

Cerebellar tissues from 1 to 15-day old puppies were cultivated on coverslips by the plasma-embedded roller tube method, employing a culture medium consisting of 50% human cancerous ascitic fluid, 5% chick embryo extract, 45% Gey's balanced salt solution, 300 mg% glucose and 1,000 u/ml penicillin. Carrel or Porter flask type cultures were additionally prepared.

After an incubation at 37° C for 7 to 14 days, the cultures were added with mouse-passaged Japanese B encephalitis (JBE) virus and reincubated at 35° C. Following the infection, contents of the virus in culture fluid were measured, and the cultivated nervous cells were observed by staining (Jacobson's May-Grunwald-Giemsa method or Bodian's silver impregnation method) or by phase-contrast microscopy.

JBE virus, Gl strain, multiplied in the tissue cultures; the highest virus titer of the infected culture fluid was approximately 10^4/ml in mouse-intracerebral LD_{50}, which was usually attained 3 to 5 days after the exposure to virus. The cultivated virus was neutralized by the anti-JBE immune rabbit serum.

The Purkinje cells in the infected cultures showed degenerative alterations which were recognized as morphological and tinctorial changes of the nuclei, dendrites, neurites, neurofibrills, etc. These changes were regarded to be caused specifically by the virus when compared with the findings noted in the control cultures incubated just the same as the infected cultures but receiving no active virus or a normal mouse brain homogenate.

(Hotta, Ohyama, Musashi, and Liao.)

Cultivation of 17D strain yellow fever virus in primary trypsinized animal cell cultures (Hotta, Ohyama, Fujita, and Yamada.)

The 17D strain yellow fever (YF) virus multiplied in primary trypsinized kidney cell cultures from chicken, guinea pig, hamster hog, and Japanese monkey, but apparently not in rabbit kidney cell cultures. The longest periods in which active virus was detected in the infected culture fluid and the highest viral titers as expressed in mouse-intracerebral LD_{50}/0.02 ml were as follows: chicken kidney cells, 30 days, $10^{3.5}$; guinea pig kidney cells, 14 days, $10^{3.25}$; hamster kidney cells, 36 days, $10^{4.25}$; hog kidney cells, 50 days, $10^{3.75}$; and monkey kidney cells, 43 days, $10^{3.5}$. The 'infective spectrum' for cell cultures and the comparatively slow rates of multiplication which 17D virus showed, resembled those of dengue virus.

The 17D virus was transmitted through a number of hamster kidney cell cultures. Infectivity for mice was shown with the 44th passage's culture fluid representing 10^{-88} dilution of the starting inoculum. The cultivated virus retained its mouse-infectivity throughout the subcultures.

The infected hamster and monkey kidney cells exhibited degeneration visible under an ordinary light microscope. The cellular degeneration was prevented by antiserum from rabbits immunized with the original 17D virus. The in vitro neutralization paralleled the conventional mouse brain tests. A tendency was shown that ID_{50} values of 17D virus for hamster kidney cell cultures were higher than LD_{50}'s for 3-week old mice, and that the cellular degeneration in cultures appeared earlier than the death of mice. Similar results were obtained in either experiment using the mouse brain passaged virus or the commercial chick embryo vaccine virus.

Extraction of infective ribonucleic acid fractions from brains of mice infected with yellow fever and western equine encephalitis viruses (Takehara and Hotta):

From brains of suckling mice infected intracerebrally with yellow fever virus (17D strain) and western equine encephalitis virus (Rockefeller Institute stock strain), RNA fractions were extracted by the cold phenol method by Gierer and Schramm (1956). Examples of the results obtained are as follows:

Virus	Inoculum	LD_{50}/0.02 ml (-Log) in Suckling mice	LD_{50}/0.02 ml (-Log) in Adult mice
YF	Original	5.50	5.50
	RNA	2.50	$<$1.17
	Original	4.50	4.50
	RNA	1.63	$<$0.75
WEE	Original	7.75	7.00
	RNA	2.24	$<$1.68
	Original	8.25	6.25
	RNA	2.17	$<$1.49

The infective RNA fractions were inactivated by exposure to RNAase, but apparently not by DNAase or immune rabbit serum.

Virus	Inoculum containing*	LD_{50} (-Log)**
YF	RNA plus saline	2.38
	" " RNAase	0[x]
	RNA plus saline	2.17
	" " DNAase	2.38
WEE	RNA plus saline	Ca. 2.00
	" " RNAase	0
	" " DNAase	Ca. 2.00
	RNA plus saline	Ca. 1.50
	" " immune serum	2.25

* RNA was exposed to enzymes in final concentration of 50 µg/ml, at 27° C, for 10 minutes.

**In suckling mice.

[x]No active virus was detected.

REPORT FROM DRS. ALFRED M. PRINCE AND NUBUO HASHIMOTO 406TH MEDICAL GENERAL LABORATORY, JAPAN

Isolation of arthropod-borne viruses directly in tissue culture by the plaque technique from chick embryo fibroblasts:

It has been found that Japanese encephalitis virus may be isolated directly from wild mosquito suspensions by the plaque technique on chick embryo fibroblasts.

Although this technique is frequently slightly less sensitive than suckling mice inoculation for mouse passaged laboratory strains of virus, it has been found to be more sensitive for the majority of strains existing in nature.

Wide variations have been observed in the ratio between plaque titers and suckling mouse titers of wild strains isolated from mosquitoes. Data are being prepared on the analysis of virulence of plaque produced clonal strains having wide differences in the above ratio.

Analysis of the neutralizing antigen of Japanese encephalitis virus strains by kinetic neutralization technique:

It has been found that when the kinetic neutralization technique (Dulbecco, McBride) is applied to Japanese encephalitis virus and homologous antisera, utilizing the plaque technique, extremely high "resistant fractions" are encountered. Frequently, high titer sera produce less than 50-75% final neutralization. These characteristics have prevented the use of the kinetic neutralization technique for comparison of antigenic structure of different strains.

It has been found that the above results are partially due to the high rate of dissociation between virus and homologous antibody under the conditions employed. In addition, there is evidence that antisera change the antigenic site, prior to dissociation of antibody, to render it resistant to neutralization by the original antibody. This dissociation can be avoided by using alkaline pH in the neutralization mixture and in diluents.

These findings make possible accurate comparison of strains of Group "B" arbor viruses from different regions in Asia.

Two approaches are being employed: the kinetic neutralization technique of Dulbecco and McBride, whose application to the Japanese encephalitis virus system has been pioneered in this laboratory by Hashimoto; and immunization challenge experiments employing mice immunized by subcutaneous inoculation of killed and live vaccines, and challenged intracerebrally with varying doses of diverse virus strains.

The following results have been obtained so far:

1) Formalin inactivated Nakayama strain vaccines, commercially produced, have been found to produce incomplete protection following intracerebral challenge.

2) Nakayama strain killed vaccine produces considerably greater protection against homologous challenge virus than it does against all other strains of Japanese encephalitis virus so far tested.

3) When live virulent 1957 strains of Japanese encephalitis virus were employed for immunization, protection is considerably greater against homologous type challenge viruses than can be produced by killed virus vaccines challenged with homologous virus. Furthermore, protection afforded by live virulent 1957 strains is considerably greater against homologous types of challenge virus than against the Nakayama prototype.

4) Attenuated 1957 virus vaccines show considerably greater protection against challenge with homologous viruses than protection against the prototype Nakayama strain. Protection by these vaccines is also significantly greater against the homologous strain than it is against heterologous strains isolated during 1957, 1958, and 1959.

In conclusion, the above results indicate that significant variation exists in the antigens of diverse strains of Japanese encephalitis virus, as has been suggested by several workers. Evidence has been presented that if safe and effective live vaccines can be perfected, these may be expected to be more effective than the presently employed killed vaccine. Preliminary studies on some candidates for possible use as live vaccines have been presented. It is suggested that large-scale investigation into antigenic variation of Japanese encephalitis virus strains from different regions of Asia is called for at the present time.

REPORT FROM DR. GORDON FIELD
DEPARTMENT OF MEDICAL ENTOMOLOGY
MEDICAL GENERAL LABORATORY (406)
U. S. ARMY MEDICAL COMMAND, JAPAN

Entomologic studies on Japanese B encephalitis have been oriented towards laboratory testing of the thesis that the local vector, Culex tritaeniorhynchus Giles, might serve as the overwintering vehicle for JEV. During 1961, the virus was re-isolated in the Department of Virus and Rickettsial Disease from a pool of 17 laboratory infected females that had been held at approximately 15° C for 143 days. These mosquitoes were infected initially by permitting them to feed ad libitum on cotton wicks saturated with JEV in 20% calf serum - 5% dextrose water. Of equal importance as survival of JEV in mosquitoes and to the likelihood of mosquitoes in nature carrying the virus through the winter months, is the successful hibernation of mosquitoes that have had a blood meal. Adult females have been given blood meals and induced to enter hibernation. Survival periods at reduced temperature (15° C) in excess of 4 months have been obtained. A source of carbohydrate (sugar-water, cut fresh fruits and vegetables, and/or rotting fruits and vegetables) appears to be necessary for survival in studies conducted at this laboratory.

Infected adult C. tritaeniorhynchus were obtained from 25 last instar larvae that had been held overnight (approximately 18 hours) in 20 cc of 20% calf serum - 5% dextrose in water with a titer of JEV of 4.5×10^6 plaque forming units (PFU)/ml. The larvae were then placed in a larger volume of clear water with larval food and permitted to pupate. The adults

were held at insectary conditions (80° F, 85% RH) for 17 days following emergence with 5% sugar-water their only source of food. JEV was isolated from 3 of 9 mosquitoes with titers of $> 4 \times 10^5$, 4.2×10^4, and 6.5×10^4 PFU/mosquito respectively.

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

Up to 1960, at the virology section of the Institute of Microbiology of Sassari, studies have been made on:

1) Some biological aspects of arbor viruses. Pathogenicity for experimental animals, viability and resistance of RSSE and Semliki Forest viruses; behavior of Semliki Forest virus in tissue culture; cross immunity between group A arbor viruses.

2) Presence of neutralizing antibodies for RSSE virus in Jugoslavian refugees. The sera of 139 refugees from Jugoslavia, where, in the preceding years, outbreaks of tickborne meningoencephalitis had been reported, were examined. Six and a half per cent of the sera showed neutralizing antibodies for RSSE virus.

3) Eventual presence of louping ill virus neutralizing antibodies in the sheep population of Sardinia. On account of the considerable diffusion of Ixodes ricinus in the Mediterranean regions and the frequency of encephalitis cases in lamb, a serological investigation was carried out in sheep of Sardinia in order to demonstrate the eventual presence of neutralizing antibodies for louping ill virus. All the sera examined gave negative results.

Program for the year 1962:

After 1960, a virology section has been created at the Institute of Microbiology of Parma. Our program contemplates a systematic study of the presence of arbor viruses in the Italian regions, and particularly in the Po Valley and in the island of Sardinia. During the year 1962, a considerable number of sera will be examined by the hemagglutination inhibition reaction. These sera will be taken from the inhabitants of Sardinia, where, especially until the antianopheles campaign conducted by the Rockefeller Foundation, there was a considerable quantity of potential vectors.

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

Studies on epidemiology of tick-borne encephalitis in the eastern part of Austria, 1960:

1) Serological investigations on patients with infections of the CNS. In the Eastern part of Austria where tickborne encephalitis has occurred since 1927 (proved since 1956), at least three blood samples were collected from all patients hospitalized with an infectious disease of the CNS. The sera were examined by a combined method of neutralization (in tissue cultures), hemagglutination inhibition, and complement fixation tests. The following results were obtained by our method:

Proved cases of tickborne encephalitis	44
Presumed cases of tickborne encephalitis	74
Other infections	143
Total	261

This total of 261 cases represents about 2/3 of all cases with infectious diseases of the CNS reported to the sanitary board in this area. All cases were considered as proved if they displayed: 1) conversion or significant rise of antibodies in the CF; 2) significant (4-fold at least) rise in NT or HI. As presumed cases were considered: all cases with negative CF but primary high level of antibodies in HI or NT without significant rise. Conversion in the CF occurs between the 16th and 23rd day of disease in all proved cases. Contrary to that in all proved and presumed cases antibodies in NT and HI were always present in the first serum sample already.

2) Investigations on ticks (Ixodes ricinus) collected in the district of Neunkirchen, where all cases with infections of the CNS were examined completely by virological and serological methods since 1956. In the forest of Pottschach starved ticks of all three stages were collected, determined, pooled, and injected ic and ip into mice to isolate the virus.

Time	Place	Larvas	Nymphs	Pool	Isolated Strains	Virus-proof in
May	Pottschach	50		1	-	-
			100	2	1	first passage
Jun	"	500		5	-	-
			100	2	-	-
Jul	"	700		7	4	second passage
						"
						"
						"
			50	1	1	second passage
Aug	"	376		4	-	"
			15	1	1	third passage

In the neutralization test, all strains were identified as tickborne encephalitis viruses. The antigenic properties did not differ from the Austrian reference strain

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

On October 17, 1961, the agreement was signed between WHO and Institute of Virology, Czechoslovak Academy of Sciences, Bratislava, Czechoslovakia, on establishing the Arthropod-borne Virus Regional Reference Laboratory/RRL/ for Central Europe at the Institute.

This agreement was made according to the policy of the WHO to insure an exact laboratory diagnostic work of arbor virus infections in the area of Central Europe. The function of the laboratory will be mainly:

1) To identify and study arthropod-borne virus strains isolated in its area of coverage.

2) To prepare reference sera and antigens from viruses isolated in its region and to provide small amounts of these sera and antigens to collaborating laboratories.

3) To maintain prototype and reference strains. These strains should be made available to other reference laboratories.

4) To collect and disseminate epidemiological and technical information on arthropod-borne viruses, in cooperation with WHO Headquarters.

5) To receive fellows sent by WHO for training purposes in such numbers as the available facilities permit.

The RRL is attached to the Department of neuroviral infections, Laboratory of ecology of tickborne encephalitis virus at the Institute of Virology. Both diagnostic and ecological work will be carried out in the RRL. Some of the recent investigations of the members of this laboratory are summarized in following presentations.

References:

D. Blaskovic: Tick-borne encephalitis in Europe: Some aspects of epidemiology and control. Transactions of the New York Ac. Sciences Ser. II. Vol. 23, No. 3, 215-232, 1961.

Biology of viruses of the tick-borne encephalitis complex. Papers presented at the Symposium held in Smolenice, Czechoslovakia, in October 1960, Publishing House of the Czechoslovak Academy of Sciences, 1962 (in press).

Study of the Relation of the Birds to Natural Foci of Tick-borne Encephalitis (M. Gresikova, M.D.):

In the summer of 1957, the migration territory of water fowl in East Slovakia has been investigated. In these field experiments, a neurotropic virus had been isolated from the Garganey (Annas querquedula) which was identified as the tick-borne encephalitis (TE) virus.

Investigations on experimental viremia and immunogenicity are being continued with other species of birds in sera of which neutralizing antibodies against the TE virus complex were found. Following birds have been tested: Falco tinnunculus, buzzard (Buteo buteo), great-tit (Parus maior), blackbird (Turdus merula), and pheasant (Phasianus colchicus). The birds were inoculated with a Czechoslovak strain of TE virus subcutaneously, intracerebrally, and the pheasant additionally by the intramuscular route. Blood for examination of viremia has been collected from the 1st to 18th day at 24-hour intervals. At the same intervals, the birds were killed and their brains and internal organs (spleen, liver, pancreas) removed for isolation of the virus. Antibodies have been studied by a neutralization test in HeLa cells and in mice.

Studies on the relation of the tick-borne encephalitis virus to different species of vertebrate hosts are important because these birds are infested with larvae and nymphs of ticks and they have a close trophic relation to small mammals. The birds Buteo buteo and Falco tinnunculus showed after subcutaneous and peroral infection no clinical signs of disease. No virus was isolated from the blood or from organs. The intracerebral inoculation of the virus caused no clinical signs of disease, although histological changes were observed in some of the brains investigated. Antibodies were present in one bird after alimentary infection and in all animals infected intracerebrally.

Experimental pathogenicity of TE virus for Parus maior and Turdus merula has been studied, representing most frequent species of birds in our natural foci throughout the year and with heavy infestation by ticks. After subcutaneous administration of TE virus, the birds showed no clinical signs of disease. The results of isolation experiments from the blood and other organs of the infected birds were negative. Virus neutralization tests with the sera of Parus maior and Turdus merula and TE virus were negative too. A further study of neutralizing antibodies in birds captured in a natural focus of infection confirmed these negative results.

Only in Phasianus colchicus virus neutralizing antibodies could be demonstrated after administration either of high doses of virus or by repeated inoculation of low doses of TE virus. The TE virus was not found in the blood and internal organs.

Because of the opinions of many workers according to which birds are playing a significant role in the ecology of TE virus, we undertook a study of the viremia in some species of birds in the sera of whose antibodies have been found. After subcutaneous inoculation of TE virus a clinically inapparent infection has been induced. Viremia did not occur and virus was not present in the central nervous system. It was therefore concluded that Falco tinnunculus, Buteo buteo, Parus maior, Phasianus colchicus can hardly be regarded as reservoirs for TE virus in nature.

Experimental hibernation of the Tick-borne Encephalitis Virus in Engorged Larvae of the Tick Ixodes ricinus L. (J. Rehacek, Ph.D.):

The problem of tick-borne encephalitis virus hibernation under the natural conditions prevailing in Czechoslovakia has not yet been solved, although Benda (1958) has shown that the virus can survive for considerable periods in ticks kept at +4° C. Therefore investigations were carried out to determine whether the virus can overwinter in ticks.

The engorged larvae of Ixodes ricinus L. were chosen as the model. The larvae were infected by engorgement on white mice with viremia and placed in an iron cylinder 50 cm. high and 6 cm. in diameter, which was loosely packed with alternate layers of grass, leaves and debris in order to allow free movement of the ticks throughout the cylinder. The top and bottom of the cylinder were covered with dense brass gauze. The cylinder was placed vertically in the soil of a natural habitat of I. ricinus ticks, its top being at the ground level. Hibernation was started on November 25, 1958, and finished on March 7, 1959.

It was found that the tick-borne encephalitis virus survived in engorged larvae for 102 days under natural conditions of hibernation. The virus was recovered by mouse inoculation of suspensions of engorged larvae prepared 6 days and 57 to 88 days after hibernation. Virus was also recovered by the bite of nymphs moulted from the larvae. It is assumed that the overwintering of the tick-borne encephalitis virus in ticks is one of the ways it survives in nature during the winter period.

References:

Benda, R.: J. Hyg. Epid. Microbiol. Immunol. 2, 314, 1958.

Transmission of tick-borne encephalitis virus by fleas. (J. Rehacek, Ph. D.)

The isolation of tick-borne encephalitis virus from fleas and their hosts (Taytsch and Wroblewska, 1958, Federov et al., 1959) stimulated the author to study the possible role of fleas in the transmission of this disease.

Fleas from moles' nests were chosen, since they are easily available and are parasites of a whole series of known reservoir animals of the tick-borne encephalitis virus. The Hypr strain of tick-borne encephalitis virus was used (Pospisil et al. 1954).

First of all, the time for which the virus persisted in the species Ct. assimilis was determined. In this experiment, flea-infested mice were kept in glass tanks measuring 10 x 15 cm. When the mice died, they were removed and the fleas were in the tank. The virus was found in the fleas only while they fed on hosts with viremia up to approximately 24 hours after sucking infected flood. It was no longer determined in the fleas after this period. The experiments were carried out three times, using 600 fleas.

In the second set of experiments, the transmission of tick-borne encephalitis virus by fleas to healthy mice was studied. The experiments were carried out in glass aquaria measuring 25 x 15 x 15 cm, which were divided in two by double wire netting with a 0.5 cm. mesh and an intervening space of 5 cm. The fleas and mice with viremia were placed in one of part of the aquarium. When the mice died, i.e., on fourth day, healthy mice were placed in the other compartment and the fleas transferred to these. The dead mice were then carefully removed. Thirty-one experiments were carried out, using over 3,600 fleas, most of which belonged to the species Ct. assimilis and the others to the species Ct. agyrtes, Ct. bisoctodentatus, and Hystrichopsylla talpae. As soon as the fleas stopped sucking the blood of mice with viremia, they were allowed to feed on healthy mice (58 altogether) for up to 14 days. The results of these experiments were negative.

References:

Federov, Yu.V., Izolkin, N.I., Tyushakova, I.K. (1959): Med. par. par. bol. 28, 149-152.

Pospisil, L., Jandasek, L., Pesek, J. (1954): Lek. listy 9, 3-5.

Taytsch, Z.F., Wroblewska, Z. (1958): Przegl. Epid. 12, 339-353.

Persistence of tick-borne encephalitis virus in hibernating bats and green lizards (M. Gresikova, M.D.)

We examined the possibility of survival of the tick-borne encephalitis virus (TE virus) in hibernating animals. Bats were selected as the model since neutralizing antibodies against TE virus had been found in their sera.

The experiments were carried out during the winter with 120 hibernating bats of the species Myotis myotis, Barbastella barbastellus, and Plecotus auritus from the caves of Plavecke Podhradie, Driny, and Harmanec, Czechoslovakia. The bats were placed in wire cages in the containers made from a dense nylon mesh. The cages containing the bats were placed into a cave near a small lake with a constant temperature of 8° C and a 100% degree of humidity. The bats were inoculated subcutaneously with Hypr strain of TE virus. After 23-30 days the bats were transferred to room temperature. Most of the bats showed no marked clinical signs of disease after hibernation. The course of the infection was studied during and after hibernation.

TE virus was found in the blood and brain of individual bats from the 7th to the 12th day and in some cases up to the 23rd day. When the bats were transferred to room temperature the virus was present in their blood from the 1st to the 7th day, in their spleen and liver up to the 11th day, and in their brain up to the 14th day. Viremia lasted for 26 days in the infected bats including the hibernation period, but in the brain the virus persisted for up to 31 days. In some cases the LD_{50} of the virus in the blood reached values of over $10^{4.5}$ to $10^{6.5}$ after hibernation. It is therefore assumed that virus multiplied in the bats.

The relation of lizards to the TE virus was not previously known, although lizards were found in natural foci of TE, where they are mainly infested with the larvae and nymphs of Ixodes ricinus L. and other species of ticks. The pathogenicity of the TE virus for Lacerta ricidis was therefore studied. After administering large doses of the virus, the viremia occurred and the virus was localized in the central nervous system. In four experiments viremia was studied in 20 lizards kept at room temperature. To these animals 100 and 10.000 LD_{50} tick-borne encephalitis virus was administered subcutaneously, each dose in two experiments. In the lizards inoculated with 10.000 LD_{50} viremia lasted from the third to the seventh day and from the fourth to the fifth day respectively. The titre of the virus in the blood ranged from 10^1 to 10^2. No viremia was determined in the lizards inoculated with 100 LD_{50}.

The conditions of hypothermia for survival of the virus were also studied. Twenty lizards were infected with TE virus. The inoculum was administered subcutaneously in the antebrachial region and contained 64.000 LD_{50} of virus. The infected lizards were kept for 28 days in a refrigerator at 8° C. They were rigid, took no food, and seven died during hypothermia. On the 28th day, blood was collected from 13 survivors to determine antibodies. Neutralization test with the sera was negative.

From the first to the tenth day after being kept at low temperature, viremia was studied in every individual lizard at 24 hour intervals. The results of isolation experiments were negative.

The lizards were killed at 6th, 8th, 10th, 12th, 14th, and 21st day. The virus was not found in the central nervous system and in the spleen. It is therefore concluded that Lacerta ricidis could not be a reservoir of TE virus during the winter period.

References:

Gresikova-Kohutova, M., Albrecht, P.: Experimental pathogenicity of the tick-borne encephalitis virus for the Green Lizard, Lacerta viridis, Laurenti 1768, J. Hyg. Microb. Epid. 3, 258, 1959.

Rehacek, J., Nosek, J., Gresikova, M.: Study of the relation of the Green Lizard, (Lacerta viridis Laur.) to natural foci of tick-borne encephalitis. J. Hyg. Microb. Epid. 5, 366, 1961.

Nosek, J., Gresikova, M., Rehacek, F.: Persistence of tick-borne encephalitis virus in hibernating bats. Acta virol. 5, 112, 1961.

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

Results of a 3-year study of Tahyna virus.

After the isolation of the first 5 strains of the virus Tahyna in Czechoslovakia from Aedes vexans and Aedes caspius in 1958 (Bardos-Danielova), the following program for further research was set up: 1) What kind of vertebrates is the reservoir of the virus in nature, 2) What are the biological vectors of the virus Tahyna in nature, 3) What is the role of the virus Tahyna in the human pathology. At the same time, we undertook a serological identification study of this new arbor virus for Europe. In our institute, we have demonstrated that virus Tahyna is not a member of the A and B group of arbor viruses (Bardos, Cupkova, Sefcovicova). Dr. J.S. Porterfield from the Medical Research Council in London showed that this virus does not belong to the C, Bunyamwera, and Bwamba groups. Dr. J. Casals from the Rockefeller Institute in New York was able to classify this virus as a new member of the California encephalitis group.

Birds are the main reservoir of most mosquito borne viruses. This was the reason why in 1959 we have begun to serologically investigate our birds. This work was done in cooperation with ornithologists (Dr. Balat, F. and Hudec, K.). The paper disc method recommended by Karstad and coworkers was used. The eluates were tested in an ic NT according Smithburn in young mice. We found substances neutralizing Tahyna virus in 18-20%.

After experimental s. cut. inoculation of nestling birds and adult birds (positive in the above tests) no virus could be isolated from their blood and no virus neutralizing antibodies were formed (Dr. Simkova, A.). Birds do not appear to be reservoirs of the virus Tahyna in nature.

We found in serological tests of mammals that horses, pigs, and cows living in an area of mass occurrence of mosquitoes have virus neutralizing antibodies in 60%. In white mice, golden hamsters, rabbits, and pigs a viremia was produced and antibodies were formed. The viremia was most pronounced in rabbits and a minimal amount of virus was needed (LD_{50}) in the inoculum to get a viremia of 3-4 days duration with a titer of 3-4 log units (0.03 ml in young mice) (Dr. Simkova). The viremia in pigs was of very low level and it could be ascertained only after ic inoculation of two-day-old suckling mice. Virus neutralizing antibodies were detected in the serum of infected pigs earlier than hemagglutination inhibition antibodies. A serological survey of hares living in an area of yearly mass occurrence of mosquitoes revealed virus neutralizing antibodies in 14 out of 20 tested.

Rabbits and hares are most probably the main reservoirs of the virus Tahyna in nature.

The isolation of the Tahyna virus from the mosquitoes Aedes vexans 30 days after their experimental infection and the transmission of the virus from viremic suckling mice to nonimmune suckling mice indicates that Aedes vexans is a biological vector of Tahyna virus in nature (Simkova, A., Danielova, V., Bardos, V.).

In 1960 we have isolated 3 more strains of Tahyna virus in other areas and thus we were able to calculate the minimal infectivity rate of Aedes vexans mosquitoes in this locality of Czechoslovakia. It was in 1958 and 1960 0.34/1000 mosquitoes.

The titer of Tahyna virus in the original mosquito suspensions of 1960 were in one case 2.0 log units LD 50/0.03 ml in young white mice and in the second case less than 0.5 log units LD 50/0.03 ml.

Trying to get a picture of the role of virus Tahyna in human pathology, we tested human sera of some inhabitants of Czechoslovakia collected by random selection. People living in areas of yearly mass occurrence of mosquitoes had virus neutralizing antibodies in 30.3%, in areas of occasional mosquito occurrence only in 13.3%, and in mountain region only in 2.4%. In the neighboring Hungary and Austria were antibodies present in 50.5 to 61.9%. In Finland, Poland, Rumania,

Yugoslavia, Bulgaria, Albania, Italy, and Uganda, the per cent of positive was less than 10.0%. No virus neutralizing antibodies were found in the sera of inhabitants tested in Holland, Israel, Turkey, Union of South Africa, Australia, and New Zealand.

Trying to find the clinical manifestations in human beings after infection with Tahyna virus, we have concentrated our attention to feverish illnesses occurring every year in summer months in areas of yearly mass occurrence of mosquitoes. From every patient sent to the hospital, blood samples were taken at the admission to the hospital and in the reconvalescence. Some of the results obtained thus far indicate that in some patients suffering from respiratory disease a rise of neutralizing antibodies against Tahyna virus was observed. Further investigations concerning the significance of the Tahyna virus as a human pathogenic agent are in progress.

REPORT FROM DR. H. A. E. VAN TONGEREN PROFESSOR OF MICROBIOLOGY, STATE UNIVERSITY, LEIDEN, AND NETHERLANDS INSTITUTE OF PREVENTIVE MEDICINE LEIDEN, HOLLAND

Netherlands-New Guinea study:

As part of a continued program of a serological survey for the presence of antibodies in sera of autochtonous Papuans against the viruses of Murray Valley and Japanese B encephalitis, Dengue I and II, no antibodies against these viruses could be detected in the serum samples of 405 adult Dani's living in the Baliem valley in the Central Highlands.

Of the Merauke area (southwestern coastal region), table 1 shows the distribution of MVE virus antibody rate by age in the Papuan population and table 2 in the Indonesian group of inhabitants, who in general have resided in that area during their lifetime.

A trial to determine whether either MVE or Jap B encephalitis virus is prevalent was inconclusive. In the first 100 Papuan sera antibody against both Dengue I and II virus were lacking. This indirectly confirms the observation that the possible vectors (Aedes aegypti, A. albopictus, A. scutellaris, and A. polynesiensis) of the dengue viruses have never been found in this part of Dutch New Guinea.

Central European encephalitis study:

Of 48 teals (Anas crecca), 2 shovelers (Spatula clypeata), 2 wigeons (Anas penelope), all migrating birds from the eastern or northeastern part of Europe, serum samples have been collected by puncturing the wing vein. None showed neutralizing antibodies against the Graz strain of CEE virus.

Table 1
Distribution of MVE Virus Antibody Rate by Age (Papuans)

age groups	no. tested	number positive	perc. pos.
0-5	2	1	(50+ 35.4)
6-10	24	18	75
11-20	26	22	84.6
21-30	48	41	85.4
31-40	10	9	90
41-50	10	10	100
51-60	6	5	83.3
≥61	5	5	100
Total	131	110	84

Table 2
Distribution of MVE Virus Antibody Rate by Age (Indonesians)

age groups	no. tested	number positive	perc. pos.
0-5	0	-	-
6-10	37	13	35.1
11-20	56	29	51.8
21-30	48	27	56.25
31-40	27	13	48.14
41-50	34	18	53
51-60	20	11	55
> 60	44	31	70.4
Total	266	142	53.4

REPORT FROM DR. E.A. FREUNDT
STATENS SERUMINSTITUT, COPENHAGEN, DENMARK

During recent years the Rickettsia and Virus Department, Statens Seruminstitut, Copenhagen, has been engaged in studies on the possible occurrence in this country of the RSS complex and other arbor viruses.

Serological Survey

1) RSSE viruses. Using hemagglutination inhibition, complement fixation, and neutralization tests, a survey was carried out on sera from animals and man collected in different areas of Denmark. The sera were tested against a Central European (Czek B3) and an English louping ill strain.

So far, evidence of RSSE virus activity was obtained from one area only, viz. in Bornholm. This is a small rocky island situated in the Baltic, south of Sweden, and a very popular summer holiday resort.

A summary of the overall results obtained with the HI and neutralization tests is shown in the following table. With a few exceptions, all positive sera contained both HI and neutralizing antibodies, and most of them in fairly high titres. In addition, low levels of CF antibodies were detectable in the majority of the human positive reactors, while the serum specimens collected from deer (Capreolus capreolus) were frequently anticomplementary.

HI and Neutralization Tests with RSSE Viruses

	BORNHOLM			Other parts of DENMARK	
Sera tested	No. sera	No. positive	Per cent positive	No. sera	No. positive
Deer	29	24(+3)[x]	83	248	0
Cattle	135	4(+2)[x]	3	134	0
Forest workers	39	11	28	-	
Cases of meningo-encephalitis, etc.	12	8	(67)	418	0
Other population groups	508	7	1.4	305	0

[x]Sera positive in HI or neutralization tests only.

The fairly high incidence of positive sera found in deer and forest workers from Bornholm is particularly noteworthy. On the other hand, the percentage of positive reactors in cattle is remarkably lower than that found in other endemic areas such as in Sweden and Finland.

To date, no acute case of tickborne meningoencephalitis in Bornholm has come to our attention in the course of the present series of investigations. Therefore, a retrospect serological survey was carried out on

sera collected in December 1961 from 12 persons who had within the preceding 4-year period been admitted to the county hospital of Bornholm with a diagnosis of acute meningoencephalitis, representing about 1/3 of the total number of cases of unknown etiology recorded from that period. As appears from the table, RSSE antibodies were found in 8 persons, all of whom had been bitten by ticks shortly before onset of disease. A typical biphasic course had been observed in 4 cases. The clinical course was fairly mild, a transient paralysis of the shoulder region being observed in one case only.

The 508 inhabitants of Bornholm who yielded 1.4% of positive reactors (see table) represent about 1 per cent of the total population of the island.

Comparative HI and CF titrations of a number of positive sera from Bornholm against the Czek B3 and the louping ill strain suggested a more close relationship of the responsible virus with the Central European than with the louping ill group of strains.

2) Group A arbor virus. A preliminary survey of sera from cases of meningoencephalitis and related conditions collected from the whole country for antibodies against Group A arbor virus was negative.

3) Removal of nonspecific serum inhibitor. In the course of the survey for HI antibodies, evidence was obtained of certain limitations of the kaolin adsorption method for the removal of nonspecific serum inhibitors of arbor virus hemagglutinins. Further studies in this field are in progress.

Attempted virus isolations

About 600 hungry ticks (Ixodes ricinus) were collected in the forests of Bornholm in June-July and September 1961. Pools were inoculated intracerebrally into suckling mice, 3 blind passages being carried out. No virus was recovered.

REPORT FROM DR. N. OKER-BLOM
HEAD, DEPARTMENT OF VIROLOGY
UNIVERSITY OF HELSINKI, FINLAND

Tick-borne meningo-encephalitis (TBE) has been studied in Finland since 1954 and from 1957 by a group in this laboratory comprising Dr. M. Brummer-Korvenkontio, Dr. L. Kaariainen, Dr. N. Oker-Blom, Dr. A. Salminen, and Dr. P. Weckstrom. The main endemic regions of TBE are

in the southwest and the southeast of the country although sporadic cases have been diagnosed also in other regions in the south. Studies performed during the last years have been concerned with methods and theoretical problems as well as with the epidemiology and the ecology of the disease. Some of the main results are summarized below.

Methods

A metabolic inhibition test with louping ill virus and a strain of TBE virus isolated in this country in 1957 has been worked out and compared with the conventional tissue culture tube test and neutralization tests in mice. There seems to be a good correlation between the methods. Non-infectious hemagglutination antigen with tickborne encephalitis virus has been prepared in tissue culture. The same method has also been applied for the production of antigens with other arboviruses of group A and B.

Theoretical problems

The non-specific inhibitors to TBE virus hemagglutinin have been shown to be composed of a mixture of two lipids, one of which is free cholesterol while the other may be either a phospholipid or free fatty acids. Similar inhibitors seem to be active also to all the other types of arboviruses of groups A and B so far tested. In studying the difference in agglutinability between hen and cock erythrocytes, it was shown that treatment of cockerels with oestrogenic hormones resulted in complete or almost complete disappearance of the agglutinability of their erythrocytes by several type A and B group arboviruses. The effect of hydrocortisone on the yield of louping ill virus and reovirus has been tested. Addition of hydrocortisone to infected cell cultures has an inhibitory effect on the multiplication of both viruses. Hydrocortisone seems to exert a greater inhibitory effect on the synthesis of louping ill virus than on the homologous interference that possibly occurs.

Epidemiology and ecology

During the past years, two strains of TBE virus have been isolated from acute phase blood of two patients with diphasic meningo-encephalitis. Altogether 16 agents pathogenic for infant mice have been isolated from about 10.000 ticks collected in the endemic regions. Several of these agents seem to be typical TBE viruses. Some of the agents differ, however, from the aforementioned in that they are difficult to adapt to adult mice. They may represent variants of the "true" TBE viruses and are under study.

Serological surveys have been carried out on human and animal sera from different parts of the country. Among the results obtained, it may be mentioned that in the endemic Aland archipelago in the southwest about 13 per cent of the population has HI antibodies to TBE virus. The distribution of immune persons on the different islands is highly different, however, changing from 0 to 25 per cent. This difference between islands is still better reflected in the immunity status of domestic animals and wild life showing a higher degree of immunity. Thus, immunity of sheep and cows on the highly endemic Kumlinge island is of the order of 50 to 100 per cent. The same seems to hold true also of small mammals.

In the apparently less endemic region in the southeast the corresponding numbers of immunes are for human beings 0.8 and for cows 8.8 per cent as tested in the conventional neutralization test in tissue culture.

Ecological studies in the highly endemic Aland archipelago showed that HI antibodies to TBE virus could be demonstrated besides in human sera and in sera from domestic animals and small mammals also in two out of four sera from blackbirds (Turdus merula) and in one of two sera from red-breasted mergansers (Mergus serrator). Birds may thus participate in the dissemination of virus from island to island.

Further studies of the epidemiology and ecology include a serological survey of about 7,000 human sera and about 2,600 cattle sera collected by random from the whole country. In addition, 150 blood samples and organs from migrating birds have been collected in the archipelago for determination of TBE antibodies and for virus isolation attempts.

In addition to the studies mentioned above, about 2,500 mosquitoes of nine species have been collected in a region in South Finland within which one person has been shown to have antibodies to Tahyna virus. The serological tests have kindly been performed by Dr. V. Bardos, Bratislava. From one pool of Aedes cinereus mosquitoes, an agent pathogenic for infant mice was isolated. The identification of the agent is in progress.

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

During 1959, 1960, and 1961, field work has been undertaken in attempt to define vectors and reservoirs of Powassan virus in two areas of northern Ontario, Powassan-North Bay and Manitoulin Island, where there is evidence of clinical and/or subclinical evidence of human infection with Powassan virus. Neutralizing antibody to Powassan virus has been found in sera from 11 of 1008 residents of northern Ontario including 5 of 194 from Powassan-North Bay, 5 of 157 from Manitoulin Island and 1 of 657 residents of other districts.

In the Powassan district, sera from 10 of 123 mammals or birds including 6 of 29 chipmunks (Tamias striatus) and 4 of 30 red squirrels (Tamiasciurus hudsonicus) neutralized Powassan virus. Antibody was detected in animals collected during summer and fall of 1959, spring and fall of 1960, and early spring and fall of 1961, but immature animals collected during late spring and early summer of 1961 did not have antibody. Small numbers of larval and nymphal forms of Ixodes sp. ticks (I. angustus, marxi or cookei) were removed from 2 squirrels taken in September 1960 and from 1 squirrel each in April, May, August, and September 1961. Powassan antibody was found in the tick-infested squirrel taken in September 1961. ~~Powassan antibody was found in the tick-infested squirrel taken in September 1961~~. No virus was isolated from any of these ticks, nor from other ectoparasites such as fleas, nor from blood clots of 56 animals.

On Manitoulin Island, studies undertaken during 1960 and 1961 in conjunction with Dr. Keith Ronald of Ontario Agricultural College, Guelph have the following results. Neutralizing antibody to Powassan virus was found in sera from 6 of 76 squirrels, 14 of which carried Ixodes sp. ticks, 2 of 32 chipmunks, 5 of 326 snowshoe hares of which all but 16 collected during winter were heavily infested with Haemaphysalis leporis-palustris ticks, and 0 of 118 other mammals. Powassan virus has not yet been isolated from wild-caught ticks.

Further field investigations will be undertaken in attempt to extend knowledge regarding vectors and reservoirs at Powassan and on Manitoulin Island during 1962.

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

The results of the 1961 Massachusetts study in cooperation with CDC are reported elsewhere in this newsletter by Richard O. Hayes. Thirty-five unidentified agents and 3 WE strains were found by the Greeley laboratory while no unidentified agents and 27 WE isolates were found by the Massachusetts laboratory from 1959 and 1960 arthropods collected in this state. The different proportions of isolates are not readily explained by selection of material. It seems possible that the methods used for isolation (mice in Greeley; chick embryo tissue cultures in Mass.) may have been selective.

It is interesting that 26/27 WE isolates in 1959 were from Culiseta melanura. The degree of WE activity in 1961 in Massachusetts is to be explored by performing HI and plaque reduction tests on 328 sera from human volunteers, 333 domestic fowl, and possibly also the bloods of 300 or more wild birds. EE neutralizing antibody tests on these sera have been completed.

The study of the duration of HI and neutralizing antibodies reported in the last newsletter is nearing completion. The disappearance of HI antibody in inoculated pigeons in 6 months was followed by a slow fall in neutralizing antibody titer over the next year and a half when it was detectable only in low dilutions. On the other hand, chickens still have HI and neutralizing antibody in high titer 2 years after inoculation with EE virus.

The utility of the HI test for field surveys for antibody is being investigated. The first question we asked ourselves is: "Is it an efficient screening procedure for antibody of EE and WE?". Under certain circumstances, the answer is no. Thus, of 78 crows of undetermined age, only 21 had HI antibody for EE while 40 had neutralizing antibody.

HI and Neutralizing Antibodies:	Gardiner Turkeys bled 11/17/59	
Group I	negative in both tests	25
Group II	HI: + WE range 1/40-1/640, confirmed by neutralization as WE	13
Group III	HI: + WE & EE cross reactors confirmed by neutralization as WE	2
Group IV	HI: + WE or EE or both, neither EE nor WE neutralizing antibody	10

	Number	HI E	HI W	Neut. PR E	Neut. PR W
Group III					
	1167	640	320	-	+
	1170	160	320	-	+
Group IV					
	1160	320	80	-	-
	1174*	640	80	-	-
	1180	320	40	-	-
	1203*	640	640	-	-
	1190	640	640	-	-
	1161	40	<20	-	-
	1164	160	<20	-	-
	1169	80	<20	-	-
	1171	160	<20	-	-
	1172	20	<20	-	-

*confirmed by reference method of acetone extraction

The second question in regard to the field utility of the HI test is: "Does its well known cross reactivity, so helpful in the study of viral relationships, limit its usefulness as an indication of activity of a particular virus?". Our answer illustrated by the 50 turkeys in the table is yes. The high titers in the absence of neutralizing antibody (by plaque reduction) suggest the possibility of related agents rather than EE or WE as incitors of antibody.

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

Studies on the ecology of EEE have continued during 1961 in two main areas: (A) Bionomics of mosquito-vector populations in the field, and (B) the study of mosquito biology in the laboratory.

A. Field studies.

(1) Collections were conducted throughout the 1961 mosquito season to follow the development of the mosquito population and to determine unusual buildup of potential vector species. Heavy snow accumulation melted rapidly in the spring and, because of more than average precipitation, breeding places were flooded This accounted for an

abundant crop of springtime mosquitoes consisting chiefly of Aedes abserratus, A. stimulans, and A. canadensis. However, as the season progressed, the occurrence of semi-drought resulted in a diminishing mosquito population. The late summer and fall mosquito populations were considerably less than those during the previous three years.

(2) Special study of larvae from breeding places in a Connecticut suburban woodland community was conducted (in collaboration with Dr. Leonard Parente and Mr. Harold Jaynes, Hamden Dept. of Health) to determine the proportion of mosquito breeding sites that produced potential encephalitis vector species.

From 180 breeding places located throughout the town, 267 samples of larvae were collected and identified during the summer season. Of the 14 species represented, the five most frequently encountered were species that are either known capable of transmitting eastern or western encephalitis virus, or ones from which virus has been isolated: Culex restuans (64/267), C. pipiens (55/267), C. territans (54/267), C. salinarius (44/267) and A. vexans (23/267) constituted 89.5 per cent of the samples (267) taken. It was somewhat surprising to find C. restuans more commonly encountered than C. pipiens. Likewise, C. territans was almost as frequently represented as C. pipiens in the suburban woodland residential areas. In view of the fact that virus has recently been isolated from this species, it would seem pertinent to learn more about its ecology.

C. salinarius (44/267) and A. vexans (23/267) were the two other potential vector species frequently encountered. Though particular attention was given to scouting for Culiseta melanura breeding places, none was encountered.

(3) Study was initiated of mosquito larvae that develop during the late summer and fall season. The purpose was to determine ecologic relationships of mosquito larvae to other aquatic animals and to birds that frequent the aquatic habitat. In many of the swampland sites where late summer mosquito larval populations build up, there was an interesting association with aquatic snails--particularly Melanupus bidentalis, the salt marsh snail--that was considered worthy of further study.

(4) In cooperation with the State Board of Fish and Game and the Yale University Arthropod-borne Virus Laboratory team, special investigations were conducted at the Albert Soli Pheasant Farm in Plainville, Conn., because of the occurrence of EEE activity there during 1959. The farm was visited twice each week from mid-August until October 28 to observe its 7000 pheasants and to collect mosquitoes. Two New Jersey light traps were operated one night each week, and

biting collections were attempted on each visit. Due to the unusually dry mid- and late summer weather, and the initiation of a mosquito control program in the town, very few mosquitoes could be found. None were taken in light traps during August and September, and virus activity that occurred in one of the pheasant pens was, therefore, unexpected. When the first pheasant deaths occurred late in the season (Oct. 2) the vicinity of the farm was searched for diurnal resting places and larval breeding sites. No adult mosquitoes were found, but larvae of C. restuans were collected in one abandoned rain barrel. While it cannot be said that no mosquitoes existed prior to and during the epidemic of EEE at the farm, it is certain that during the month prior to and throughout the duration of transmission the mosquito population was at an extremely low level.

B. Laboratory study on mosquitoes.

(1) In the laboratory, study was initiated to elucidate the snail-mosquito larvae relationship. Larvae of C. restuans and A. aegypti were set up in 2 gallon aquarium tanks with snails (Lymnaea spp.) to simulate conditions observed in the field where there were high larval populations concentrated in diminishing aquatic sites as the water receded in the fall season. In this situation, snail feces became an attractive source of food for the mosquito larvae.

(2) In study of adult mosquitoes in the laboratory, a convenient procedure utilizing chicken egg embryos has been developed for feeding blood and virus inoculum to mosquitoes.

The chorio-allantoic membrane of the chicken egg, after 9 days' incubation, provided an excellent source of blood for mosquito feeding in laboratory experiments. A window cut through the shell over the air sac exposed the membrane where a rich blood supply in numerous arteriols, venules, and capillary network is present in the mesodermal layer. When this was placed in contact with the screen covering of mosquito cages, the mosquitoes obtained blood by probing through the screen into the living membrane.

When the embryo was removed from the egg, the inside of the egg washed and inoculum substituted (10% mouse brain in Hank's B.S.S. diluted in 5% sucrose solution), A. aegypti females became engorged with it by probing through the chorio-allantoic membrane.

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

Virus Sub-populations Comprising Various Strains of WEE:

Variations in antigenic and biologic properties often exist between isolates of a given arthropod-borne virus. In order to better define these variations and using WEE as a model, the virus populations which comprise ten WEE strains were "disassembled." The virus strains were plaque purified in chick embryo cell cultures and stock virus and immune serum prepared from each clone. Then, each unpurified strain was plated in the presence of immune serum prepared from its plaque counterpart. Viruses not entirely suppressed by the immune serum would form plaques. Virus sub-populations were recovered step-wise in this fashion until the development of all plaques was suppressed by the single and/or combined immune sera prepared from each plaque population which had emerged.

The results of these analyses indicated that most of the WEE strains under study were composed of two and often more virus sub-populations, usually interrelated antigenically within and between strains but distinguishable by the method of analysis described. Comparison of sub-populations between strains has revealed both qualitative and quantitative differences. Evidence is being accumulated which seems to indicate that the antigenic as well as biologic properties inherent in each WEE strain studied are a reflection of and are definable by the nature and concentration of the virus sub-populations of which each strain is comprised.

Hemolytic Activity by Eastern and Western Equine Encephalomyelitis Viruses:

During the course of assaying tissue culture preparations of EEE and WEE for hemagglutinating activity, it was observed that hemolysis of chick red blood cells also occurred in addition to hemagglutination. Dr. Karabatsos, of our laboratory, has pursued this initial observation and found that hemolytic activity of virus containing culture fluids could only be revealed after freezing and thawing and only slightly after dialysis. The hemolytic activity was inhibited by specific viral antibody. Both hemagglutinating and hemolytic capacities were destroyed by heating the viruses at 56° C for 30 minutes. Slight changes in the pH at which assays for both activities were performed, resulted in a significant loss of hemolytic activity. Titration of WEE virus in the presence of calcium ions or treatment of the virus with trypsin or ether reduced hemolytic activity without significantly changing the hemagglutinating capacity of the virus.

Treatment of EEE virus under identical conditions yielded results which differed significantly from those obtained with WEE virus. These differences were interpreted as being due to a greater binding of EEE virus by non-specific inhibitors present in the infected tissue culture fluids. Treatment of the red blood cells with receptor destroying enzyme did not affect either the hemolytic or hemagglutinating capacities of both viruses, whereas trypsin treatment of the red blood cells resulted in an increased hemagglutination titer.

REPORT FROM DR. ROBERT P. HANSON
DEPARTMENT OF VETERINARY SCIENCE
UNIVERSITY OF WISCONSIN, MADISON, WISCONSIN

For the second consecutive year in Wisconsin there appears to be a decrease in the prevalence of eastern viral encephalitis based both on attempts to isolate the virus and serological surveillance.

Chicken embryo lethal or mouse lethal agents were not isolated from 7 birds, 4 mammals, and 53 reptiles and 194 pools of arthropods. The arthropods included the following genera with the number of species given in brackets: Aedes (9) Anopheles (3), Culex (3), Culiseta (1), Mansonia (1), Culicoids (4), Cnephia (1), Eusimulim (2), Simulim (2), Hippelates (1), Dermacentor (1), and Ixodes (1).

Virus neutralization tests for eastern viral encephalitis (EVE), western viral encephalitis (WVE), vesicular stomatitis virus - New Jersey serotype (VSV-NJ), vesicular stomatitis virus - Indiana serotype (VSV-Ind) and quail bronchitis virus (CELO) were performed in chicken embryos and in tissue culture. Only species of which one or more individuals possessed antibodies are listed in the table.

Virus Neutralization
Number positive/number tested

	EVE	WVE	CELO
Bronze Grackle	0/4	1/4	
Pheasants	8/21		
Ruffed Grouse	1/9		
Black Billed Cuckoo	1/1		
Turkey	1/20		65/100
Beaver	1/7		
Otter	1/2		
Fox Squirrel	1/1		
Deer	3/400	0/400	

In cooperation with Wayne Thompson of Wisconsin State Laboratory of Hygiene, one human case of clinical encephalitis was investigated. This individual was a Negro woman employed in a poultry processing plant. Acute phase serum taken in March when her illness was diagnosed and convalescent serum obtained in May and September had a complement fixation titer of 1-64 or higher for eastern viral encephalitis.

Acute and convalescent serums obtained in Wisconsin in the summer of 1961 from four horses reported to have encephalitis were negative for EVE and WVE antibodies. Convalescent serums from eight other horses were also negative for EVE.

Over 4,500 arthropods of 13 genera were collected from light traps and from animal bait traps. Turkeys, pheasants, chickens, doves, and two species of snakes were used as animal baits. Turkeys were most attractive. The snakes did not attract any biting arthropods.

The presence of a leucocytozoon parasitemia did not appear to augment the susceptibility of a group of experimental turkeys to eastern viral encephalitis. Resistance of pheasants to eastern viral encephalitis increased rapidly with age. During the first three weeks the chicken embryo lethal dose and the subcutaneous lethal dose for the pheasant were approximately equivalent. At three months even 10,000 embryo lethal doses were not sufficient to regularly kill pheasants.

Ground squirrels inoculated with EVE and placed in hibernation developed in some instances a persisting infection. The virus grew more slowly in hamster kidney cells at low temperatures than at 37° C.

A virus (590) was isolated in mice in June 1955 from the brain of a cow that had died of what was suspected to be rabies. The virus was tentatively identified at CDC (Kissling) as vesicular stomatitis virus. The USDA at Beltsville (Mulhern) did not concur in the identification.

The virus was cytopathogenic for HeLa cells (titer 10^6), pathogenic for chicken embryos (titer 10^6) and for mice (i. c. titer 10^7). Vesicular lesions were produced by intradermal inoculation of the foot pad of guinea pigs, the tongue of a steer and tongue of a chicken. The lesion in the chicken tongue was atypical. The steer inoculated on the tongue died in seven days. Some of the inoculated guinea pigs died. VS is not known to be lethal to these animals unless it is inoculated directly into the brain.

Antiserum to 590 neutralized VSV-NJ and eastern viral encephalitis. Antiserum to VSV-NJ and antiserum to EVE failed to neutralize 590. It appears that the culture 590 is a mixture of two viruses and was probably

a mixture in the second mouse passage. VSV-NJ was not being studied in the laboratory in which the isolation was made; it is not a latent virus in mice and consequently the only source would appear to be the brain of the cow. This would be the first known isolation of VSV from the CNS of a naturally infected animal. Possibly concurrent infection with EVE contributed to death of the original cow and the experimental inoculated steer. Study of what appears to be an instance of viral synergism is continuing.

A continuing program of surveillance of arbovirus activity in southeast Georgia is carried on by the Epidemiology Service, Georgia Department of Public Health, with the cooperation of this laboratory. Arthropods were identified and virus isolations attempted in cell cultures or mice at the Virus Research Laboratory, 1101 Church Street, Waycross, Georgia. Eastern encephalitis virus was isolated from a pool of 23 Culiseta melanura collected August 16, 1960, in Brantley County, Georgia. An equine case of encephalitis had occurred on an adjacent farm August 1 1960 but attempts to isolate virus from the brain of the diseased horse were not successful. Pools of arthropods collected in this area from April to October 1961 are now being processed. To date, eastern encephalitis virus has been isolated from a pool of 30 Culiseta melanura collected July 31, 1961, and from a pool of 20 Culiseta melanura collected August 9, 1961, at a point approximately three-fourths mile from the site of the 1960 isolation. Other viral agents have been isolated from a pool of 9 Culiseta melanura collected August 9, 1961, from a pool of 20 Culiseta melanura collected August 9, 1961, and from the viscera of a freshly hatched chicken which died after exposure to arthropods August 9-11, 1961, in this same area. Identification of these latter agents is still incomplete. All isolations from Culiseta melanura were made from pools of unengorged females.

REPORT FROM DR. WALTER R. GUSCIORA, ENTOMOLOGIST BUREAU OF VETERINARY PUBLIC HEALTH NEW JERSEY DEPARTMENT OF HEALTH, TRENTON, N.J.

A narrowing down in the choice of test specimens employed in an inquiry into possible EE and WE infection in non-haemophagous insects:

It is generally accepted that fresh-water swamps, where Culiseta melanura breeds, serve as enzootic foci for the transmission of eastern encephalitis. The Taunton, Massachusetts, EE field laboratory, for example, has presented strong evidence to support this idea with its results of HAI antibody tests with sera collected from sentinel chickens at intervals during one season:

Sentinel Locations	Per Cent of Sera with HAI Antibodies		
	Aug 3-11	Aug 17-25	Oct 12-16
Site 1 (center of an enzootic swamp habitat)	19	35	65
Site 2 (edge of swamp)	0	14	35
Site 3 (1/2 mile from swamp)	0	7	36
Site 4 (hillside farm 2 miles from swamp)	0	2	35

Transmission in the swamp was nearly twice as great when compared to sites outside the swamp.

The Yale Section of Epidemiology and Preventive Medicine has attempted to demonstrate the presence of EE virus in non-hemophagous insects captured in the vicinity of infected pheasant pens with negative results. However, Yale experiments have shown that the larvae of certain non-hemophagous insects are susceptible to infection by a number of arboviruses. It is therefore conceivable that some unknown epidemiologic aspect might be involved in the natural history of the EE virus which may include non-hemophagous insects.

The Yale Section has shown that nestling birds may be infected by the ingestion of larvae of non-hemophagous insects. One successful experiment was as follows: An approximately 12-day old robin was fed with larvae which were inoculated two weeks previously with EE virus. The bird developed viremia followed by paralysis and on the 12th day became moribund and was sacrificed. EE virus was recovered from the brain and liver. On this basis, it is reasonable to assume that a predatory insect may become infected if it ingests another infected insect and in addition, that a nestling bird may also become infected under natural conditions.

Suggested choice of test specimens: That the following three highly predaceous insects (that is, predators which feed on any insect they can physically cope with and on any insect which may come their way) be tested for EE and WE viruses: Robber flies (Asilidae), dragon-flies, and water-striders. The collections should be carried out within fresh water swamps of known EE enzootic areas. The fresh-water swamp ecotype would presumably have the highest infective rate for predatory insects. To indicate that dragon-flies (mosquito hawks), robber flies, and water striders can become infected by way of their natural predatory

habits would add an important link to the limitedly known epidemiologic chain. It would also show that the range of infectivity may exist throughout a broad arthropod spectrum and, also, that aside from the mosquito-bite route a higher EE reservoir potential in birds may be built up by the ingestion of infected insects. All of the above would stem from the hypothesis that these viruses may have their fundamental cycles in various aquatic organisms within enzootic foci of infection.

REPORT FROM FLORIDA STATE BOARD OF HEALTH JACKSONVILLE, FLORIDA

After a year of unusual quiescence, arbovirus activity was again apparent in Florida with a series of interesting outbreaks in the fall of 1961. EE virus activity was confirmed in pheasants and wild birds and SLE or a closely related group B virus was demonstrated in humans, mosquitoes, domestic and wild birds.

During October, a severe die off occurred in a flock of pheasants in Brevard County on the Central-East Coast section of the State. The preliminary diagnosis of EE infection was reported by the Florida Department of Agriculture Animal Diagnostic Laboratory in Kissimmee. This was based on histologic examination and inoculation of eggs with brain material. The subsequent investigation by the Florida State Board of Health field and laboratory team involved the collection and examination of 97 domestic bird sera, including quail, pheasants and turkeys; 50 wild birds, 9 mammals, one snake and 58 pools of mosquitoes. Using a "wet chick" screen and serial mouse passage with SN identification of the isolates, the virologic laboratory of the Florida State Board of Health reported nine isolations of EE; eight of these from pheasants and one from a bluejay. A surveillance for cases in humans and large animals in the surrounding area was carried out with negative results.

On the western side of the state in the Tampa Bay area, an unusual number of human encephalitis cases were noted from October through early December. A total of 25 cases occurred with seven deaths. Of the total cases, six were in Sarasota County, ten in Manatee, and nine in Pinellas. The latter county was the site of the extensive SLE-like outbreak in 1959 involving 68 clinical cases. CF and HI antibody studies were carried out by the Florida State Board of Health and Communicable Disease Center, USPHS, virology laboratories using sera collected from 20 of these individuals. Six demonstrated a 4-fold or greater rise in titer against SLE antigens in testing paired sera; 10 showed either a significant titer on a single serum or a stable or falling titer on paired sera. All sera tested were negative against EE, WE, Dengue 2, Murray Valley, and LCM antigens. No viral isolations were obtained from brain tissue collected from three of the seven deceased patients. Four of

the seven fatal cases were autopsied and all revealed the typical histopathological findings of acute viral encephalitis.

Certain epidemiological associations of interest were noted amongst the cases. All were white, 15 were female and 13 over the age of 65. The youngest was a 13-year old white male. In Pinellas and Manatee Counties, there was a rough geographic concentration of cases, in both instances associated with fresh water mosquito breeding sites. Domestic chicken flocks were found on the premises of two households and sera from these birds indicated recent infection with SLE virus.

Extensive collections from the biologic environment of human cases were carried out by field teams of the State Board of Health and Communicable Disease Center, U. S. Public Health Service. Despite an extensive drought, a moderate number of mosquitoes were obtained as late as the second week in December. These were predominantly A. crucians., C. nigripalpus, and C. salinarius. These pools have been screened in wet chicks and suckling mice and to date a single pool of mixed Culex species has yielded a viral agent identified by the virology laboratory of the Florida State Board of Health as belonging to the "B" group.

Although the annual fall migration of birds had passed before the outbreak, several different species of wild and domestic birds were caught and bled. To date, no viral isolation has been reported by the Communicable Disease Center laboratory. However, there is considerable antibody against a Group B agent closely related to SLE in sera collected from mammals and birds in a zoo at the approximate geographic center of the Pinellas County cases. There were also EE antibodies found in chickens in St. Petersburg and Sarasota and WE in a parakeet in the above mentioned zoo. Final reports are not yet available from the Communicable Disease Center but information as per telephone conversation indicates serological evidence of infection with the same viruses in these animals.

During 1961, studies were also undertaken to detect evidence of arbovirus in mosquitoes collected in the vicinity of Vero Beach, Florida, and Myakka River basin. Over 1,000 mosquitoes were collected from the latter area in August and 20,000 mosquitoes from Vero Beach during the summer months of July, August, and September. To date, no viral isolations have been obtained from these mosquito pools by suckling mice inoculation.

An application for an NIH grant to permit more intensive year-round investigation of virus-mosquito relationship was approved but could not be funded due to the general reduction of NIH research monies.

Submitted by Mr. John A. Mulrennan, James E. Scatterday, D. V. M., Nathan J. Schneider, Ph. D., A. L. Lewis, D. V. M. and James O. Bond, M. D., Florida State Board of Health.

REPORT FROM DR. ARTHUR N. GORELICK, VIRUS & RICKETTSIA DIVISION, U. S. ARMY CHEMICAL CORPS BIOLOGICAL LABORATORIES FORT DETRICK, FREDERICK, MARYLAND

I. Immunological Studies on Arboviruses.

A study on the immunologic overlap among arboviruses has been initiated recently by Dr. W. P. Allen in these laboratories. Emphasis has been placed particularly on (1) the degree and spectrum of cross-protection demonstrated in animals that have been immunized with viable arboviruses and (2) the relationship between protective and serologic overlap. Preliminary investigations have been concerned with protective overlap among Group A viruses, particularly the viruses of Chikungunya, Mayaro, Semliki, Venezuelan equine encephalitis (VEE), Eastern equine encephalitis (EEE), Sindbis, and Western equine encephalitis (WEE). The strain of Chikungunya virus had been through 172 mouse brain passages and was lethal for adult mice. Neither Mayaro nor Sindbis virus was lethal for adult mice.

Results thus far have indicated that cross-protection among Group A viruses in mice is extensive, often exceeding that which would be expected from the ability of prechallenge serums to neutralize the challenge viruses. The accompanying table summarizes some of these results. In these experiments mice were challenged three weeks after immunization. The data collected indicated that: (1) immunization with any of the Group A viruses tested, except WEE, elicited substantial protection against the lethal effects of Chikungunya and Semliki viruses; (2) immunization with attenuated VEE virus or EEE virus protected mice against challenge with all but WEE virus; (3) immunization with Sindbis virus resulted in detectable protection against all the challenge viruses and was the only immunizing virus to cross-protect against intracerebral challenge with WEE virus; (4) substantial immunity against all challenges resulted when mice were simultaneously immunized with Sindbis and attenuated VEE viruses, and (5) correlation of protective with serologic overlap occurred only among mice immunized with Mayaro or Chikungunya viruses. No protective overlap between Group A viruses and viruses of other serologic groups has yet been observed, but these latter studies were limited. When mice, immunized with Chikungunya or attenuated VEE viruses, were challenged three months after immunization, the degree of cross-protection appeared to be slightly less than that found

three weeks after immunization. The spectrum of cross-protection, however, remained unchanged.

II. Heat Inactivation of Two Arbor Viruses and Their Derived Infectious Nucleic Acid Complexes

The infectious ribonucleic acid (IRNA) from the viruses of Venezuelan equine encephalitis (VEE) and Eastern equine encephalitis (EEE) have been successfully isolated in fairly high titre (10^6 - 10^7 plaque-forming units/ml). In comparing the characteristics of these two substances, Dr. L. A. Mika, J. E. Officer, and A. Brown made the following interesting observations.

The rates of inactivation at 50 C (over a 7-hour period) were determined for the following virus moieties of VEE and EEE: (1) partially purified intact virus, (2) IRNA complexes isolated from (1) above, (3) hot phenol-extracted infected chick embryo and (4) cold phenol-extracted infected chick embryo. The following results were obtained: The inactivation curve of intact VEE virus was biphasic, i.e., initially slow then increasing steeply in slope. The curve for the less resistant EEE virus was also biphasic, but phases were reversed when compared with those of VEE virus. Curves describing the recoverable IRNA from both heated intact virus preparations above were complex, requiring further investigation. Additional experiments show, thus far, that inactivation curves of hot phenol-extracted infected tissue for both viruses (presumably containing both precursor and virus IRNA) were biphasic, i.e., initially slow then increasing in slope. By contrast, the curve for cold phenol-extracted infected tissue (containing only precursor IRNA) was monophasic, approximating the steeper portion of the curve for the hot phenol-extracted tissue. Two possible explanations are that precursor IRNA, and IRNA from the intact virus are either different IRNA species or one species combined with moieties of differing complexity. A comparison of all the curves leads to the following additional conclusions: (1) The difference in heat resistance between the two viruses can be ascribed to differences in the lability of their lipoprotein coats. (2) Heat inactivation is usually first directed against the lipoprotein coat and then against the nucleic acid.

PROTECTIVE AND SEROLOGIC OVERLAP AMONG GROUP A ARBOR VIRUSES IN MICE

IMMUNIZING VIRUSES	VIRUS ROUTE[a]/ DOSE[b]/	PC[c]/ SN	PRECHALLENGE ANTIBODY AND RESPONSE TO CHALLENGE																			
			Chikungunya IC			PC SN	VEE SC			PC SN	EEE IC			PC SN	WEE IC			PC SN	Semliki IP	
			10^4	10^3	10^2		10^5	10^3	10^1		10^4	10^2	10^0		10^4	10^2	10^0		10^4	10^2
Chikungunya		+	4[d]/	4	4	±	0	1	0	-	0	0	0	-	0	0	0	+	3	3
VEE-attenuated		-	4	4	4	+	4	4	4	-	3	3	4	-	0	0	0	-	4	4
EEE		-	3			-		4		+		4		-		0		-		3
WEE		-	2			-		0		-		0		+	4	4		-		0
Mayaro		+	4	3	4	±	4	4	4	-	0	0	2	-	0	0		+	4	4
Sindbis		-	4	3		-	4	3	3	-	1	1	2	±	3	3	4	-	3	4
VEE-attenuated and Sindbis			4				4	4	4		3	2	4		0	1	4		4	4

a. IC, SC, and IP refer to intracerebral, subcutaneous, and intraperitoneal routes, respectively.
b. Approximate number of $ICLD_{50}$ for 10 to 14 gm mice.
c. Ability of prechallenge immune mouse serums to neutralize 100 LD_{50} of respective challenge virus.
d. Relative protection of immune mice:
   4 = 75 to 100 per cent)
   3 = 50 to 75 per cent) difference in survival between principals and controls following challenge
   2 = 25 to 50 per cent)
   1 = 10 to 25 per cent)
   0 = less than 10 per cent

REPORT FROM DRS. ROBERT W. McKINNEY AND GEORGE R. FRENCH, VIROLOGY DIVISION, U.S. ARMY MEDICAL UNIT, FORT DETRICK, FREDERICK, MARYLAND

Work has been progressing in this laboratory toward development of a procedure to prepare safely, non-infectious arbovirus CF and HI antigens.

Non-infectious CF and HI antigens have been prepared for VEE, WEE, EEE, YF, and TF-21 strain Langat virus.

Using Trinidad strain VEE as the pilot virus, the problem has been approached at two levels, i.e., (1) development of a closed system for acetone-ether extracting infectious mouse brain, and (2) inactivation of VEE virus with Beta-propiolactone.

(1) A closed system for acetone-ether extraction of infected mouse brain:

In view of the hazards associated with preparing these antigens, it was felt desirable, if not necessary, to develop a closed system for this procedure. A glass apparatus utilizing a sintered glass filter has been developed, tested, and shown to be satisfactory for this purpose.

This apparatus allows the entire procedure to be carried out under negative pressure from the time the infected brain material is homogenized until the extracted antigen is resuspended and ready for clarification. The apparatus remains closed throughout the entire procedure, and personnel are never exposed to the potentially contaminated waste products of acetone and ether. The design includes provisions for controlling temperature.

(2) BPL inactivation of VEE:

Using the guinea pig and suckling mouse as isolation hosts, a lethal dose of BPL was determined for Trinidad strain VEE virus. It was found that the minimum concentration of BPL required to completely inactivate virus in a 25% suspension of suckling mouse brain in borate saline - 0.05M Na_2HPo_4 pH 8.9 - was 0.07% (vol./vol.).

Within the range of 4°C to 37°C, temperature was found to have no effect on the concentration of BPL required to inactivate VEE virus. However, our data indicate that inactivation at the lower temperature results in a more stable antigen. The pH could not be demonstrated to have any effect on BPL levels required within the range of pH stability of the virus (pH 7.0-pH9.0). We now routinely prepare 25% whole mouse

brain antigens in borate saline-phosphate buffer pH 8.9 and acetone-ether extracted antigens in a similar buffer at pH 8.7. Inactivation is accomplished with a final concentration of 0.2% BPL at 4°C. This quantity is approximately three times the minimum inactivating dose, and is employed to provide a greater margin of safety. Antigens inactivated with this quantity of BPL are equal in quality to those treated with the minimum dose. The above indicated pH's are designed to yield a final product of pH 7.2.

These antigens when prepared as described above are (1) serologically identical to untreated antigens; (2) are comparable to untreated antigens in stability; (3) can be dried in the frozen state or stored at -50°C; and (4) are as high titered (CF and HA) as untreated antigens.

We feel the glass apparatus, when used in combination with BPL materially reduces the hazards normally associated with preparation and utilization of arthropod-borne serologic antigens, and at the same time yields a completely satisfactory product.

Manuscripts are now in preparation describing the methodology and equipment utilized in these procedures.

REPORT FROM DR. ALEXIS SHELOKOV, CHIEF LABORATORY OF TROPICAL VIROLOGY, NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES BETHESDA, MARYLAND

Following the demonstration of CPE and plaque production in pilot experiments employing Bunyamwera group viruses in a monkey stable (MS) cell line, master pools of each of the 8 prototypes were prepared from harvests of the 1st and working pools from the 2nd TC passages. All viruses produced CPE and virus concentrations of 5 to 7 logs in 72 hours, or less. In terminal dilutions of tube titrations, complete development of CPE required 4 days. CPE characteristics were similar for all members except Kairi. Mouse LD-50 and TC CPD-50 of TC harvests compared favorably. One of the conditions necessary for maximum infectivity titers by TC CPD-50 seems to be the use of "young" cell cultures - 3 to 6 days incubation after preparation from cell suspension.

Thus far, studies of the biology of Bunyamwera virus in MS-cell cultures indicate a generation time of 2 hours or less, and relative thermostability. Direct measurement of virus adsorption onto MS

cells has not produced anticipated results and is under further study. The current program is directed toward plaque purification of virus strains in the group.

Pilot studies with Oriboca, Caraparu, Murutucu, and Marituba of group C have also been promising. CPE and virus production of 5 to 7 logs were obtained in 72 hours, or less, after infection of MS-cell cultures. CPE was similar to the Bunyamwera group, but terminal dilutions required one more day for complete development than with the Bunyamwera group.

Strain L cells adapted to serum-free 2X Eagle's medium by Marchant (strain L(M)) have been infected with Bunyamwera virus and passed serially for a total of 9 passages. Relatively high dosage (1/50 multiplicity or greater) has produced 4 to 6 logs virus concentrations and CPE in 48 to 72 hours. Pilot studies of these harvests as antigens for immune ascitic fluid production are in progress.

Carrier cultures of Dengue 1 and Japanese encephalitis viruses in human skin cells have been transferred from NMRI (with Dr. N. H. Wiebenga) and continued at LTV. Considerable difficulty has been experienced in maintaining "healthy" cultures consistent with the experience of the past four years (variations in physical environment and facilities?). Virus has been persistently recovered from both cultures, but without apparent modification of pathogenicity (neurotropism in suckling mice). Virus reproduction of these TC adapted viruses creates no significant disturbance of MS-cell cultures.

The Arthropod-borne Virus Section of LTV in Bethesda accepted a responsibility to the Subcommittee on Virus Reagents to prepare immune mouse ascitic fluids (according to the protocol modified at MARU) for production of polyvalent Bunyamwera grouping reagent. Initial studies with the Bunyamwera prototype virus are in progress. The Section is also responsible for part of a collaborative study to explore the feasibility of ascites production in guinea pigs and of the polyvalent immune ascitic fluids with Bunyamwera and B groups. So far attempts to produce ascites have not been successful in the "general purpose" GP strain. An inbred strain said to be more susceptible will be next used.

The Takatsy "microtitrator" technique for HI tests has been compared with standard tube and lucite plate using several viruses of group A, B, and C. The results are promising, particularly with goose rather than one-day-old chick erythrocytes. Recently most of

the exploratory work has been with the Bunyamwera viruses; unfortunately, so far the HA antigens are at low titers.

Because to our knowledge only a limited serological study of Puerto Rican population has been made (Casals, 1956) we welcomed the opportunity of a collaborative study with Dr. D. Mendez-Cashion, University of Puerto Rico School of Medicine. To establish a serological base line, 150 sera from healthy adults are being CF and HI tested in our Bethesda and Canal Zone Laboratories with a battery of Group A, B, C, Bunyamwera, and a few other antigens. The results will determine the selection of antigens for testing of the paired sera from a large group of well studied pediatric patients to explore the possible role of arboviruses in the etiology of ill-defined neurological disease in children.

Dr. Jacob A. Brody, formerly of MARU-LTV in Panama, is now on a 6 months' official US-USSR Exchange Fellowship in Moscow. He is assigned to Professor Chumakov and Dr. Levkovich of the Institute of Poliomyelitis Research and Neuroviral Infections to work with laboratory and epidemiologic aspects of tick encephalitis and RSSE virus complex. He is initially involved in Soviet HA techniques and application of his experience with filter paper discs to HI procedures.

REPORT FROM DR. FRANCIS B. GORDON
DIVISION OF VIROLOGY, NAVAL MEDICAL RESEARCH INSTITUTE
BETHESDA, MARYLAND

Following Dr. Hurlbut's studies on growth of arthropod-borne viruses after parenteral inoculation in various species of arthropods, we have continued our interest in growth of these viruses in other than mammalian and avian cells. We are currently working with a line of fish cells derived from gonads of trout. (Wolf and Dunbar, 1957, P.S.E.B.M. 95: 455) The cells are regularly grown at 19 C but will tolerate higher temperatures. We have started a program of tests with representative arthropod-borne viruses of several groups to determine whether growth will take place in the fish cell line with or without a cytopathic effect, and whether passage under such conditions will alter the character of the strains. A temperature of 24 C is used, at which the cells can be kept for long periods in the same culture with little attention.

This investigation has not progressed sufficiently far to allow conclusions to be drawn although preliminary results are encouraging. We expect to be able to report more definitely on this study in a later issue.

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

The work at Pennsylvania State University on birds will emphasize the census methods and breeding studies. Probably the main species will be red-winged blackbirds as well as starlings. Work will be continued in a marsh on the productivity with an attempt to see whether intraspecific fighting limits the population. It is hoped to reduce fighting by feeding the birds tranquilizers in nature. The expected result would be an increase in the population. While an increase in the population may not be desired, nevertheless, this approach will help in our understanding of what holds down populations.

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

Japanese B Encephalitis

The attenuated vaccine pool for man has passed all safety tests including monkeys and chimpanzees and will now be tested in man. This attenuated strain appears to remain stable in passage at 24° C but tends to revert to mouse virulence in serial passage at 37° C. This strain has a definite T° marker, not growing at all at 40°, while the original isolate and the Nakayama strain grow essentially as well at 40° as at 37°C.

Dr. Rhim in our laboratory has now developed a method of producing excellent plaques in hamster kidney cells with JBE virus, attenuated, wild and mouse adapted. The plaques are larger and clearer than those in chick embryo cells. Titration and plaque reduction neutralization are readily performed and one plaque is apparently the result of a single infectious viral particle.

Thai Hemorrhagic Fever Studies

Isolates from patients of 1960 have now been worked up with the following results: BH-50 and BH-28--dengue type 4; BH-570 and BH-40--dengue type TH-36 (? type 5); BH-10--dengue type uncertain but resembles most closely type TH-Sman (? type 6 or even 7).

Two as yet unidentified types of virus have apparently been isolated from patients of 1960. Two strains of each were isolated, BH-20L and

BH-33, representing one type and BH-20S and BH-32 representing the other. BH-20S and BH-20L came from the same serum. They have distinctly different incubation periods and do not appear to be antigenically related. They are not dengue or chikungunya related.

T-55 from 1958 Aedes aegypti is dengue type 3.

Dengue Virus Studies

Fluorescent antibody has been used successfully to demonstrate dengue virus antigen in mouse brain and in tissue culture using an established strain.

The pathology of suckling mice following inoculation of a low and a high dilution of the four recognized dengue types is being studied by Dr. John Craighead of Dr. Dammin's laboratory in a cooperative study with us.

Singapore Dengue Virus

Strains S-843 and S-601, Dr. Lim's isolates from Singapore in 1960, have been shown in our laboratory to be most closely related to dengue prototype strains TH-36 and TH-Sman, respectively, from Bangkok rather than type 2 (New Guinea C) and type 1 (Hawaiian).

Bangkok Mosquitoes of 1961

From mosquitoes collected by Dr. Rudnick during our visit in Bangkok in 1961, we have now isolated 3 viruses from Aedes aegypti and two other probables, one from the same species and one from C. quinquefasciatus. Nineteen sixty-one was a year of low incidence for hemorrhagic fever in Bangkok and these viruses do not behave like dengues or chikungunya.

REPORT FROM DR. CHARLES L. WISSEMAN
DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE
BALTIMORE, MARYLAND

Four variants of type 1 dengue (Hawaiian strain) have been tested in human volunteers for the purpose of ascertaining the degree of attenuation. All four were found to be attenuated in comparison with the unmodified parent strain. Three of the strains produced rash and mild systemic manifestations. One failed to produce fever, rash, or

significant systemic manifestations. All strains elicited HI antibodies in low titer. All stimulated the production of dengue 1 neutralizing antibodies which appeared between the seventh and fourteenth day following vaccination. A second volunteer study was undertaken with the 1-D strain which had been eventually free of side-reaction in the first study. Twenty health young adult males were inoculated intradermally with either 20,000 or 200,000 suckling mouse i.c. LD_{50} of the attenuated strain. For all practical purposes, they remained free of symptoms or rash. Only on the most careful questioning and examination on a daily basis was it possible to detect signs indicative of an infectious process. HI antibodies again were produced. Viremia studies and the determination of neutralizing antibody response are in progress. It is planned shortly to test resistance to infection with an unmodified strain and to determine conversion rates and reaction rates in larger numbers of volunteers.

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

This is the first report from this department to the Information Exchange. Work which has been going on for many years has been concerned with studies of group B arboviruses in various types of cell and tissue cultures. Most of the work has been done with the New Guinea B strain of dengue-2 virus, previously adapted to mice by Schlesinger and Frankel. Unless otherwise specified, this report will deal with this agent derived from the 56th to 60th suckling mouse passage. Following is a summary statement of progress during the past 12 months.

1. Cytopathogenic effects (CPE) in KB cell cultures: Primary infection of KB cell tube cultures (various clone-derived sublines) induces characteristic CPE which ultimately leads to detachment from the glass of all but a few cells. The latter invariably give rise to regrowth of persistently infected carrier cultures (see 2). The CP titer depends on the medium used for dilution and adsorption of the input virus. When virus is diluted in PBS-10% serum or in buffered gelatin with TRIS (BGT), all at pH 7.4, the CP titer is 100-300-fold lower than the LD_{50} titer in mice. With BGT pH 7.0-7.2 as diluent, the two methods of titration give almost equivalent results, but both are 5-10-fold lower than the LD_{50} at pH 7.4. Maximum CP titers are reached when virus is adsorbed for 2-3 hours at 30°C before addition of medium. Monolayers infected at an input multiplicity of 0.05 CPD/cell

yield a maximum of about 30 LD_{50} per cell after 6-8 days. Efficiency of primary adsorption under these conditions is probably low, as demonstrated by the fact that cells in infected cultures continue to multiply for 3-4 days after infection.

2. Chronically infected KB cultures: Whenever infected KB tube cultures are kept beyond the time of maximum CPE, regrowth of cells occurs in clonal fashion. The newly arising cell clusters eventually degenerate in turn, and similar cycles of regrowth and degeneration repeat themselves. After a few such cycles, the cell population becomes denser, and degeneration and regrowth occur simultaneously in the same tube. These cultures are granular and "messy" -- full of debris. The process has continued in a set of tubes for over 2-1/2 years. The phenomenon is independent of the initial input multiplicity, and all sublines tested have shown the same capacity for conversion to the carrier state. Whenever tested for the presence of virus, culture media of such carrier cells have yielded $10^{4.4}$-10^{6} LD_{50} per tube or 0.3-2.0 LD_{50} per cell. Plaque titrations (see 4.) have yielded about 5-6 PFU per cell.

3. Effect of agar or agar extract (AE) on CPE and growth of dengue-2 virus: Despite the CPE under liquid medium just described, no plaques have been obtained in KB cell monolayers overlaid with agar medium. In fact, attempts with dengue-1, dengue-2, and yellow fever 17D viruses on a large variety of primary and established cell lines (including duck and chick embryo fibroblasts) have failed to give indication of reliable plaque formation under agar overlays. Such overlays inhibit CPE and prevent virus multiplication. Aqueous extracts of 0.9% agar gel inhibit not only CPE but also hemagglutination and lead to a reduction in LD_{50}. Additional information on this inhibitory principle contained in AE will be summarized below.

4. Plaque formation by dengue-2 and other group B arboviruses under methyl cellulose (MC) overlay): Agar has been replaced in the overlay medium by 2% MC (Methocel, 4000 centipoises, Dow Chemical Co.) as described by Rapp et al. (Proc. Soc. Exp. Biol. Med. 101, 289, 1959). The overlay contains, in addition, double strength Eagle's basic medium (1955 brand) and 10% heated horse serum. KB monolayers tolerate this medium exceedingly well. MC can be poured at 4°C, gels somewhat at 37°C. However, it remains too fluid to invert the plates repeatedly and for long enough to do plaque counts. Therefore, we add a second overlay of neutral red agar with the same nutrient except that 5% heated calf serum is used instead of the HoS (empirical--gives best plaques). The second overlay is added 2 days before plaques are expected.

Dengue-2 plaques are seen first after 5-6 days. They grow to 2 mm in diameter and are clear and easy to count. Maximum counts are obtained on day 8 or 9, but 6-7th day counts are 85-95% of maximum. Dengue-1 or yellow fever 17D require a longer time, but more work has to be done on these. Trinidad 1751 strain of dengue-2 behaves like New Guinea B. Early mouse brain passages of New Guinea B are as efficient as later ones. SLE JapB, and MVE viruses give clear and large plaques after 4-5 days.

The efficiency of plating of dengue-2 and SLE, JapB, or MVE viruses is roughly equivalent to the mouse LD_{50}; that of the others needs improvement. Averages per gram of suckling mouse brain are:

	PFU ($\log_{10}$)	LD_{50} ($\log_{10}$)
Dengue-2	7.9	7.9
JapB	9.4	9.8
SLE	9.9	10.5
MVE	9.8	10.0
Dengue-1 (Hawaii)	6.2	7.9
Yellow fever 17D	7.5	8.2

A mouse hyperimmune (obtained from Dr. Wisseman) and a monkey anti-human-dengue-2 serum suppress plaque formation specifically at titers of 1:320-1:640.

Maximum adsorption of dengue-2 PFU out of BGT pH 7.0-7.2 requires 2 hours at 37°C. Adsorption at 28° C reduces plaque count to 60% of that at 37°. Excellent correlation of plaque counts with virus dilution plated indicates that each plaque is formed by a single infectious particle.

Plaque formation by dengue-2 virus under MC is inhibited by aqueous agar extract added directly to the MC.

5. Preliminary identification of dengue-2 inhibitor in agar: The inhibitory effect of agar extract on plaque formation by dengue-2 virus is apparently due to instantaneous complex formation between virus particles and a factor in the AE. The resulting inactivation is partially reversed by dilution of the AE-treated virus out of the AE. Treatment of agar with soluble DEAE-dextran as described by Liebhaber and Takemoto, or absorption of AE with DEAE reduces or eliminates the inhibitory principle. The findings suggest that it may be identical with the sulfated polysaccharide(s) of Takemoto and Liebhaber.

6. Partial purification of dengue-2 virus: Most of the virus grown in KB cell monolayers under liquid medium is recovered in the medium, and repeated washing of the monolayers for 30 minute periods with pH 9 borate buffer (Hallauer) or sonication of the cells fails to release significantly more virus. Virus in medium can be cleaned by 2 consecutive extractions with fluorcarbon, followed by cycles of centrifugation at 30,000 rpm for 90 minutes. One hundred-fold concentration has been achieved, and reasonably good banding of the virus has been obtained in cesium gradients. This work is in progress. Conditions for quantitative recovery of concentrated virus, both in terms of PFU and HA units, have been worked out.

7. Growth kinetics of dengue-2 virus in KB cells: One-step growth curves of dengue-2 virus have been constructed from experiments in monolayers and in suspension cultures of KB cells initiated with concentrated virus. They reveal perfect parallelism between PFU and HA at an average ratio of $10^{4.5}$. The rate of multiplication is slow in that the peak is reached only after 40 hours (first measurable increase at 20-24 hours). The yield is also low, i.e., about 35 PFU/cell between 30 and 72 hours after infection.

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

Bird and mammal sera collected in the latter part of 1960 and early part of 1961 in the high plains area of Texas have been tested by hemagglutination-inhibition against WE, EE, and SLE viruses. The species of birds found positive for either or both WE and SLE were English sparrow, downy woodpecker, and domestic pigeon. Twelve other species tested were negative or equivocal. Eleven species of small mammals were tested. One eastern gray squirrel and one jackrabbit were positive for WE. Antibodies to SLE were found in the black-tailed prarie dog, cotton rat, and gray wood rat. Tests performed on bird and mammal sera collected in 1961 from other parts of Texas were negative. Sera from a non-sentinel flock of chickens in the lower Rio Grande valley area showed WE virus activity had occurred there prior to August. Sera from other non-sentinel and sentinel chickens from various areas of the state remain to be tested.

Confirmed human cases of arthropod-borne encephalitis in Texas during 1961 have occurred mainly in the high plains area where there were ten cases of WE and six cases of SLE. One case of WE occurred

in El Paso. The virus of SLE was recovered from the brain of a fatal case in San Antonio. In Corpus Christi an early case of SLE occurred with an onset date of May 27.

Five cases of WE in horses were confirmed from the high plains and central Texas areas.

During 1961 over 15,000 mosquitoes, in a total of 536 pools, have been tested in suckling mice for virus. These came from eight areas of the state. Five genera and twenty species were represented in the collections. The only positive pools found were Culex tarsalis from the high plains (Lubbock-Plainview) area. So far, three isolates have been identified as WE virus; four have been identified as SLE virus; and some twenty isolates remain to be identified.

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

Studies on the susceptibility of insectivorous bats to experimental infection with various arbovirus strains is providing evidence that these animals could be involved in the biological life cycles of these viruses. The response of three species of insectivorous bats, two deep hibernators (Myotis l. lucifugus and Eptesicus f. fuscus) and a quasi-hibernator (Tadarida b. mexicana) to experimental infection with various strains of JBE and SLE virus indicates that the degree of laboratory (mouse) adaptation of the virus strain influences its infectivity for a particular bat species. The high mouse passage Nakayama and Hubbard strains of JBE and SLE viruses, respectively, are in general less virulent than recent mosquito isolates. In addition, viral assay of various tissues of experimentally infected bats indicates that the animals may serve as reservoirs of these agents through viral invasion of brown adipose tissue, an hypothesis which has been proposed by us as a reservoiring mechanism for rabies virus. Studies still in progress on the effect of environmental temperature on the course of infection in hibernating bats indicate that the progress of the viral infection is suppressed when animals are maintained in a torpid state and that the infection can be activated on transfer of the animal to a warm environment. JBE virus has been detected in the brown fat of infected animals in simulated hibernation. Experiments on the antibody response in bats experimentally infected with JBE virus indicate that (a) virus may be detected in low titer in blood samples containing neutralizing antibody; (b) that antibodies detectable by the CF and HI techniques currently in use are not regularly produced; and (c) that antibody synthesis apparently

does not occur in the torpid animal. The cultivation of bat brown adipose tissue in vitro is providing a basis for studying arbovirus replication in this tissue at the cellular level. Studies on the effect of the gravid state of lipogenesis in bat brown fat as it relates to viral proliferation in this tissue are in progress. Transplacental transmission of JBE virus has been demonstrated in the Mexican free-tailed bat.

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

I. Report from Greeley, Colorado, Dr. A. D. Hess, Dr. L. C. LaMotte, and James V. Smith.

High Altitude Transmission of WE and SLE

During 1961, sentinel chicken flocks were maintained at various elevations from 4,700 to over 10,000 feet. HAI antibody rates at the end of the season indicated high Group A (presumably WE) virus activity up to the maximum elevations. Transmission at the upper elevations (8-10,000 feet) occurred in the complete absence of Culex tarsalis, the predominant mosquito species being Culiseta inornata and Aedes pullatus. Considerable numbers of biting snipe flies (Symphoromyia) were also collected in sentinel shed traps. Although no transmission of SLE occurred at the maximum elevations, Group B HAI antibodies (presumably SLE) were present in flocks above 9,000 foot elevations where there was also no evidence of C. tarsalis. These data along with those obtained during 1960 indicate that something other than C. tarsalis is serving as an enzootic vector of WE and SLE.

Relationships of Temperature to Encephalitis Transmission Rates

The Statistics Section of CDC has made an analysis of the Greeley data on WE and SLE transmission rates and temperatures using regression lines calculated by the method of least squares. By this method, the index of 50DD >70° F (date when first accumulated) gave the best fit for WE, which shows a negative relationship with temperature. Using analysis of variance, the slope for WE in the St. Vrain sentinel area at Greeley was significant at the 5% level. Using an index of 15DD >70° F, the WE data from Bakersfield showed a similar relationship to that from Greeley, no real difference being demonstrated between the two regression lines. As might be expected, the index end points appeared much earlier at the more southern latitude of Bakersfield.

The index of 10DD>75° F gave the best fit for SLE, which shows a positive relationship with temperature in the Greeley area. The slope of the SLE regression line was also significant at the 5% level. The SLE data from Bakersfield did not show a positive relationship with temperature. This also might be expected, since at this more southern latitude temperatures are probably never low enough to serve as a limiting factor.

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

(The activities at the Taunton Field Station are a cooperative endeavor of the Encephalitis Section, Communicable Disease Center, USPHS, and the Division of Communicable Diseases, Massachusetts Dept. of Public Health.)

Granular formulations of dieldrin and heptachlor were applied during the summer of 1961 to 1/4-acre study plots in a Massachusetts fresh water swamp at the rates of 0.5 and 1.0 pound of active ingredients per acre. Heptachlor was more effective than dieldrin in controlling C. melanura larvae. Essentially complete control was attained for 62 days in the plot treated at the rate of 1.0 pound heptachlor per acre. The results indicated that studies on winter applications of granular heptachlor are needed, and that consideration is merited regarding the use of granular formulations of insecticides for the emergency control of C. melanura larvae during outbreaks of eastern encephalitis.

Virus isolation tests were completed during 1961 on the arthropods collected in 1959 and 1960. Fifty isolations of virus were obtained from the 24,968 specimens tested from 1959. Of these, 27 were identified as WE and the identity of 23 remains undetermined. All of the 1959 virus isolations were from mosquitoes. Fifteen virus isolations were obtained from the 10,848 specimens tested from 1960. Of these, 3 were WE virus and the remaining 12 are not yet identified. Three of these unidentified isolates were from insects other than mosquitoes: 1 from Psychodidae and 2 from Tabanidae. The possible significance for human disease of these 35 unidentified agents isolated from the 1959 and 1960 arthropod specimens remains to be studied as soon as time and facilities permit.

During 1961, a total of 910 wild birds were collected and banded. One isolation of WE virus was obtained from the 468 wild bird blood samples selected for virus tests, and this virus was from a catbird collected September 11, 1961 in Pine Swamp. Of 313 blood samples selected for antibody tests, 23 were positive for EE antibody (10 of 68 spot-checked migrant birds and 13 of 245 nesting season samples).

Two of the birds positive for antibody a black-capped chickadee and a catbird, were immatures; thus, they were infected in 1961. No antibody conversion from negative to positive was detected among the banded birds, and it was not possible to determine which, if any, of the 21 remaining positive birds also were infected in 1961.

A sharp rise in the number of immature catbirds was noted during the August 1961 netting periods. That is, the adult to immature ratio which was 1 to 1 in July became a 1 to 11 ratio in August. Since the number of young birds observed in the August ratio is thought to greatly exceed the average catbird brood, the large number of immatures captured in August may have been composed of the young produced in the swamp plus an influx of young birds from the surrounding area or possibly by reverse migration from further south. This large rise in the immature population of catbirds occurred during the peak of the mosquito population. These data give insight into the combination of phenomena that may lead to an outbreak of arthropod-borne encephalitis. Thus, a large population of young susceptible birds serving as hosts of the virus and a large population of mosquitoes to transmit it from host to host would enhance the possibility of the eventual involvement of humans in the virus cycle.

III. Report from Plainview, Texas - Bruce Francy.

Pre-treatment surveys have been completed for carrying out the field experiment on control of St. Louis and western encephalitis at Hale Center, Texas. There is a total of approximately 1, 200 acres of potential mosquito breeding areas within a three-mile radius of the town. All of these areas will be treated with residual larvicides beginning in February 1962. The effectiveness of this operation in reducing encephalitis transmission rates will then be observed, using Plainview and Abernathy as untreated comparison towns.

IV. Report from Wenatchee, Washington - Virgil I. Miles.

Larvicidal control of mosquitoes in an area of approximately 17 square miles effectively reduced mosquito production. 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. The difficulty of providing adequate larvicide coverage of cornfield habitats resulted in considerable mosquito production the last 2 weeks of August, after a heavy growth of "watergrass" had developed.

In spite of the reasonably effective control of mosquito production, populations of adult female C. tarsalis at the center of the area were only slightly reduced until late July and exceeded the populations in comparison areas the first 2 weeks of August. There was circumstantial evidence of infiltration of female C. tarsalis into the controlled area from uncontrolled areas, as has been experienced at the Bakersfield, California, field station.

Evaluation of the effect of vector control on virus transmission rates awaits tests on sera of sentinel chickens for 1961.

Evaluation of the effect of vector control on virus transmission rates awaits tests on sera of sentinel chickens for 1961.

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

Ecology of Mosquito-Transmitted Viruses

WESTERN EQUINE ENCEPHALITIS VIRUS

Objectives of study

Overwintering - role of latent infection in birds and hibernating C. tarsalis.

a) Latent infection in birds.

If latent infection in migrating birds carries virus from the South, one should expect to find latent infection in birds even in the Arctic region. Collections were made by members of the Arctic Health Research Center during May, June and August in Alaska (Table 1).

Table 1

Wild Bird Sera & Tissues Submitted by the Arctic Health Research Center, 1960

	Sera Neutralization Test	Tissues Inoculated into Suckling Mice
Alcidae	22	127
Anatidae	2	1
Buteoninae	3	2
Compsothlypidae	2	8
Fringillidae	13	21
Hirundinidae	12	34
Laridae	2	2
Rynchopidae	1	1
Scolopacidae	0	1
Stercorariidae	2	0
Turdidae	1	2
Tyrannidae	3	5
Totals	63	204

WEE antibodies were not found in 63 sera examined and no virus was isolated from the tissues of 207 birds.

Dr. Sladen collected sera from 31 birds on St. Paul Island, Alaska, and none had WEE antibodies (Table 2).

Table 2

Wild Bird Sera Submitted by Dr. Sladen from St. Paul Island, Alaska, 1960

Species	Sera Neutralization Test
Least auklet	16
Red-legged kittiwake	1
Ruddy turnstone	2
Cormorant	3
Horned Puffin	4
Tattler	1
Thick-billed murre	3
Pac. fulmar	1
Total	31

There is no indication from these data that WEE virus gets to Alaska.

Sera obtained during October 1960 from 173 birds at Vale, Oregon (Table 3) showed the presence of antibodies only in English sparrows and redwing blackbirds. Species such as the white crowned sparrow and the goldfinch were collected in large enough numbers so that the negative results indicate that they are not involved in transmission of WEE virus. In looking for latent virus infection, emphasis should probably be laid on the English sparrow and blackbird tissues. Tissues from 53 of these species were examined without any virus isolation.

Table 3

Species	No. Tissues Inoc. into Mice*	No. Sera Examined for Antibodies	No. Pos. Screen Neut. Test
Song sparrow	28	11	
White crowned sparrow	91	53	
English sparrow	44	29	9
Savannah sparrow	4	3	
Flicker	3	4	
Goldfinch	78	51	
Oregon junco	2	1	
Ruby crowned kinglet	3	1	
Redwing	9	6	2
Magpie	1		
Robin	1		
Redstart	1		
Thrush	1		
McGilvery warbler	1		
Audubon warbler	4	1	
Dove	5	1	
California quail	2	2	
Pine siskin	6	6	
House finch	1		
Goldfinch or white crowned sparrow		4**	
	285	173	11

*No virus was isolated from any tissue
**Labeling mixup

b) Hibernating C. tarsalis.

Vale, Oregon. In this region it is now possible to follow C. tarsalis throughout the year. During the summer of 1960, there were 10 isolations of WEE virus from 1449 C. tarsalis (collected Aug. 9-11). During the winter and early spring of 1961, 2478 C. tarsalis were collected with no isolation of virus. During the summer of 1961 there have been 18 isolations of WEE virus made from 2017 C. tarsalis (collected 7/27 through 8/2). Mosquito collections were not made in the early summer at Vale but they were made in Council, Idaho, nearby. The first isolation here was made from a 7/5/61 collection. It is very obvious that the hibernating, late spring and early summer, C. tarsalis population differs from the summer population as regards virus content. Observations were also made as to when C. tarsalis began to emerge from hibernation and take blood (Table 4).

Table 4

Number of Mosquitoes Attracted to Chicken Baited Traps Taking Blood

	No. Specimens Trapped	Percentage Taking Blood
March 9-18	130*	2%
March 19-31	1115	31%
April 13-27	459	61%
May 12-28	152	95%

*Approximately.

Early in March about 2% of mosquitoes coming out of hibernation took blood. In late May 95% of the C. tarsalis population took blood. During April and May enough mosquitoes are taking blood so that a virus cycle should be established if either the mosquitoes or birds are carrying virus providing the average temperature is high enough to permit virus multiplication in the mosquito. Results to date give no evidence that hibernating C. tarsalis or latent infection of birds carries virus through the winter.

c) Other mechanisms for maintenance of virus.

1) In North Dakota during the summer of 1960, as mentioned in the last newsletter, only 2 isolations of WEE virus were made from 5374 C. tarsalis. It has since been found that none of 24 sentinel

chickens developed antibodies. Also, in Minnesota adjacent to North Dakota (Crookston and Fergus Falls) 5% of 105 sentinel pigeon sera received from Dr. Olson showed a neutralizing index of 50 in a screen neutralization test. There was then no good evidence of a C. tarsalis-bird cycle in this area. It appears possible that there is some other mechanism maintaining WEE virus to which C. tarsalis has access to account for the slight amount of virus activity observed. Work so far done indicates that virus activity is at the same low ebb in North Dakota the summer of 1961. The evidence of WEE virus activity in western irrigated areas during 1960 and 1961, while it is at a low ebb in the north central portion of the country, is difficult to account for if one assumes that virus is being carried northward from an infected southern area.

2) Snake work - Garter snakes infected by mosquito bite September 1960 carried virus through the winter and viremia was found in some snakes until well into June 1961 but no infected snakes have been found in nature.

ST. LOUIS ENCEPHALITIS VIRUS

To date no isolation of SLE virus has been made in 1961 in Oregon or Idaho although there is ample evidence of WEE activity.

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

Two human cases of Western equine encephalitis were identified in 1961, one in August and the other in September. Twelve cases of horse encephalitis caused by Western equine virus were identified by serological tests; 3 occurred in July, 3 in August and 6 in September. There were five human cases of St. Louis encephalitis where a definite diagnosis could be made by demonstrating a fourfold or greater rise in CF antibodies. One of these occurred in July, 3 in September, and 1 in October. There were three additional cases where a presumptive diagnosis of St. Louis encephalitis could be made on the basis of CF and neutralization tests. Two of these occurred in August and one in September. The blood serum specimens from cases of horse encephalitis were tested against both Western and St. Louis viruses by the CF test. One horse which developed clinical signs of encephalitis in October and recovered was negative for Western equine virus infection

by the CF test but showed a fourfold rise in titer of CF antibodies for St. Louis virus. Both the acute and convalescent serum specimens neutralized >2 logs of St. Louis virus. This indicates that this case of horse encephalitis was caused by St. Louis virus or some other group B virus. There were no additional cases of Colorado tick fever since the last report.

In addition to the virus isolations described in the last report, Hart Park virus was isolated from a tissue pool from a nestling house finch, Carpodacus mexicanus, collected in Kern County, July 17, 1961. Kern Canyon virus was isolated from one of 26 apparently normal Myotis yumanensis, collected from a bat colony at Borel Power Station, Kern Canyon, July 19, 1961. The prototype strain of Kern Canyon virus was isolated from a bat of the same species collected at this same site on June 9, 1956. Rabies virus was isolated from the brain, brown fat, lung, and pectoral muscle of a Mexican freetail bat, Tadarida brasiliensis, found paralyzed in Kern Canyon on July 19, 1961.

A total of 39 pools of Culex tarsalis mosquitoes collected in Kern County during a period of high virus activity in June, July, and August 1956 were tested for virus in 1961. Twelve virus strains were isolated, 9 of Western equine virus, 2 of St. Louis virus and 1 of Hart Park virus. These specimens had been stored in sealed glass tubes in a dry ice chest for more than five years.

Blood serum specimens have been collected from 75 horses kept on ranches at an elevation of about 5000 feet ASL on the Great Basin Plateau of Modoc County. About 50% of these were positive for HI antibodies for WE virus and 15% for SLE virus. All of the sera which were positive by the HI test were also positive by the virus neutralization test. We are now testing the HI negative sera and have found several which neutralize > 2 logs of WE or SLE virus. In the last report, it was noted that a striped skunk fed mice infected with Western equine virus subsequently developed an asymptomatic infection as shown by the demonstration of viremia. A blood serum specimen taken four months after exposure neutralized > 2 logs of WE virus but was negative for CF and HI antibodies for WE virus.

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

A second year of intensive Culex tarsalis larval control has been carried out in a 28 square mile area of Kern County, California. This is a cooperative program with the Kern Mosquito Abatement District. The objective of this program was to interrupt the basic transmission cycles of WEE and SLE viruses. Evaluations of the populations of larvae and adult males indicated an approximate 95 per cent reduction in C. tarsalis emergence in the control area. In spite of this reduction, the adult female C. tarsalis population continued at a relatively high level. Virus infection and transmission rates of the C. tarsalis population and antibody conversion rates in chickens exposed in bait traps or in sentinel flocks showed no significant difference between the controlled and comparison areas. It was tentatively concluded that massive infiltration of female C. tarsalis into the controlled area sufficed to maintain virus transmission at a high level.

Flight range studies on C. tarsalis substantiated that in this type of agricultural marginal desert area a much larger region would have to be under control to prevent infiltration of vectors and continuing virus transmission. Marked C. tarsalis which were released at a number of peripheral points outside the intensively controlled area infiltrated in significant numbers to the center of the area. Recoveries of marked females at distances of 3 to 5 miles from release points were not uncommon and the maximum recovery was at a distance of 9.6 miles. Data are now being analyzed and will be reported in detail at a later date.

Blood-meals from C. tarsalis and other mosquitoes collected in all periods of the year are now being identified by precipitin tests. Tests of 74 blood-meals from Culiseta inornata collected in winter months reveals that 70 fed on mammals, 1 on a bird, and 3 had insufficient antigen for identification. The general pattern of C. tarsalis feeding at all seasons is one of over 90 per cent of feeding on birds and the remainder on mammals. In the first 300 samples, none had fed on cold-blooded vertebrates. Preliminary screening indicates that feeding is on the predominant species of birds at each collecting site.

REPORT FROM DR. ALBERT RUDNICK
INTERNATIONAL CENTER FOR MEDICAL RESEARCH AND TRAINING
HOOPER FOUNDATION
UNIVERSITY OF CALIFORNIA MEDICAL CENTER
SAN FRANCISCO 22, CALIFORNIA

Singapore Hemorrhagic Fever (in cooperation with Professor K. A. Lim, Department of Bacteriology, Faculty of Medicine, University of Malaya in Singapore):

Hemorrhagic fever appeared in Singapore, as a newly recognized disease, in epidemic form in 1960. Following the peak of the epidemic, but while cases were still occurring, a mosquito survey was initiated for the purpose of attempting virus isolations and to determine the relative incidence and distribution of potential vectors. Over 12,000 live adult mosquitoes were collected from October to January. These represented over 40 species of 8 genera. The most common urban mosquitoes were Culex pipiens quinquefasciatus, Aedes aegypti, and Aedes albopictus. The most common suburban and rural mosquitoes were Culex pipiens quinquefasciatus, Aedes albopictus, Anopheles vagus, Culex gelidus, C. tritaeniorhynchus, and Mansonia uniformis. Adult Aedes aegypti were collected inside houses in the urban areas and only rarely beyond the Singapore city limits. Within the urban areas, A. aegypti was most common in the poorer, more crowded residential areas and was collected in over 50% of the house stations examined. Aedes albopictus was collected in large numbers in both urban and rural situations but was associated with vegetation, so that it was most generally found in forested or garden areas. It was, therefore, less common in the poor crowded urban areas than in the better residential or suburban and rural areas. Culex pipiens quinquefasciatus was common throughout the island in association with human habitation. C. gelidus and C. tritaeniorhynchus, although present in the city in numbers, were more common in rural areas.

The disease has been associated etiologically with dengue by Professor Lim on the basis of serological studies and virus isolations from patients' sera. Two of these isolations have been identified, one as dengue I or closely related, and the second as dengue II or closely related.

Over 6,000 adult female mosquitoes of various species have been tested for virus isolation in suckling mice. From these at least six agents have been isolated, five from Aedes aegypti and one from Aedes albopictus. All appear to be dengue or dengue related viruses on the

basis of immunity developed in passage mice to intracerebral challenge of 100 LD_{50}'s of adult adapted dengue virus. Five agents are still in the process of adaptation to mice, while the sixth has been adapted and is ready for identification tests.

It appears that the virus isolations will support the conclusion that Aedes aegypti was the primary vector involved in the Singapore epidemic.

Thai Hemorrhagic Fever (On invitation from and in cooperation with Dr. W. McD. Hammon, Department of Epidemiology and Microbiology and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh Pennsylvania; The Ministry of Health, Thailand, and the SEATO Medical Laboratory, Bangkok.):

Further studies of the vectors of Thai Hemorrhagic fever were initiated in August. A mosquito collecting program was conducted in the metropolitan Bangkok area and communities near the Bangkok area. Over 8,000 live adult mosquitoes were collected by several methods. Over 5,000 female mosquitoes representing 17 species of five genera are being processed for virus isolation in both the Pittsburgh and San Francisco laboratories.

As in the 1958 survey, Aedes aegypti was found to be common and widespread in the urban areas and Aedes albopictus rare.

Buffalo Disease of Unknown Etiology

(In cooperation with Dr. S.K. Chong, Veterinary Research Institute, Ipoh, Federation of Malaya).

A disease among Malayan swamp buffaloes was first observed in Kluang in 1956 and reappeared in 1959 and 1960. Fatality was high with a total of 76 deaths recorded as of February 1960. Acute blood from two natural cases inoculated intravenously into normal buffaloes resulted in reproduction of the disease (Dr. Chong). Extensive bacteriological and protozoological studies were either negative or did not suggest any etiological relationship (Dr. Chong).

Acute sera from natural cases and experimental cases are being studied in this laboratory.

Ecology of Dengue Viruses

Field and laboratory studies of the ecology of the dengue viruses are expected to be initiated at the Institute for Medical Research, Kuala Lumpur, Malaya, during the latter part of 1962.

REQUEST FROM DR. S. EDWARD SULKIN, CHAIRMAN, COMMITTEE OF LABORATORY INFECTIONS AND ACCIDENTS OF THE AMERICAN PUBLIC HEALTH ASSOCIATION

Reporting Laboratory Infections and Accidents

In order that a permanent Committee on Laboratory Infections and Accidents of the American Public Health Association may more effectively carry out its activities, it is requested that instances of laboratory-acquired infection, particularly those occurring within the last ten years, be reported to this committee. As much information as possible about each case is desirable, such as type of work involved, probable mode of infection, the agent, the type of disease, the manner in which the diagnosis was confirmed, the outcome of the infection, and the type of personnel involved. If so desired, the information provided will remain anonymous. If the case has been published, the reference should be indicated.

Information should be sent to the Chairman of the Committee, Dr. S. Edward Sulkin, Department of Microbiology, The University of Texas Southwestern Medical School, Dallas, Texas.