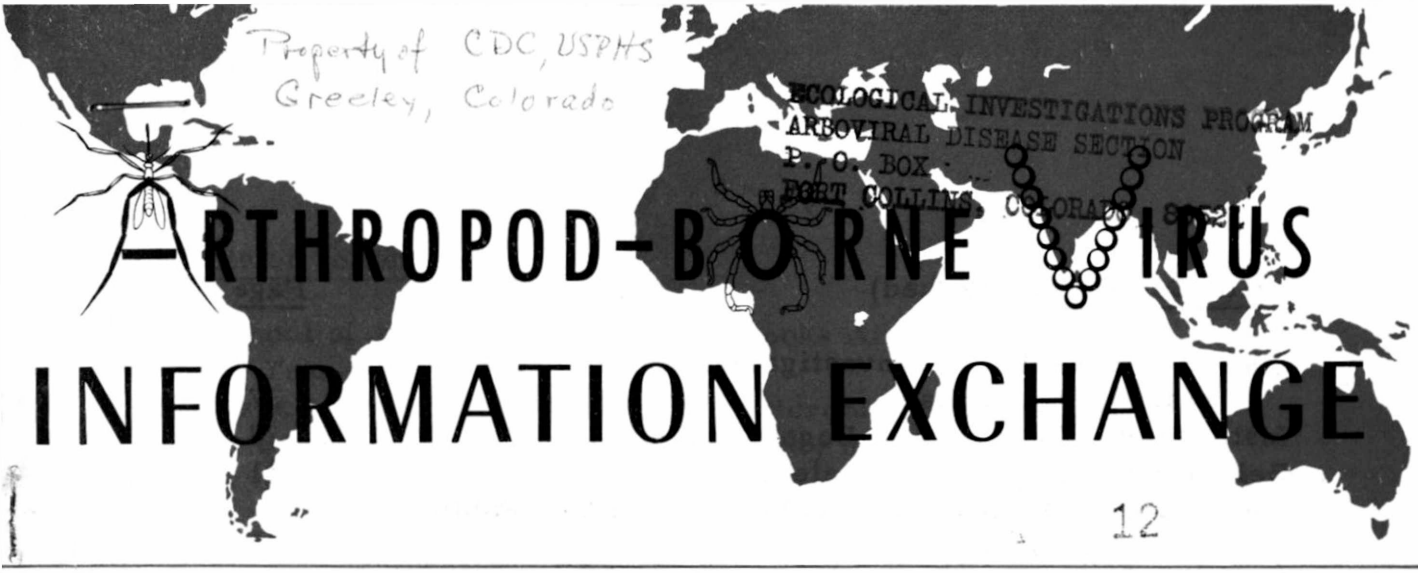

ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

Number Four October 1961

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Summary Report of the April 9 Atlanta Meeting of the Gould House Group

Since most participants in the Arbovirus Information Exchange have already received the Summary of Actions of the April, 1961, meeting of the Gould House Group of American Investigators of Arthropod-borne Viruses, and because of its length, it is not reproduced in this issue of the newsletter. This is the meeting where activities of the Subcommittee on Information Exchange were opened to international participation and an American Committee on Arthropod-borne Viruses was established. Any participants of the Information Exchange who have not received a copy of this Summary Report and who are interested in the activities of that meeting may request a copy by writing to Dr. Telford H. Work, Editor, Arthropod-borne Virus Information Exchange, Virus and Rickettsia Section, Communicable Disease Center, Atlanta 22, Georgia, U.S.A.

INTRODUCTORY NOTES FROM THE SUBCOMMITTEE ON INFORMATION EXCHANGE

At the last meeting of the so-called Gould House Group in Atlanta in April, several decisions were made which affected the Subcommittee on Information Exchange. For administrative effectiveness, the former Gould House Group was succeeded by a smaller committee designated as the American Committee on Arthropod-borne Viruses. This latter committee now serves as the parent committee for the Subcommittee on Information Exchange (see Summary of Actions of the April 1961 meeting of the Gould House Group). Another decision that affected this subcommittee was that its activities, including both the Catalogue and the Newsletter, be expanded to international coverage. Also the modified form of the questionnaire for registering a virus in the Catalogue was approved, which cleared the way to proceed with the further assembling of the Catalogue.

Since the April meeting, this subcommittee has been engaged in carrying out these directives. This has involved the selection of and sending out of invitations to persons in foreign countries to participate in the Newsletter and to invite them to register any new viruses that should qualify for inclusion in the Catalogue. The conditions for participation in the Newsletter and instructions for registering new viruses in the Catalogue were covered in form letters.

The selection of foreign participants and the method of communication with them are being developed in collaboration with the WHO. For areas that are covered by Regional Reference Laboratories designated by the WHO, the director or some person he may designate in the laboratory, is being utilized as "correspondent" through whom the exchange of information is channeled. So far, Regional Reference Laboratories have been designated in the Soviet Union, Czechoslovakia, England (for Europe and parts of Africa), and Australia to cover both Australia and New Zealand. In areas for which no Regional Laboratories have been designated, correspondence is being carried on directly by the subcommittee with foreign participants. A selection of foreign participants is still proceeding but at the time of this writing, 74 investigators operating in 37 different nations have been

invited to participate. Among these are 13 Americans stationed abroad. The number of recipients of the Newsletter in the United States now stands at 64, making a total of 138.

After the approval of the new form for registering viruses in the Catalogue, special emphasis was directed toward obtaining full registration of all the known arthropod-borne viruses, and invitations were circulated to register "new" and unpublished viruses that should qualify for registration. The objective of securing full registration of the well-known viruses has not yet been reached, but great progress has been realized and a total of 93 viruses have now been registered. In the registration of the published, well-known viruses, an attempt is made to have the person or his collaborators who were responsible for the discovery of the virus make the registration. Failing in this, an investigator who has been devoting major attention to the virus and who is familiar with recent literature is asked to make the registration. This procedure has required time and has accounted for the delay in registration of some of these viruses.

As for the unpublished viruses, the responsibility for registration lies with the discoverer. The duty of the committee goes no further than to extend the invitation to register. The main problem in the registration of new viruses, besides obtaining adequate data on their characteristics, is to decide whether the virus differs sufficiently from previously registered viruses to be regarded as a new species or type. This subcommittee has avoided acting as a judge in this respect and has limited its function to exchange of views among investigators who have had an opportunity to work with the virus in question. Since at present the most generally accepted criteria for classification of viruses relate to their antigenic characteristics, the opinion of Dr. Jordi Casals, who has been the outstanding leader in this field, is frequently sought, and it is a pleasure to acknowledge his collaboration and very helpful advice.

It may be anticipated that the exact bases of classification and the magnitude of divergence from other related viruses that are required for regarding a virus as a distinct entity worthy of separate designation will continue to be a matter of discussion for some time to come. Certainly it is not the function of this subcommittee to lay down rules or make decisions on the acceptance or rejection of a virus for registration; and in order to be guided in this respect, it is hoped a special committee will eventually be

appointed and assume the responsibility of rendering decisions on acceptance or rejection of a virus for registration in the Catalogue. This reference committee should also have the responsibility of reviewing from time to time the Catalogue and make recommendations on viruses that should be deleted.

Clearly, a virus catalogue, to be of value, must be kept current, not only for the registration of new viruses as they are discovered, but also new information concerning the viruses registered should be currently supplied. It was and still is hoped that this responsibility will be assumed by the person registering the virus, but it is questioned whether this source alone is dependable, especially as many of the investigators reporting viruses may be in field laboratories where library facilities are inadequate for keeping abreast with the literature. As an accessory source of information, arrangements have been made to reproduce and distribute abstracts of articles on arthropod-borne viruses from Biological Abstracts. Reproduction of abstracts is now in progress and it is the intention to send out with the Catalogue at the end of October abstracts going back to at least January 1960, and shortly thereafter for the years 1959 and 1958, and possibly 1957. The Director of Biological Abstracts, G. Miles Conrad, has not only been kind enough to grant us this permission, but also generously supplied us without charge back numbers of Section C for the past four years and will send us bimonthly issues as they appear.

These abstracts will be reproduced in the same size print as the original on 3 x 5 inch heavy paper slips. With the thought that an index system for filing would make them more readily useful, each abstract will carry an index code at the top left hand corner and will be accompanied by a supply of index cards. Thus, with the code number and the flag cards, filing is a simple clerical procedure. It will here suffice to say that the index system uses as a framework the main rubrics in the questionnaire for registering viruses in the Catalogue in order that cross reference or inter-transfer of information may be facilitated.

A similar arrangement has been made through Dr. Gordon Smith and Dr. Charles Wilcocks for the utilization of abstracts in future issues of the Bulletin of Hygiene and of Tropical Diseases if it seems desirable, but the initial issue will include only abstracts from Biological Abstracts.

For more current and unpublished information, recourse may be had to the Newsletter.

This fourth issue of the Newsletter is the first that was opened to international participation. The response has certainly vindicated the unanimous decision to share this facility with arbovirus investigators of all nations. This issue marks the achievement in the short period of two years of a truly international sharing of scientific knowledge of timely interest and importance.

Members of the Subcommittee on Information Exchange have recently had the opportunity of visiting a number of laboratories and investigators which previously have been somewhat removed in time and geography from sources of information about one of the most rapidly developing fields of medical and biological science. It is worthy of note to mention here the sincere appreciation expressed by these investigators individually and collectively to the members of the original Gould House Group who freely and generously approved the extension of this mechanism of communication and information exchange to all serious investigators of arthropod-borne viruses.

The increase in number of contributing countries to eighteen and contributing investigators to thirty-nine attests to the international coverage of the newsletter.

Subcommittee on Information Exchange

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REPORT FROM JOAN B. DANIELS, VIRUS SECTION
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MASSACHUSETTS DEPARTMENT OF PUBLIC HEALTH
(IN COOPERATION WITH DR. RICHARD O. HAYES,
TAUNTON FIELD STATION, ENCEPHALITIS SECTION
TECHNOLOGY BRANCH, USPHS, CDC)

Duration of HI and Neutralizing Antibodies for EE

In order to interpret the absence of antibodies in long-lived species as well as the specificity of HI antibodies, the following sera are being studied: human sera collected from 10 persons 3 to 22 years after infection with EE; 3 human sera found positive by the neutralization test by plaque reduction (PR) in a survey by Dr. Robert A. MacCready of 328 volunteers from the endemic area; sera from inoculated animals collected monthly and made available by Dr. Richard O. Hayes. The latter group comprised 5 chickens, 4 pigeons, and 2 wild rabbits which had been viremic and which had neutralizing antibody in every bleeding including the present (19 months). Two wild rabbits, 5 frogs, and 4 squirrels which did not become viremic nor develop antibody served as controls with each test for the first 6 months.

All 13 human sera were positive for neutralizing antibody (PR) in spot tests of 1/5 dilutions against 60-120 plaque forming units (PFU) of virus.

Titrations of PR antibody are incomplete but the values at present available have not indicated any great dissociation between HI and neutralizing antibody. The latter, however, is a more sensitive test.

Four persons infected with EE in 1938 had in 1960 HI titers of 40, 80, 160, and 320, respectively. Two have been titered for PR antibody (80-100% reduction of PFU in controls). Patient B was positive PR in 1/320 dilution (HI 80) and patient L. F. was positive PR in 1/640 dilution (HI 320).

Two persons infected in 1955 had in 1960 HI titers of 40 and 160.

Four persons infected in 1956 had in 1960 HI titers of 160, 20, 40, and 160.

Three persons found positive by PR in the 1961 survey gave the following titers:

Donor	HI	PR
P. P.	40	160
A. Mc.	<20	>5; <80
S. W. *	160	320

Among the inoculated animals, chickens showed a slow decline in HI antibody from 640 or greater to 40-320 in the later bleedings. Two late bleedings have PR titers of 1/640 and 1/1280 (HI 160).

Wild rabbits showed a similar slow decline of HI antibody from highs of 320 and 640 to 160 over a 12-month period.

In general, consecutive bleedings of the same animal gave HI values within a twofold dilution. There were two exceptions to this. On two occasions bleedings of one species or another showed negative HI readings (but positive PR neutralization) when titers of bloods of other species at the same time remained high. The negative HI bleedings were preceded and followed by high titer bloods in the same animals. No explanation has been found in reviewing the procedures used in collecting bloods or performing tests in the laboratory.

The second exception is the behavior of antibody in pigeons. Between the 43rd and the 90th day, all four pigeons showed a rapid decline in HI antibody from 320, 320, 640, 80, to 10, <20, 40, <10, and thereafter HI antibody was undetectable except for an occasional low reading. PR antibody still remains positive in spot tests at 1/5 dilution in the 19-month bleedings. These birds should prove particularly interesting to study any possible dissociation of HI and neutralizing antibody when data becomes available on PR titers.

* S. W. is a sibling of a 1956 fatal case.

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

Florida Study:

As part of a continued program of a survey for arthropod-borne viruses (arboviruses) in southcentral Florida referred to

in three previous newsletters, collections limited to bird bloods were again made during August 1961. One hundred forty-eight plasmas representing thirteen bird species were subjected to virus isolation and antibody level studies. As in past years, the plaque technique utilizing primary cultures of chick and duck embryo was employed for virus isolation. The hemagglutination-inhibition test was used for antibody screening.

One confirmed virus isolation was made from the plasma of a purple grackle. A 1:10 dilution of this plasma contained 30 duck embryo plaque forming units per ml. Upon passage of the plaques in infant mice, the average survival time (AST) was 2-3 days. Identification of this agent is currently in process.

A summary of the antibody studies on bird plasmas collected in 1960 and 1961 is shown in the following table for the purpose of comparison.

HI Reactors with Plasmas from Birds Captured in 1960 and 1961 in the Same Area

	No. plasmas examined		PLASMA REACTORS WITH ANTIGENS			
			WEE (RI)	WEE (HJ)	EEE	SLE
1960	420	No.	43	95	87	32
		%	10.2	22.6	20.7	7.6
1961	148	No.	0	5	4	8*
		%	0	3.4	2.7	5.4

*6 of 8 plasma reactors were from immature birds

It would appear by the serologic data that the group A arboviruses were generally more active in the bird population sampled in 1960 than in 1961. Moreover, there was found to be a 6-12-fold decrease in 1961, as compared to the 1960 survey, in the incidence of plasma reactors from grackles, blue jays, red-winged blackbirds, ground doves, and towhees, using EEE, WEE, and WEE (HJ) antigens.

Essentially no significant difference was detected in the number of SLE or related group B plasma reactors in the two sampling years, except that all of the positive plasma reactors in 1960 were from

mature birds and in 1961 the reactors were mostly from immature birds. It is not improbable that group B arbovirus activity had been relatively recent in the 1961 bird population sampled, even though, as yet, a group B virus isolation has not been made to support this.

South Carolina Study:

In order to fill in another segment of the geographical belt of the eastern seaboard not presently covered by our group or others carrying out arbovirus field studies, collections were made during July and August, 1961, in an attempt to survey for arboviruses in the Beaufort area of South Carolina. Virus isolation and/or antibody studies were undertaken utilizing mosquitoes, and bird, equine, and human bloods collected in the field.

The following table shows the number of specimens collected, the number examined for antibody and for virus, and the number of virus isolations made. It should be noted that only about one-half of the mosquito pools have been examined for virus to date.

Specimen	No. Collected	No. Examined for Virus	No. Examined for antibody	No. Virus Isolations
Bird plasmas	109	108	109	0
Mosquito pools	471	280	0	7*
Horse sera	15	15	15	0
Human sera	36	36	36	0
Totals	631	439	160	7

*Plaque-forming and mouse pathogenic agents

Virus isolations. Of the 280 mosquito pools, representing 17 species of mosquitoes, examined for virus thus far, some 15 chick or duck embryo tissue culture plaque-forming agents have been recognized. Seven of these agents, when inoculated into infant mice, produced paralysis and death. The AST in mice for most of these agents was 5-7 days.

No virus isolations were made from bird, horse, or human bloods.

Antibody levels. The following table shows the number of bird plasma and human and horse serum reactors to WEE (RI strain), WEE (HJ strain), EEE and SLE viruses as measured by the HI test:

Sample	No. Tested		POSITIVE REACTORS TO WEE (RI)	WEE (HJ)	EEE	SLE
Bird plasma	109	No.	2	2	4	26
		%	1.8	1.8	3.7	23.8
Human sera	36	No.	0	0	1	8
		%	0	0	2.8	22.2
Horse sera	15	No.	3	3	5	1
		%	20.0	20.0	33.3	6.6

It is of some interest to note the high percentage of SLE HI reactors in bird and human bloods and the apparent inactivity, as manifest by the serologic tests, of the group A arboviruses in the bird and human sample populations. Group A arbovirus antibody levels in horse sera can be attributed to vaccination.

Stronger evidence to support the serologic results of group B virus activity is pending identification of the virus isolates from mosquitoes.

REPORT FROM THE STATE OF NEW JERSEY DEPARTMENT OF HEALTH TRENTON 25, NEW JERSEY

The 1961 Arthropod-borne Virus Study by the New Jersey State Department of Health:

The Division of Laboratories under Assistant Director Dr. Martin Goldfield, and the Bureau of Veterinary Public Health, under Dr. Oscar Sussman, are conducting an Arthropod-borne virus study which will include four separate study areas. Three of these are located within the 1959 EE outbreak area where human, horse, and pheasant cases occurred. The fourth area is located purposely inland within the great Swamp, near Morristown, where horse and pheasant epizootics occur and where no human cases are known to have occurred.

Dr. Martin Goldfield will conduct the laboratory and some field aspects, which involve the collection target of 500 native human blood samples with personal histories, early in the season, from each study area, with additional blood sampling to follow. The laboratory aspects include the testing of sentinel chicken, bird, and human sera, and speciated pools of mosquitoes.

Dr. Raymond E. Kerlin, Assistant Chief of the Bureau of Veterinary Public Health, with the aid of one medical and three veterinary students, one at each of the four study areas, will supervise the veterinary aspects. Mr. Walter R. Gusciora, entomologist, Arthropod and Rodent Control Section of the Bureau, with two biology students, one to be assigned per two study areas, will supervise the entomologic aspects. Mr. Ernest M. Mills, Assistant District Agent, of Fish and Wildlife Service, Branch of Predator and Rodent Control, U.S. Department of Interior, will trap small mammals for blood studies.

Birds will be netted four days per week at each study area; blood samples will be drawn and the birds will be banded, released, and recaptures noted. Mosquitoes are to be trapped for one light trap day per study area per week and one resting box trapping day per study area per week. Nine light trap stations plus seven resting box stations (six boxes at each station) will be established within each study area. In addition, a mosquito truck trap, the type introduced by the State of Florida, will be employed. Chicken blood samples will be collected on a bi-weekly basis from locations near each study area and checked for reactivity to determine whether they may or may not serve as indices of viral activity. The operation began on June 5 and will end about October 31.

Dr. Jeff Swinebroad, Chairman, Department of Biological Sciences, Douglass College, Rutgers University, will act as a consultant on ornithologic aspects in addition to his bird population study. Blood-engorged mosquitoes will be stored for later submittal to Dr. Roy Chamberlain, Virus and Rickettsia Section, CDC, for host blood meal determination studies.

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

A training grant has been approved by the Public Health Service for work at the Pennsylvania State University under the direction of David E. Davis to develop research persons who can work as a

member of a team. Opportunities are available at the graduate and post-doctoral level for ornithologists or mammalogists to conduct research and take courses that will prepare them to examine the role that birds or mammals may play in arthropod-borne diseases. In addition, provision is made for less experienced persons to work during the summer. The objective of this program is to fill the gap that now exists between those who are competent in ornithology and mammalogy and those who wish to use biological information to solve epidemiological problems.

REPORT FROM MAJOR FRANK G. FAVORITE
CHIEF, MEDICAL ENTOMOLOGY DIVISION
U.S. ARMY ENVIRONMENTAL HYGIENE AGENCY
UNITED STATES ARMY MEDICAL SERVICE

Our progress in 1961 has been the collection of bird samples from 212 birds collected by Japanese mist nets on the Edgewood, Maryland peninsula. Of these, 94 have been screened in weanling mice for virus. All have been negative. The remaining samples are being held at -60° C either as whole bloods or separated fractions of serum and modified Alsever's solution diluted red cells until we have time for processing. (If we recover no more than 1.0 ml of blood from a bird, the sample is held as whole blood).

Along this line of effort, we have finally adopted cardiac punctures vs other methods such as jugular and wing vein bleedings to obtain blood samples from birds. Many workers, including myself, have previously adhered to these latter methods, but we now find we can bleed even small birds (sparrow-size and under) by cardiac puncture, recovering 0.7 to 1.5 ml of blood with better than 90% success. "Success" is defined as obtaining a blood sample with minimum contamination and having the bird depart under its own power after 30 minutes holding.

We are also shifting to the method of Dr. E.A. Meyer (using seamless cellulose tubing) in separating serum from blood. Thus far we have limited experience but hope untimately to improve the recovery of bird serum both qualitatively and quantitatively.

Bird Blood Samples Collected as of 18 Aug. 61

( ) No. of samples inoculated into weanling mice as of 18 August 1961

Family	Species	Sex Undetermined Immature	Mature	Mature	Mature
Cuculidae	Yellow-billed Cuckoo		1 (1)		
Picidae	Flicker			1 (1)	5 (1)
	Hairy Woodpecker		2 (2)	1	2 (1)
Tyrannidae	Crested Flycatcher		4 (1)		
	Wood Pewee		3		
Hirundinidae	Barn Swallow		1		
Corvidae	Blue Jay		9		
Paridae	Carolina chickadee		1		
	Tufted Titmouse		1		
Mimidae	Mockingbird		2 (2)		
	Brown Thrasher		6		
	Catbird		7		
Turdidae	Robin	3 (2)	16 (7)	1	7 (5)
	Wood Thrush		6 (2)		
	Olive-backed Thrush		2		
Sturnidae	Starling	19 (11)	26 (19)		
Vireonidae	Red-eyed Vireo		1		
Ploceidae	House Sparrow	2 (1)		7 (4)	8 (4)
Icteridae	Purple Grackle	2	29 (19)	11 (3)	7
	Rusty Blackbird		1 (1)	1 (1)	
	Red-winged Blackbird	1 (1)		8 (7)	1 (1)
Thraupidae	Scarlet Tanager			1	
Fringillidae	Common Goldfinch		1 (1)	1 (1)	1 (1)
	Towhee		1	1	1
	Song Sparrow		1		
Totals		27 (15)	120 (49)	33 (17)	32 (13)
Grand Total	212 (94)				

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

We have had three cases of equine encephalomyelitis in horses in Maryland since September 1. We have isolated virus from the brain of one of these animals. We have also obtained serological confirmation on one case of the disease in a pony in southern Delaware.

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

Personnel news from the Bethesda LTV component includes: Dr. A. Shelokov returned to NIH from Middle America Research Unit, Canal Zone, with Dr. Henry Beye replacing him as Director, MARU, while Mr. C.J. Gibbs continues as Acting Head of the Bethesda Arthropod-Borne Virus Section; Dr. N.H. Wiebenga transferred in June from Naval Medical Research Institute to assume responsibilities of senior staff member; Dr. R.V. McCloskey, NIAID Research Associate (at one time with Dr. H.M. Morgan of Rochester) joined LTV staff in July. With the arrival of new professional personnel, the program is being reviewed and expanded.

Studies on the typing of arthropod-borne viruses with combination antiserum pools in suckling mice IC neutralization test recently have been concerned with selected Group A viruses (EE, WE, Mayaro, Sindbis, and Uruma). Findings thus far indicate that: (1) the test, employing pooled hyperimmune rabbit antisera, is group specific; (2) broad cross-reactivity within Group A is enhanced when the combination includes the serum homologous to the virus being tested; (3) the dilution resulting from the pooling of specific antisera has not been critical in the tests conducted. Experiments are underway to determine the CF and HI patterns obtainable with these antiserum pools.

A simplified technique for producing arthropod-borne virus CF antigens is being developed. Mouse brain suspension prepared in borate-KCl-buffer (pH 9.0) is stored for 24 hours at 4° C and then centrifuged at low speed. The resulting CF antigens have been of high titer, specific and not anticomplementary. The antigens are easily rendered non-infectious by exposing the virus suspension to ethylene oxide gas for 4 hours in a BenVenue sterilizing chamber, apparently without a deleterious effect on the titer. Potent antigens have been already produced with EE, WEE, VEE, SLE, MVE, and YF (Asibi, French neurotropic and 17D) virus strains.

Two lots of HKTC-propagated EE virus (Panama 101 strain) were treated with formalin, held at 37° C for 3 days and at 5° C for 6-10 days; then tenfold dilutions, beginning with 1:10, were inoculated IC into guinea pigs and mice. No symptoms attributable to live virus had developed after inoculation with either lot.

Fourteen days after the two vaccine injections a week apart (GP - intradermally, mice - IP), the animals were found to be resistant to 2 logs of a heterologous EE virus strain (horse isolate from Cambridge, Md.) when challenged IC with tenfold dilutions. Still incomplete tests of antibody response indicate that mice developed significant titers of NT antibodies (GP not yet tested); guinea pigs did not develop CF titers (mice not tested); neither species had shown any increment in HI antibodies.

REPORT FROM DR. PAULINE H. PERALTA, ACTING HEAD VIRUS SECTION, MIDDLE AMERICA RESEARCH UNIT BALBOA HEIGHTS, CANAL ZONE

MARU VIRUS SECTION, LABORATORY OF TROPICAL VIROLOGY, NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

In the course of studies on medically important viral diseases of the American tropics, the Virus Section of MARU allots at least 50% of its effort to investigations involving arthropod-borne viruses.

A continuing activity of the Section is the virological work-up of clinical cases brought to our attention by outside physicians. Although enteroviruses are the most frequent isolates, the virus of Venezuelan equine encephalomyelitis (VEE) was isolated for the first time in Panama as a result of this activity. The virus was recovered from serum obtained April 20, 1961, from an acutely ill Panamanian boy a few hours before his death. By the time the identification of this isolate was made in early May, one of the MARU staff members was already hospitalized and virus had also been recovered from his serum. Two other members of the staff who had been working closely with the second isolate became ill in rapid succession.

Gorgas Memorial Laboratory in Panama joined us to study the ecology of VEE virus in the area of Canito where the deceased patient resided. The area is located on an arm of Gatun Lake, the large artificial lake through which the channel of the Panama Canal passes. Field collections were carried out during the month of May.

In June, a program of vaccination of MARU personnel was begun, using attenuated VEE virus (TC-80) vaccine kindly made available by Cols. Tigertt and Berge of USAMRU, Ft. Detrick, Maryland. By mid-August, 43 persons had been vaccinated. There were a few (mild) reactions.

Laboratory studies at MARU are still in progress. These include isolation attempts from Canito arthropods and sentinel chickens, from specimens collected from the fatal case as well as from the three infected laboratory workers, and from daily serum specimens obtained from the first eight vaccinees. Serological tests are under way to determine the VEE antibody status of residents and domestic animals of the Canito area, the pattern of antibody development in the three persons accidentally infected in the laboratory, and antibody responses in all vaccinees.

September 1961 marks the beginning of the third year of a 3-year project in which MARU is collaborating with Gorgas Memorial Laboratory to investigate the ecology of the arthropod-borne viruses of the tropical rain forest in Bocas del Toro Province, Panama. Thus far in the project, thirty-one isolates have been recovered in this laboratory from arthropod pools--ten from Phlebotomus sandflies and twenty-one from mosquitoes. The rate of isolation from the former continues to exceed by far the overall rate of isolation from the latter.

In recent months, progress in identification has gained considerable momentum. Five isolates--four of them from Phlebotomus--are strains of Indiana type vesicular stomatitis virus (VSV). Following a recent exchange of prototype immune sera and ascitic fluids with Trinidad Regional Virus Laboratory and Belem Virus Laboratory, it was possible to relate seventeen isolates (which, on the basis of CF tests with MARU antigens and antisera only, fall into four groups) to four viruses recognized in Trinidad and/or Brazil: Una, Guaroa, Wyeomyia, and Guama.

Six closely related Phlebotomus agents and two isolates from Bocas mosquitoes have not yet been related to any other viruses; the last isolate is too recent to have been tested. The accompanying table outlines progress in identification of the 31 isolates.

As another result of the exchange of immune fluids, one of three isolates recovered from mosquitoes in the 1958-59 study of the ecology of Eastern equine encephalomyelitis virus in the Pacora-Tocumen area east of Panama City has been found to be closely related to Melae virus. Still unidentified are the other two mosquito isolates mentioned, as well as an arthropod-borne virus (JW-10) recovered in July 1960 from serum obtained from a febrile participant in jungle warfare training conducted by the U.S. Army on the Isthmus.

For a number of months we have been preparing immune ascitic fluids in mice according to modifications of a method in the literature, in which Freund's adjuvant and heat-killed staphylococcus are employed in addition to virus. So far it appears that such ascitic fluids prepared against arthropod-borne viral agents will be very useful for identification procedures in this laboratory. Currently, various inoculation schedules are being evaluated (with Ilheus as the virus) in an attempt to arrive at a routine immunization procedure.

Despite isolation attempts from over 300 lots of acarina specimens collected in Panama by Drs. Brennan and Yunker, on temporary assignment here from the Rocky Mountain Laboratory, Hamilton, Montana, no viruses have been recovered to date in a suckling mouse system

Progress in Identification of Virus Isolates from Arthropods, Bocas del Toro, Panama

MARU data as of 1 September 1961

Arthropod-borne virus group	Virus	MARU Prototype Number	No. of Isolates	Breakdown of isolates by genus from which isolated	
				Number	Genus
Group A	Una	BT-1371	6	2	Aedes
				4	Psorophora
Bunyamwera	Guaroa	BT-1122	1		Anopheles
	Wyeomyia	BT-219	4	1	Culex
				3	Psorophora
Guama		BT-640	6		Culex
Unidentified Viruses		BT-2132	1		Culex
		BT-2368	1		Psorophora
		BT-2579	1		Psorophora
VSV Indiana		BT-78	5	1	Culex
				4	Phlebotomus
Unidentified group		BT-104	6		Phlebotomus

REPORT FROM DR. H. C. BARNETT, CHIEF, DEPARTMENT OF ENTOMOLOGY, WALTER REED ARMY INSTITUTE OF RESEARCH WASHINGTON, D. C.

Studies on the laboratory transmission of Japanese encephalitis virus by Oriental culicine mosquitoes have been continued. While no "dosage threshold effect" has been observed in the Culex species

studied thus far, it was demonstrated that Aedes albopictus did not become infected or infective unless it was fed on a viremic host circulating more than 4.0 logs of JE virus. A similar response was noted in the closely related species, Aedes aegypti. In these experiments, mosquitoes were tested after extrinsic incubation periods of from 9 to 50 days. Because of their high infection thresholds, it is probable that the vector potential of A. albopictus and A. aegypti is much lower than that of such species as Culex gelidus, Culex tritaeniorhynchus, and Culex pipiens.

A comparison was undertaken of transmission rates of JE virus achieved by two subspecies of Culex pipiens of different geographic origin, one from Japan, and the other from Maryland. Two strains of JE virus, FM 380 and T 485, both at low mouse passage levels, were used in this study. Batches of the two strains of mosquitoes were fed simultaneously upon chicks circulating 4.0 to 5.0 logs of one or the other strain of JE virus. The rates of transmission to chicks for mosquitoes infected with FM 380 when tested after 17 days extrinsic incubation were: 6 out of 13 (46%) of the Japanese C. pipiens pallens and 5 out of 11 (45%) of Maryland C. pipiens pipiens. Mosquitoes infected with T 485 virus tested after the same extrinsic incubation period transmitted as follows: 9 out of 20 (46%) Japanese C. pipiens and 7 out of 14 (50% Maryland C. pipiens. Studies are currently underway with both virus strains fed to mosquitoes at lower dosage levels.

Work on virus isolates from sandflies and patients suspected of having sandfly fever, described in previous reports, has been continued. Dr. Fred R. McCrumb of the University of Maryland School of Medicine, who is collaborating with us in the identification of these agents, reports success with the complement-fixation test using hyperimmune sera prepared in adult mice. Studies have also been undertaken at WRAIR on the growth patterns of these viruses in a variety of tissue cultures of human origin.

REPORT FROM DR. ROY W. CHAMBERLAIN, ARBOVIRUS UNIT VIRUS & RICKETTSIA SECTION, COMMUNICABLE DISEASE CENTER, ATLANTA, GEORGIA

Field Studies:

The bulk of field effort this past season has been expended on the Big Cypress Seminole Indian Reservation, 45 miles south of Clewiston in south Florida. One of the stimuli for this study was

the finding by Work in 1960 that a high proportion of the Indians on the reservation possessed VEE antibodies (Information Exchange No. 3, April, 1961.)

The area was surveyed in March, 1961, to plan comprehensive bird and mosquito collections for the ensuing season and a few preliminary samples were taken. Commencing in April, intensive bird collecting was done by mist nets and mosquito collecting by New Jersey light traps, portable miniature light traps, chicken-baited lard can traps, and human biting catches. These have continued at least two weeks per month since their initiation. Approximately 20 sentinel mouse litter exposures have also been made each month. Over 100 chickens also serve as sentinels.

The sampling areas include several different habitat types, i.e., vicinity of Indian "chickees", grassy marsh, cypress and custard apple swamps, oak-palm hammocks, pine woods, pasture land, and woods bordering cultivated vegetable fields. The following table summarizes the vertebrate catches and testing to mid-September. Approximately 500 birds collected since that time are not included in the table and most of these are still on test. One of these, a black and white warbler, collected September 20, has been found virus positive, but the isolate awaits identification.

No definite isolations have thus far been made from the vertebrate samples other than the bird mentioned above, although several specimens are doubtful positives and await further checking.

Mosquito populations were light in April and most of May because of unusually dry weather. Despite this, however, over 5000 mosquitoes were obtained. In late May, with the coming of rains, mosquito production increased, and it has been possible to collect approximately 100,000 mosquitoes each month, June through September. October collections are not yet completed at time of writing, but are running considerably lower than in September. Full scale mosquito collecting will be made again in November, but will be reduced or discontinued thereafter.

To date, 472 mosquito pools have been tested in suckling mice, comprising collections made from March to the first of June. A total of 934 pools have been tested in 1/2-day-old chicks, including collections made to mid-June. From these pools, two possible virus isolations have thus far been made, one from Culex melansconion

collected May 20-22, and another from C. nigripalpus, taken June 14-16. These have not yet been verified or identified.

Approximately 5000 additional mosquito pools await testing and probably will not be completed until Spring, 1962.

No isolations have been made from the sentinel suckling mice.

The South Alabama study site, reported upon in the last Information Exchange, was only spot-checked this past season to verify continued EE and WE virus activity in birds and mosquitoes. Two of 217 birds collected in August yielded EE virus, one an oven bird and the other a wood thrush. Mosquitoes collected in July were virus negative. An October mosquito collection will be made and tested.

Summary of Field Collections and Laboratory Testing of Big Cypress Blood Specimens for Virus Isolations

	SPECIMEN ORIGIN				TESTING		
Dates	Birds	Mammals	Reptiles	Amphibians	Wet Chicks	suckling mice	DETC*
Mar 30-31	41		1				x
Apr 17-22	170	3	1	1			x
May 5-14	284	91	9				
May 20-24	34						
Jun 7-20	354				x		x
Jun 14-Aug 29 (Austin)	550	17				x**	x
Jul 8-17	310	1					x
Aug 30-Sep 8	104	50	10	6			x
Sep 9-13	48						x
	1892	162 (includes 35 calves)	21	7	354	216**	1597

*DETC - Duck embryo tissue culture overlays

**Inoculation of suckling mice started with specimens collected July 19, 1961, and goes through specimens collected Aug. 17, 1961.

REPORT FROM DR. WILLIAM L. POND, DEPARTMENT OF MEDICINE, UNIVERSITY OF MIAMI MEDICAL SCHOOL MIAMI, FLORIDA

A new arthropod-borne virus research program was recently initiated in the Department of Medicine, University of Miami Medical School, in Miami under a long-term grant from the National Institute of Allergy and Infectious Diseases of the Department of Health, Education, and Welfare. The program will be concerned with the laboratory and clinical investigations of arthropod-borne virus diseases and is under the joint direction of Dr. William L. Pond and Dr. N. Joel Ehrenkranz.

REPORT FROM DR. LOUIS S. GRANT, PROFESSOR OF MICROBIOLOGY, UNIVERSITY COLLEGE OF THE WEST INDIES KINGSTON 7, JAMAICA

Brief Account of Sphere of Interest:

This laboratory is interested in investigating the prevalence of arthropod-borne virus diseases in Jamaica and in studying the epidemiology of these diseases in this island.

Summary of Past Work (1953 and 1956-61):

In 1953, during the course of an epidemiological survey by Dr. M. M. Sigel and Dr. L. S. Grant among school children and adults for the prevalence of Lymphogranuloma Venereum antibodies, some of the sera were examined for St. Louis virus antibodies by Dr. M. M. Sigel--at that time Virologist at the Children's Hospital in Philadelphia. Sigel, in reporting to U.S. Navy by whom the funds were provided for these studies, records that in some of the areas, mainly May Pen and Vere, antibodies for St. Louis virus were demonstrated in the sera of children and animals. Attempted isolation of virus from the bloods of birds and from mosquitoes were carried out by mouse inoculation, without any success.

Funds were later provided by the Rockefeller Foundation to carry out a Programme of Arthropod-borne Virus Investigation in Jamaica and this work was started in 1956.

Preliminary Serological Survey (1956-57):

Using haemagglutination, complement fixation, and neutralization tests, a preliminary serological survey was carried out on

approximately 300 sera collected in five areas of different ecology in Jamaica. These were examined at the Trinidad Regional Virus Laboratory and the Rockefeller Virus Laboratory in New York for evidence of arbovirus infection. A preliminary survey was carried out on the mosquito species prevalent in one of the areas of highest group B arbovirus infection and attempts were made to isolate virus from the species collected.

Intensive Serological Survey (1957-61):

Using haemagglutination inhibition and complement fixation tests, an intensive serological survey was carried out on about 1,000 adults and children in the Vere-May Pen area, one of the areas of most intensive Group B arbovirus infection. Analysis of this material will provide epidemiological data of Dengue and St. Louis infection in relation to age, sex, etc. Surveys were also carried out on sera from other areas closer to the virus laboratory in order to find a suitable and small area for a future programme of more intensive epidemiological work. During 1960-61 this intensive serological survey was continued at two sugar estate areas--Caymanas and Innswood near to the University College Virus Laboratory. Tests were also done on adult and children's sera in Kingston, the capital city, and at Port Royal, areas where yellow fever control programmes have been keeping the Aedes aegypti under complete or almost complete control during the past 8 years.

Investigations of animal sera (cows, mules, and horses, etc.) have been continued, and attempted virus isolation from febrile school children has been undertaken. Bloods were collected from patients with early undiagnosed pyrexias and inoculated into baby mice in order to attempt isolation of virus.

Entomological programmes were continued in the Vere-May Pen and Caymanas areas. Weekly catches of mosquitoes were carried out. These were identified, pooled, and inoculated into baby mice for attempted isolation of virus. Sentinel animals were put out in the Caymanas area at various periods during the past 2 years.

Results of Programme to Date:

The technical methods used have been those that were currently being carried out at the Trinidad Regional Virus Laboratory and the Rockefeller Foundation Virus Laboratory, New York.

(a) Preliminary Serological Survey: The sera showed antibodies for group B arborvirus and were negative for Groups A and C. In group B, neutralization tests showed only St. Louis and Dengue antibodies. For the island as a whole, the neutralization test for group B arbovirus showed protection in 200 out of 300 sera tested, the percentage being much higher in adults than in children under 14. The incidence of partial or complete protection for St. Louis ranged from 26.8% in the central mountain areas to approximately 80% in a rural area, with coastal swamp, mangrove, inland pastures and ruinate. For Dengue, partial and complete protection varied from 66% in rural village areas of high rainfall to 93% in an irrigated sugar cane area with coastal swamps.

(b) Intensive Serological Survey: (Vere-May Pen and other areas). The significant immune rates for children of different age groups as shown by HI test were as high as 40% for Dengue and 80% for St. Louis in some districts or villages in the Vere-May Pen area. In the complement fixation test these figures for children were as high as 17% for Dengue and 33% for St. Louis. For adults the HI test showed immune rates as high as 79% for Dengue and 97% for St. Louis. The CF test showed rates as high as 68% for Dengue and 70% for St. Louis. In the Port Royal area where Aedes mosquitoes have been eradicated over the past 8 years, using HI tests, there is an absence of antibody for Dengue in school children age 5-15 years but approximately 20% of these children show antibodies for St. Louis.

Current Activity & Future Programme:

1. (a) Attempted isolation of virus from P.U.O., early clinical and subclinical febrile cases, mainly in young children up to 7 years of age.

(b) Attempted isolation of virus from sentinel animals, mosquitoes, bed bugs, etc.

2. Serological and clinical followup of 50 families in an area of moderately high Dengue and St. Louis infection.

3. Study of the transfer of maternal antibodies for Dengue and St. Louis virus to infants and the duration of the antibodies in the infant and child (1-3 years followup).

4. Investigation of pure or almost pure Dengue or St. Louis areas.

5. Entomological study of insects (mainly mosquitoes) in the areas under study.

Source of Funds for Programme:

The funds for carrying out this programme 1956-60 have been provided wholly by a grant from the Rockefeller Foundation. Funds have also been provided for continuation of the work until 1965. Added to these financial provisions there has been readily available the facilities, advice, assistance, and cooperation of the staff of the Trinidad Regional Virus Laboratory, Port-of-Spain, and the Rockefeller Foundation Virus Laboratories, New York. Fellowships have also been provided for medical and technical staff to visit the Trinidad and New York laboratories of the Rockefeller Foundation for training in and study of the methods used there.

The World Health Organization has also provided Fellowships for staff to visit the Rockefeller Foundation and other laboratories for training in both general and specialized virological methods.

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

Two newly undertaken activities are both concerned with the role of rodents in virus cycles, particularly Group C and Guama Group agents.

1) The use of sentinel mice in Bush Bush Forest in the eastern part of the island led to numerous isolations of virus strains during the past rainy season. It is presumed that mosquitoes were the responsible vectors. Hence a number of experiments have been made in the design of various sorts of traps that will hold mosquitoes attracted to the sentinels.

2) In view of the suspected role of native wild rodents in these virus cycles, attempts to colonize some of them in the laboratory are in progress. To date some success has been achieved with Oryzomys laticeps and Zygodontomys brevicauda. Second generation offspring of the captive animals will be used in studies of the pathogenesis of viruses autochthonous in Bush Bush Forest.

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

The Virus Department of IVIC began its activity in July 1959 and has at present a total staff of twenty-three persons. The majority of them are directly engaged in arbovirus investigations. Apart from the laboratory for plant viruses, the department consists of a laboratory for domestic animal viruses (mainly arbor A group) headed by Dr. M. Mussgay, a laboratory for group B arboviruses (mainly yellow fever) and classically insect viruses headed by Dr. G.H. Bergold and a laboratory for Entomology under the direction of Mr. O. Suarez.

The aim of the Virus Department is the investigation of arboviruses occurring in Venezuela. Due to the background of the staff and the equipment available, emphasis is directed to the biophysical and biological characterization rather than to the Epidemiology of viruses of Venezuela.

The establishment of a mouse colony (at present about 10,000) and the equipping and staffing of the department was rather slow. Considerable difficulties were experienced in training technical help in general and for tissue culture in particular. Several cell lines are continuously maintained and chicken fibroblasts are routinely prepared once or twice a week. Contamination, particularly of local eggs, and presence of unusual air-borne germs is causing considerable trouble and necessitates the addition of several uncommon antibiotics apart from mycostatin.

LABORATORY FOR DOMESTIC ANIMAL VIRUSES.

A. A simple method for identification of equine encephalomyelitis viruses. The plaque inhibition test developed by Wecker can be used for a rapid identification of equine encephalomyelitis viruses. Chick embryo cell cultures were infected with serum samples of patients suspected to suffer an equine encephalitis virus infection. The cultures were overlaid with an agar medium containing VEE, EEE, VEE immune serum, and normal serum, respectively (serum dilution in the overlay 1:40). Comparison of the plaque diameters showed that there was only a plaque-size reduction in dishes with a VEE-immune serum overlay, indicating that the plaque-producing virus in the patient sera was VEE virus. The test is very sensitive; an immune serum with a neutralization index of $10^{1.6}$ (in mice) reduces the plaque diameters 50%.

B. Studies with VEE and Aedes aegypti mosquitoes. In Aedes aegypti mosquitoes about 200 plaque forming units (VEE) were injected. After infection, mosquitoes were taken every day, triturated and the infectivity titers measured. Up to the third day after infection, the titers increased and then remained constant for at least three weeks. Eggs from infected mosquitoes showed a reduced and delayed rate of hatching. No evidence for an ovarian transmission of VEE virus was obtained. An attenuated VEE virus strain was obtained by passages in KB cells. This strain differed from the pathogenic one in having no pathogenicity for mice after subcutaneous application and in producing smaller plaques. There existed no differences between the two strains with respect to the multiplication in mosquitoes. It seems that no back mutation occurred during the multiplication of the attenuated virus in mosquitoes.

C. Electronmicroscopic studies of VEE virus. The development of VEE virus in KB cells was investigated electronmicroscopically. On the basis of these observations, it is suggested that the virus matures on membranes which surround cytoplasmic vacuoles. Besides the 40-45 mμ virus particles, other spherical particles with a diameter of about 25 mμ were visible; these particles were found attached to membranes on which the virus matures. The VEE virus was purified by sucrose density gradient centrifugations.

D. Mayaro Virus. A post-doctorate fellow (Dr. A. Saturno) is working on the biological and biophysical characterization of Mayaro virus.

LABORATORY OF GROUP B ARBOVIRUSES

A. A viral agent was isolated from the serum and liver of Alouata seniculus shot in the fall of 1959 in Estado Cojedes, which proved to be yellow fever virus (IVIC strain). This was confirmed by the kind cooperation of the staff of TRVL. Using mice neutralization tests, it was found that antiserum prepared from the IVIC strain protects very well against TRVL and JSS strain but does not fully neutralize 17D or other African strains. On the other hand, antisera prepared from African strains neutralize completely the IVIC and other American strains.

B. Extensive electron microscopic investigations reveal clearly 17D and IVIC virus particles in the brain of paralyzed mice and in highly purified suspensions (using the density gradient technique) prepared from infected KB cells. The demonstration of the virus particles in Alouata and mice liver as well as in KB cells and chicken

fibroblasts and salivary glands of Aedes aegypti is not so convincing. Some confusion occurred by the appearance of some larger spherical particles in the salivary glands of non-infected laboratory bred A. aegypti (local strain), which were feeding on mice originating from Carworth Farm in the United States. So far it was not possible to identify this virus which is inapparent in mice. Definite identification of particles seen with the electronmicroscope in mice, insect tissues and suspensions is presently investigated by using ferritin conjugated yellow fever virus antibodies.

C. Considerable difficulties were encountered in trying to use the plaque technique with chicken fibroblasts for viruses of the B group. Results using the bicarbonate-containing overlayer of Henderson were difficult to reproduce, particularly when certain types of disposable plastic flasks (Falcon plastics) were used. The tris-containing overlayer of Porterfield appears to be somewhat better. The lack of sufficient mice and the not yet well working plaque tests have held up unduly the whole yellow fever virus project.

D. Agents isolated from some patients from Estado Tachira, who exhibited symptoms very similar to yellow fever and who finally died, could not be identified as yet.

LABORATORY OF ENTOMOLOGY

A. A large colony of A. aegypti originating from nearby Los Teques is maintained for transmission experiments. Experiments to breed Aedes ioliote and Haemagogus splendence using field collected larvae and adults are under way. Some 2,000 mosquitoes were collected in various regions of Venezuela, identified, and stored for further investigation.

REPORT FROM DR. H. GROOT, INSTITUTO "CARLOS FINLAY", BOGOTA, COLOMBIA

Neutralizing antibodies for Bussuquara, Strain Ar 41922.

Although it shows some immunological differences from prototype Bussuquara, Ar 41922 is considered to be merely a strain of Bussuquara. Tests for neutralizing antibodies for this agent were made with the sera of people who resided in the Middle Magdalena Valley within 20 miles of the place where Ar 41922 was isolated twice from wild caught Culex spp. The results are presented in the following table. The prevalence of antibodies, similar in both sexes,

is lower in the younger group (15.4 per cent of 65 children aged 0-14) than in the older group (37.8 per cent of 103 adults).

The donors, who had been vaccinated about six months before with 17D by scarification, showed antibodies for yellow fever in the proportion of 96 per cent. However, it is believed that the presence of yellow fever antibodies has not affected significantly the NT results with Bussuquara. The majority of the sera were also tested by NT with other group B agents with the following results: 7 of 155 positive for dengue 2, 19 of 158 positive for Ilheus, and 9 of 158 positive for St. Louis. No correlation was observed between the sera positive for Bussuquara and the sera positive for the other viruses.

Sera from residents in other tropical localities in Colombia were also tested with Bussuquara by NT. The results showed no positives in 112 residents in the Upper Magdalena Valley and only 1 positive in 75 sera from the Eastern Plains.

Bussuquara virus, strain Ar 41922. Results of neutralization tests on 168 persons resident in rural areas of San Vicente and Barranca-bermeja, Colombia.

Age group (years)	MALE		FEMALE		TOTAL	
	Ratio*	%	Ratio	%	Ratio	%
0 - 4	2/11	18.2	0/8	0.0	2/19	10.5
5-9	4/14	28.6	2/10	20.0	6/24	25.0
10-14	0/11	0.0	2/11	18.2	2/22	9.1
15-19	1/10	10.0	3/10	30.0	4/20	20.0
20-29	2/10	20.0	2/13	15.4	4/23	17.4
30-39	3/10	30.0	5/10	50.0	8/20	40.0
40-49	3/11	27.3	2/20	20.0	5/21	23.8
50+	4/8	50.0	3/11	27.3	7/19	36.8
Total	19/85	22.4	19/83	23.9	38/168	22.6

*Number positive/number tested

REPORT FROM DR. ROBERT E. SHOPE, THE BELEM VIRUS LABORATORY, BELEM, BRAZIL

An Epidemic of Oropouche Virus in Belem.

During April and May of 1961, the Belem Virus Laboratory had the opportunity of studying a large-scale epidemic of Oropouche virus in the city of Belem. Oropouche virus was isolated from the blood of fifteen patients and an estimated 11,000 or more cases occurred in a circumscribed epidemic focus.

Fourteen of the fifteen cases from which the virus was isolated had a uniform clinical illness. One case was asymptomatic. The illness was characterized by fever, headache, backache, muscular aches, and articular pain. Also noted at times were photophobia, dizziness, delirium, chills, and nausea. None of the patients had cough or coryza. Temperatures varied from normal to 104° F (oral) during the viremic period. Abnormalities in the physical examination other than fever and conjunctival suffusion were rarely observed. One patient had an enlarged liver.

Leukopenia was observed in nearly all cases and lasted over a period of at least four days in three patients where serial counts were done. Virus was isolated in suckling mice from the first to the fifth days of illness and viremia titers ranged from 0.8 to 4.8 log LD_{50}. The median level of viremia was 3.4 log LD_{50}.

The duration of illness rarely exceeded one week and no deaths or serious sequellae are known. All fifteen patients developed neutralizing antibodies following infection.

Anderson et al. of Trinidad, in describing the original and only previous isolation of Oropouche from human blood (Am. J. of Trop. Med. & Hyg. 10, 574, 1961), raised doubt as to whether the virus was the causative agent of the clinical syndrome of their patient. In the present epidemic, Oropouche virus was intimately associated with the illness observed and was almost certainly the etiological agent. During April, sera of 82 persons from the districts of Marco and Pedreira were inoculated into mice. Of these donors, 25 were considered ill at the time of bleeding and virus was isolated from 12 of 25. (Seven of 13 from whom virus was not isolated had either cough or coryza, whereas none of the Oropouche proven cases had these symptoms.)

Of 57 people without symptoms, virus was isolated from one. Thus 48% of people with symptoms had viremia and only 2% of those without symptoms were circulating Oropouche virus. Inapparent infection would seem to have been rare, at least in this epidemic. (It is still possible that those without symptoms were circulating virus at a level too low to be detected.)

All of the clinically recognized cases resided in Marco and Pedreira, two heavily populated boroughs of the city. It is probable that the borough of Sacramenta, which adjoins Pedreira, was also involved since of 290 children bled after the epidemic, 27% of those who resided in Sacramenta had neutralizing entibodies. In Marco and Pedreira the incidence was 26%. Those children living in the 14 other boroughs of the city had less than eight per cent incidence and most of the positives resided adjacent to Pedreira.

Why Oropouche occurred in epidemic form in a circumscribed area of the city is not known. The epidemic focus is heavily populated and of relatively low economic status. Houses are not screened and many areas of low, swampy ground existed during April which constituted the end of the rainy season in Belem. Mosquitoes were abundant. Of over 3,000 arthropods captured on human bait, nearly all were Culex fatigans. No virus was isolated from these arthropods. Animal life was sparse except for domestic birds and house pets. Organs of fifteen wild rats and twenty-three opossums, trapped in the area were negative for virus. Sera from these wild animals were also negative for neutralizing antibodies as was sera from a dog. However, two of ten sera from domestic fowl (a duck and a pigeon) were positive.

An effort has been made to estimate the number of people infected during the epidemic. The laboratory had 58 sera from Marco and Pedreira which had been collected during 1960. The donors were re-bled after the epidemic and the sera tested in pairs. Fourteen per cent were positive in both specimens indicating prior immunity. Sixty-nine per cent were negative and seventeen per cent converted from negative to positive. Assuming the sample is representative of the total population of Marco and Pedriera, or 66,169 (1960 census), then roughly 11,250 people were infected during the epidemic.

The epidemiological cycle of Oropouche virus is far from clear. The level of viremia in man would appear to be sufficient to support a human-mosquito-human cycle. The predominant human-biting mosquito found was Culex fatigans, but without virus isolation from this arthropod, it is only speculative to consider it the vector. Other questions such as the identity of the reservoir host (possibly birds) are still unanswered. One fact is clear, however--Oropouche virus as a human pathogen, which remained for several years a curiosity of doubtful significance, has proven itself potent.

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

The Arthropod-borne Virus Unit, from the Instituto Adolfo Lutz, in Sao Paulo, initiated work on November, 1960.

There are few data available by now, so this report is primarily a description of our field stations and the results obtained up to now.

We have established 3 field stations around Sao Paulo. The first one, called by us "station A", is located in a very rainy place, part of a chain of mountains which marks the beginning of a big plateau where our city is located. The place is called Borasceia and has an altitude of 800 meters and it is about 75 miles from the laboratory. The second one, called by us as "station B" is located in an uninhabited beach called Bertioga, about 80 miles from Sao Paulo, but only 5 miles from the first one. The difference in altitude between them gives marked ecological differences in the fauna and flora which was the reason why we chose these two places for our field work. The third one, called "station C" is located in the direction of the interior of the state in a place called Cotia, about 45 miles from the lab. It is located in the plateau with an altitude of about 1300 meters, near a small dam which supplies water for part of Sao Paulo.

In these three field stations, we are putting sentinel mice, collecting mosquitoes once a week using platforms at ground level and 5 and 10 meters high. The mosquitoes are captured by human bait. Larval ecology is being studied by setting breeding traps made by bamboos and Bromelias previously washed. We started trapping mammals and birds for serology and for attempts to isolate viruses.

We have been putting sentinel mice in station C since February. By the end of April, 40 strains of a virus were isolated from them. The work on stations A and B was begun on August 1st.

In May began our winter time, which is a very mild one with a temperature no lower than 4° C (40° F) during nights and mornings with an elevation in it during the afternoons. The density of mosquitoes during this time of the year is low and we have no additional isolations up to now from the sentinel mice.

The 40 strains of virus isolated from Cotia by us did not produce a hemagglutinin so almost all work to identify them is based in the CF test. All 40 strains appear indistinguishable by CF test and we consider them as different isolations of the same virus.

These strains have an AST of 3.0 to 4.0 for babies by IC and 4.0 to 5.0 for babies by IP. They do not kill adult mice by IC or IP routes.

A titration done with a 10th passage of the prototype An 232 gave us a titer of $10^{-4.5}$ and a DCA test was done with this passage using the Theiler's method. We have observed a DCA susceptibility of more than 2.5 logs.

Using CF tests and one way HI test with immune sera, we have not observed reactions with A group (EEE, Mayaro, WEE, Aura, and Una), B group (SLE, Y Fever, Ilheus), C group (Oriboca, Caraparu, and Marituba), Bunyamwera group (Cache and Guaroa). These were the available sera and antigens in our lab so this virus is not grouped up to now. Around our field station of Cotia live about 100 families and we have collected 68 sera from adults and children for serological surveys which will be run with the sera obtained from the trapped mammals.

REPORT FROM DRS. MARTHA BOXACA, A.S. PARODI, H. RUGIERO, AND R. BLAY, FACULTAD DE CIENCIAS MEDICAS UNIVERSIDAD DE BUENOS AIRES, BUENOS AIRES, ARGENTINA

Experimental hemorrhagic fever in the guinea pig (Junin Virus)

The Junin virus was isolated from human subjects in 1958 during an outbreak of hemorrhagic fever in Argentina.

Since infected guinea pigs developed most of the features of this disease as seen in human beings, we decided to study certain aspects of the experimental infection in this animal species: routes of inoculation; distribution of the virus in the blood and various organs, intensity and duration of viremia; alteration in the temperature and weight and death-rate.

Material. Strain XJ of Junin virus isolated in Junin City. It had two passages in guinea pigs, 12 in mice, and 8 in guinea pig. The titer was $10^{-5.4}$ in 0.2 ml subcutaneously. The animals weighed 250-350 grs and were observed as long as 40 days after inoculation.

Routes of inoculation. Guinea pigs were inoculated by different routes: intraperitoneal; subcutaneous; intracerebral; oral, percutaneous and in the cornea. The animals regularly developed disease after inoculation by IP, IC, sub, IN and percutaneous routes. Such animals died between 10 and 18 days after inoculation. Of four animals inoculated by cornea scarification, two died with a typical picture, one died, possibly from extraneous causes, and the fourth was eliminated after 40 days. Of 2 animals inoculated by oral route, only one died 38 days afterwards. The other remained well.

Distribution of the virus. Eight guinea pigs were inoculated with 1000 LD_{50} subcutaneously. Every 24 hours, two animals were bled and the blood was immediately inoculated IP in two guinea pigs. Each of these received 0.5 ml from each of the infected animals.

The organs were triturated on broth with penicillin and streptomycin to obtain a 10% suspension, and 0.30 ml of this was inoculated subcutaneously into each of 2 guinea pigs. The results show that the virus was first isolated from the spleen and lymphatic ganglia. Only at 72 hours after the infection was virus recovered from the other organs tested.

Viremia. All the animals show a persistent viremia from the 2nd or 3rd day until death. The virus was titrated at the 4th, 9th, and 12th, 14th days. The last bleeding was done when the infected guinea pigs were dying. During this course there was an increasing concentration of virus in blood. At the 4th day, the titer was $10^{-1.5}$, at 9th day $10^{-4.47}$, and $10^{-5.43}$ at the time of death.

Survival. Eighty-one inoculated guinea pigs were observed to determine survival time following infection. They were inoculated subcutaneously with 10^5 LD_{50} of virus. Sixty three per cent died between

11 and 15 days and 96 per cent were dead by 9 to 17 days after inoculation. Necropsies revealed characteristic pathologic picture between 6 to 17 days.

Body temperature. Body temperatures were determined in the rectum at the level of the renal artery, every day at the same hour on fasting animals. The temperature range in normal guinea pigs was 38.5 to 39.7° C. The infected animals had normal temperatures during the first seven days. After that, body temperatures tended to increase and in some animals reached 41.3, but all show a fall below normal a few hours before death.

Weight. At the same time the temperature was taken each day, the animals were weighed. The infected animals showed a significant and constant loss of weight after the 7th day.

REPORT FROM DR. J.S. PORTERFIELD, NATIONAL INSTITUTE FOR MEDICAL RESEARCH, MILL HILL LONDON, N.W. 7, ENGLAND

The main emphasis has been upon the use of chick embryo fibroblast tissue cultures in experimental studies on arthropod-borne viruses. Three different applications of plaque methods for the titration of yellow fever neutralizing antibodies have been compared. In the first method, plaque neutralization, serial dilutions of serum are incubated with 100 plaque forming units of virus for two hours at 37° C. The residual virus is determined by plaque counting, each serum virus mixture being tested on one or two petri dishes. The end point is expressed as the serum dilution which brings about a 50% reduction in the plaque count. In the second method, plaque reduction, serial dilutions of immune serum are incorporated in the agar overlay covering cells previously infected with 100 p.f.u. of virus. Plaques are counted on successive days and the titre is again expressed as the final dilution which brings about a 50% reduction in plaque count. In the third method, plaque inhibition, beads containing immune sera are applied to the surface of agar overlaying cells infected with 1000-5000 p.f.u. per dish. The highest dilution of serum which produces a detectable zone of plaque inhibition is taken as the serum titre. Unknown sera were tested by all three methods in tissue culture and the results were compared with other figures for the serum antibody titre obtained by standard intracerebral tests in mice carried out in a number of laboratories as part of a WHO collaborative assay. Using the mouse titres as a standard, the plaque neutralization test was about fifty times more sensitive, the sensitivity of the plaque reduction test was

about the same as that obtained in mice and the plaque inhibition test was five to ten times less sensitive.

In preliminary studies with Tahyna virus, obtained from Dr. V. Bardos in Bratislava, Czechoslovakia, no relationship to 25 other arthropod-borne viruses could be shown by plaque inhibition tests. After Dr. Casals had established that Tahyna virus was serologically related to California virus, these two viruses were compared by reciprocal tests in tissue culture with the results given below:

		ANTISERUM	
Method	Virus	California	Tahyna
Plaque inhibition	California	24*, 23	15.15
	Tahyna	17.16	22.22
Plaque neutralization	California	760+	160
	Tahyna	160	3000

*zone of plaque inhibition in mm. with undiluted serum
+50% plaque neutralization titre

Studies on the optimal conditions for plaque production with certain arthropod-borne viruses carried out in association with Dr. A. Isaacs and Dr. S. Baron have shown that the oxygen tension prevailing in cells under agar exerts a marked effect upon the presence or absence of plaques. There is a wide variation within different Group A viruses, Semliki Forest and WEE viruses being able to produce plaques under conditions of reduced oxygen tension which completely inhibited plaque formation with Chikungunya and O'nyong-nyong viruses. This effect has been found to be related to the interferon sensitivity of different virus strains.

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

Chromatography of arthropod-borne viruses on calcium phosphate has been continued and apart from its value in antigenic and physico-chemical analysis appears to offer the best method for preparation of haemagglutinins from viruses which do not yield them by standard methods.

Field studies have commenced of louping ill in sheep, ticks, and small mammals in Ayrshire and Invernesshire in Scotland. A number of strains have been isolated from sheep brains and from ticks (Ixodes ricinus), many of which were blood-engorged. A long-term serological and virological study of a flock of 300 sheep has commenced.

Experimental infections by Langat virus in human cancer patients, monkeys, sheep, and ticks (Ixodes ricinus and Haemaphysalis spinigera) have been studied with a view to antibody responses, pathogenesis and oncolytic effect, and to evaluate this agent as a possible live vaccine. The virus appears to be safe in sheep and lambs and to give a degree of protection against louping ill, to cause no disease in monkeys and to protect them to some degree against biphasic meningoencephalitis virus, and to cause only a mild febrile response in man.

With funds provided by WHO, Dr. Varma and Dr. Sims of the British Museum of National History collected 214 migrant and 93 resident birds near the mouth of the Guadalquivir River in Spain in April/May. Six (3%) of the migrants and 2 (2%) of the residents were found to be carrying ticks.

REPORT FROM DR. C. HALLAUER, UNIVERSITAT BERN, BERN, SWITZERLAND

The work I have done hitherto with yellow fever virus may be summarized as follows:

1. Yellow fever virus (17D-, French Neurotropic-, Asibi-strains) has been cultivated in explants of human tissue (tumor cell lines HeLa and KB, normal amnion and kidney) without any previous adaptation.

2. KB cells proved to be distinctly more suitable than all other tissue forms. In explants of this nature, infectivity titres of log 5.0 - 7.0 ID_{50} were regularly obtained after an incubation period of 4-6 days.

3. The demonstration of virus in explants is easy when based on the following criteria: a) the presence of microscopically visible tissue defects ("plaques"); b) the increasing turbidity of nutritive medium; c) the suppression of cellular metabolism, and d) the microscopic

demonstration of the cytopathogenic effect. The specificity of these tissue alterations is proved by the inhibitory action of yellow fever immune sera.

4. Culturing of yellow fever virus in human explants allows an accurate titration of the infectivity. The final titre is approximately the same as that obtained using the intracerebral mouse test. Immune sera may also be evaluated in a reliable way in such explants.

5. During the passage into KB explants, the 17D strain lost its pathogenicity for mice, the Asibi strain its viscerotropic affinity for monkeys.

6. Hemagglutinin of yellow fever virus has been isolated in human tissue culture both from the tissue (by extraction with alkaline borate buffer) and the nutrient medium (after elimination of non-specific inhibitors by Freon).

7. Hemagglutinin is detectable in the explanted tissue before any cytopathogenic effect is evident and reaches its highest concentration when the infectivity titre culminates. The release of hemagglutinin into the fluid culture phase proceeds synchronically with the progression of the cytopathogenic effect.

8. There is a distinct proportionality between hemagglutinin and infectivity titres.

These results have already been published in the Archiv f. Virusforschung 9, 428, 1959 (pos. 1-5) and 10, 268, 1960 (pos. 6-8).

Comments and suggestions:

It seems to me that the tissue culture method offers several advantages over the usual intracerebral mice test for the detection and titration of yellow fever virus and its corresponding antibody. The virus susceptibility of stabilized human cell-lines is nearly constant, that of different races of mice is not; the detection of of virus in vitro is possible within a few days (especially when the hemagglutinin extraction is performed) whereas the mice test requires usually a week or more. The only question is whether the sensitivity of the in vitro test would be--at least for the pantropic strains--the same as in vivo. This question should be clarified by appropriate field investigations.

The hemagglutination test in tissue cultures appears to be a simple and excellent procedure for the continual control of virus multiplication either in the explanted tissue or in the supernatant nutrient medium. This method should be tried also with other arthropod-borne viruses. Furthermore, by extracting yellow fever hemagglutinin at an alcaline reaction (borate buffer of pH 9.0) a rather purified virus preparation is obtained which may be further purified and concentrated by untracentrifuging at 35,000 r/min. Such preparations would be doubtless useful for electron-microscopic studies, or, if such a need exists, for purified 17D vaccines.

The fact that pantropic yellow fever virus strains loose their viscerotropic affinities in human tissue cultures in a similar manner as Theiler demonstrated in murine and chick tissue cultures is of theoretical interest since it indicates that the selection of attenuated mutants is equally possible in tissues of highly susceptible hosts. It appears therefore that the "transforming effect" is rather governed by the special physiological conditions of the explanted tissue than by its host-provenance. Worth mentioning is further the observation that 17D strains preserve or increase their attenuated quality during more than 200 passages in human tissue cultures. So, their high degree of stability is demonstrated once more.

Actually, experiments are in course for determining the infectious properties of the tissue culture virus strains in a larger number of rhesus monkeys.

REPORT FROM DR. JELKA VESENJAK-HIRJAN, SCHOOL OF PUBLIC HEALTH, "ANDRIJA STAMPAR" MEDICAL FACULTY OF ZAGREB, YUGOSLAVIA

In 1953, for the first time in Yugoslavia, human infections with arthropod-borne viruses were demonstrated in our laboratories by the isolation of tick-borne virus belonging to the CEE (Central European encephalitis) group from blood of patients.

Later the virus was isolated from ticks and again from patients. Antibodies to the virus have been demonstrated serologically in 30% of 7 species of micro-mammals, while no isolations have been made from these animals. Antibodies for CEE were found in bats by means of complement fixation. HAI tests for antibodies to Ntaya virus were negative. No viruses have been isolated from bats.

Until 1960, the disease was known only in the westernmost parts of Yugoslavia, i.e., in Slovenia (Karstic Alpine area) where it appeared endemically and epidemically. It was not known to exist in other parts of Yugoslavia.

In 1961, under improved technical conditions, new investigations were carried out and 22 human cases of the same virus disease were recorded in Croatia, a region to the east of Slovenia. The disease varied from mild serous meningitis cases to cases clinically diagnosed as poliomyelitis. The disease appeared in two geographically and climatically distinguished areas along the Adriatic coast near Zadar and in the Panonian plain. It was then found that the disease had appeared there already in 1952 as some positive reactors, hospitalized in 1952 as meningoencephalitis cases, were found. Of the total number of 126 persons examined, 22 were positive.

In the above areas, antibodies with CEE antigen were found in horses (of 31, 11 positive), cows (of 30, 2 positive) and donkeys (of 6, 1 positive) by means of complement fixation and haem. inhibition.

In the course of 1961 a major epidemic of serous meningitis, 80% of which is caused by CEE virus, has appeared in Slovenia. Definite figures have not been obtained so far.

REPORT FROM DR. M. LIKAR, VIRUS LABORATORY, INSTITUTE OF MICROBIOLOGY, UNIVERSITY LJUBLJANA, YUGOSLAVIA

Search for the Group A Antibodies in Human and Animal Sera

A number of human and animal sera were tested for the presence of the group A arbovirus antibodies by the hemagglutination inhibition technique. The results are given in Table 1.

Table 1

Serum No. of sera tested	With EEE antibodies				With WEE antibodies			
	2*	3	4	5	2	3	4	5
Human 896	14	8	4	0	15	5	1	1
Animal 148	6	3	1	1	12	2	0	0

*2 indicates titer 1:20, 3 indicates 1:40, 4 indicates 1:80, and 5 indicates 1:160.

In Table 2 the various species of animals tested in our experiments are listed and antibody titers given as above.

Table 2

Serum	No. of sera tested	With EEE antibodies 2	3	4	5	With WEE antibodies 2	3	4	5
Fowl	27	3	2	1	1	3	1	0	0
Goose	38	0	1	0	0	8	1	0	0
Ox	32	3	0	0	0	1	0	0	0
Horse	17	0	0	0	0	0	0	0	0
Pig	34	0	0	0	0	0	0	0	0

The various clinical illnesses of the patients tested for antibodies to EEE and WEE viruses are presented in Table 3.

Table 3

Clinical picture	No. sera tested	% with EEE antibodies	% with WEE antibodies
Infection of the central nervous system.	659	3.8	2.4
Febrile state of unknown etiology.	57	1.7	1.7
Respiratory infection	104	0.0	3.8
Others	76	0.0	0.0

As far as diagnostic value of our findings is concerned, we found HI antibodies for EEE and WEE viruses in the sera of some patients with infections of the central nervous system with no other detectable virus antibodies which could have any etiologic relationship with the illness of the patient.

Field Studies:

The sera from all inhabitants were collected in the village Sv. Trojica. It was found that antibodies developed for the tick-borne group of viruses without any clinical illness. The sera were collected once monthly from March this year and antibodies were detected as shown in Table 4.

Table 4

Sera taken in	% with group B CFT antibodies	% with HI antibodies for louping ill virus
March	2%	24%
April	7%	34%
May	11%	37%
June	20%	39%
July	22%	41%

The village Sv. Trojica is located in an endemic area with tick-borne encephalitis.

Ticks (Ixodes reduvius) and mosquitoes were collected at various sites around the village investigated and a number of strains isolated which have not yet been determined. In all, 6750 ticks and 470 mosquitoes were inoculated into mice for virus isolations.

Basic Research:

Various arboviruses were successfully grown in human embryonic kidney tissue cultures. As our laboratory was mainly interested in the development of a neutralization test for tick-borne complex of viruses, we had to abandon these experiments as the results were not clear cut. But the supernatant fluid of the infected cultures has given a good HI antigen.

With fluorescent antibody technique the sites of DNA and RNA in infected tissue culture cells were observed. Tick-borne viruses of various origin were used for the experiments, some isolated from ticks, others from mild or fatal cases in humans with meningo-encephalitis.

REPORT FROM DRS. R. PANTHIER AND C. HANNOUN YELLOW FEVER SERVICE OF THE PASTEUR INSTITUTE PARIS, FRANCE

At the end of the year 1960 and the beginning of the year 1961, we have received human sera addressed to us from the Pasteur Institute, Addis Ababa, Ethiopia (Dr. Serie). The sera were collected in South-western Ethiopia where a lethal epidemic rages on the east bank of the river Omo not far from its point of discharge into Lake Rudolphe. Of the first 15 sera received in December 1960, taken between the 3rd and

30th day following the onset of illness, 12 showed yellow fever antibodies. As soon as this fact was reported to the Pasteur Institute at Addis Ababa, a mission (team) was dispatched to collect other sera in the afflicted zone on the east side of the Omo River and between that zone and the Sudanese frontier to the west.

The study of antibodies of the sera taken in the recently afflicted zone permitted confirmation of the yellow fever etiology of the epidemic. The sera taken very early (first and second days) were negative in CF as well as HAI tests and in the mouse neutralization test. The sera taken later showed an early and rapid increase in the HAI antibodies. There was a less early and slower rise in complement fixing and neutralizing antibodies. These are detectable on the 4th or 5th day by prolongation of the average survival time of the mice.

Here are some results obtained with sera taken from typical cases from the middle of the epidemic focus. The exact date of onset of illness is an approximation within one or two days.

No. of days after onset of illness	CF	HAI	NT	AST
2 days	0	0	0/6	5.38
3 days	0	8	0/6	7.5
	10	10+	0/6	7.94
4 days	0	8	1/6	8.5
5 days	10	5	5/6	9.72
	0	10+	3/5	9.8
	5	10+	2/6	9.27
6 days	20	10+	3/6	9.55
8 days	AC	13+	5/5	10.
	40	12	2/6	9.22
9 days	80	9	6/6	10.
10 days	160	13+	2/6	9.22

CF, 2.5 units.

HAI. The figure 0 represents a negative result with the serum at 1:10. 1 = positive result with serum at 1:10; 2, with serum at 1:20; 3, with serum at 1:40, etc.

NT: numerator, number of mice surviving.
denominator, total number of mice surviving to the 45th day.

AST: average survival time (Bugher)

Two monkey sera are positive for inhibition of hemagglutination and for complement fixation.

The results obtained with the sera taken from the region of the right bank of the Omo river and the Sudan frontier permit it to be believed that the yellow fever virus has been active in that region at a time longer ago--positive seroprotection tests (6/6-10); inhibition of hemagglutination at a lower titer, some quite low positive reactions 5 to 20, a single reaction at 80, complement fixation tests often negative.

The epidemic is now continuing up the Omo river, the focus active in 1960 having extinguished itself spontaneously during the dry season. A strain of virus isolated in Addis Ababa has been sent to us. This strain is totally inhibited by anti-yellow fever serum.

We have been able, on the first passage of this strain into young mice (2nd passage after isolation), to prepare from the brains of these mice a hemagglutinin titrating at 1:5120.

We have not been able to study these sera nor this strain comparatively with other arbor antigens of group B, nor with other sera prepared from Group B virus. A separate laboratory reserved for the study of the arbovirus is still under construction.

REPORT FROM DR. F.N. MACNAMARA, VIRUS RESEARCH UNIT, WEST AFRICAN COUNCIL FOR MEDICAL RESEARCH YABA, LAGOS

"Cameroons dengue"

A field expedition was made in May-June 1961 to investigate a small outbreak of "Cameroons dengue" among occupying British troops in the S. Cameroons and among civilians. This disease syndrome, characterized by fever, rash, lymphadenopathy, and terminal asthenia, has been known for some years to be endemic in the Cameroons, and has been assumed to be due to an unidentified arbovirus, although it has never been investigated. On this occasion, paired acute and convalescent sera from 12 cases were tested at Yaba and one at the RFVL New York. No rise in HI antibodies to groups A, B, or C arboviruses was found. From the acute serum of 2 cases and from the stool of one, a virus was isolated in suckling mice, which on examination has been found to be a member of the Coxsackie B group.

It has yet to be demonstrated serologically that the virus was responsible for all cases in this outbreak. However, it is of interest to record that some cases of "dengue" may in fact be due to enterovirus infection.

Titration of accessory factor

Among the many variables that make the comparison of yellow fever neutralization test (YF NT) surveys done by different laboratories difficult is the arbitrary practice of inactivating or not inactivating test sera, and differences in the amounts of accessory factor added to the test in the form of fresh serum, often from different species of animals. As a step towards the standardization of the YF NT, a number of experiments have been done to see whether it is possible to titrate the amount of accessory factor present in different batches of fresh serum. This has been found to be feasible, and it has also been shown that it is possible to freeze-dry and store fresh serum preserving the accessory factor. It is hoped to evolve a standard method for titrating accessory factor, so that equivalent amounts may be added in tests.

Isolation program

A small isolation program has been initiated. This includes the inoculation of routine mosquito catches, the collection of blood from cases of undiagnosed fever, and the testing of post mortem material. This has been restricted to the environs of Lagos. To date no isolation has been made.

Serological surveys

In collaboration with Dr. and Mrs. Causey of the Rockefeller Foundation, about 500 sera were collected on an antibody survey to arthropod-borne viruses from different places in S. W. Nigeria and the Cameroons. These are being tested at the Rockefeller Foundation Virus Laboratories in New York.

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

The Virus Laboratory Unit is attached to the Dept. of Pathology and has been in operation since October 1960. It is engaged in a search for the etiologic agent of Thai Hemorrhagic Fever. This report reviews the accomplishments during the period between October 1960 to July 1961.

Virus Isolation:

During 1960, 441 cases of the Thai Hemorrhagic Fever were admitted to Chulalongkorn Hospital with the mortality rate of 5.44%.

One hundred selected specimens of acute blood sera collected one to seven days after the onset of the fever were inoculated into suckling mice for virus isolation. Eleven viruses were isolated and identified by the CF and the HI reactions according to the Clarke and Casals technic. The results can be listed as follows:

3 Dengue type 2 (Tr. 1751)
3 Dengue type 4 (H. 241)
2 Chikungunya
1 Strain closely related both to Dengue 1 and Dengue 2
1 Strain of a group B virus which is not completely characterized as yet.

Virus isolation was possible only up to the 3rd day after the onset of the fever.

Specimens from 7 autopsies consisting of various organs including heart blood were examined. Only one yielded a Dengue type 4 virus which was confirmed by Dr. Max Theiler of the Rockefeller Foundation Virus Laboratory. It came from the liver of a 4-year-old child who died 4 days after the onset of the fever. The autopsy was done 3 hours after death.

Attempts to isolate virus from mosquitoes collected on a year-round basis are now being carried out. The results are not complete as yet.

Serological Studies:

Fifty pairs of patients' sera were tested by the CF test against Dengue 1, 2, 3, and 4, Jap. B, and Chikungunya antigens. The results were quite complicated. There was little agreement between the CF titer increase and the type of virus isolated from the patient. The following table serves as an example.

Complement Fixation Tests in 5 Patients from Whom Arbor Viruses Were Isolated

Case No.	Age in Years	Virus isolation	Serum collected on day of illness	Reciprocal CF titer against DENGUE 1	2	3	4	Jap B	C*
SH 384	1-1/2	Dengue 1	1st	0**	0	0	0	0	0
			22nd	2048	1024	64	128	512	0
SH 403	9	Dengue 4	2nd	0	0	0	0	0	0
			39th	64	64	0	32	64	0
SH 428	12	Dengue 2	3rd	0	0	0	0	0	4
			30th	32	4	0	0	64	4
***SH 509	4	Chikungunya	2nd	0	0	0	0	0	0
			61st	64	0	0	0	0	0
SH 524	6	Dengue 1 or 2****	3rd	0	0	0	0	0	0
			21st	512	128	0	4	256	0

*Chikungunya virus

**Less than 4

***Neutralization index to Chikungunya virus between acute and convalescent sera: more than $10^{-3.54}$ using suckling mice i.c.

****Undetermined to date.

Jap B: Japanese B encephalitis

Nineteen cases showed a significant rise of antibody level to the group B antigens tested, 7 did not show antibody against any of the antigens tested, while the rest did not reveal significant changes in the antibody titer.

REPORT FROM DR. DORA TAN, INSTITUTE FOR MEDICAL RESEARCH, FEDERATION OF MALAYA

Since 1955, the Institute for Medical Research, Kuala Lumpur, Federation of Malaya, has studied a total of 153 tick pools from forest rodents, out of which 9 virus isolates were obtained to date. Six of these, isolated from Ixodes granulatus taken off rodents trapped at the same forest area as Langat (TP21) virus are identical with the Langat virus, a member of the Russian spring-summer encephalitis complex. The identity of the other two strains is not yet known.

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

There occurred in Singapore in the middle of 1960 an outbreak of a dengue-like illness with mild hemorrhagic manifestations. Chief complaints and findings were headache, bone and muscle pains, leucopenia, thrombocytopenia, a dengue like rash and petechial hemorrhages (about 20% of cases). There were also lymphadenopathy, splenomegaly, and abdominal pains, nausea and vomiting. There was no frank bleeding or purpura. The patients were mostly young adults and there were no deaths.

Patients' sera show high rate of conversion to dengue type 1 and both to dengue type 1 and Japanese encephalitis virus antigens by complement fixation tests. One patient (601) had antibody rise for chikungunya virus by both CF and neutralization tests. In 1960, two viruses were isolated from blood taken in acute phase of illness. These strains have been labeled S-601 and S-843 and have been typed tentatively as dengue type 1 and dengue type 2 viruses. Two other viruses also were isolated from acute blood specimens in 1961 and according to preliminary tests are also related to dengue type viruses but are different from S-601 and S-843. The viruses were isolated in infant mice and all except one 1961 strain were adapted to adult mice in 7 to 10 passages.

Mosquito (Aedes egypti) isolations were attempted in the Hooper Foundation laboratories by Dr. A. Rudnick, who reports that 4 out of 15 pools passed show evidence of presence of virus but adaptation to infant mice is still in progress.

REPORT FROM DR. IAN D. MARSHALL, DEPARTMENT OF MICROBIOLOGY, AUSTRALIAN NATIONAL UNIVERSITY, CANBERRA, A.C.T., AUSTRALIA

The arthropod-borne virus research unit being formed in the Department of Microbiology, Australian National University, will function as the WHO Regional Reference laboratory for the Australasian area and will conduct epidemiological and laboratory investigations of selected problems.

The Commonwealth Department of Health has modified its quarantine regulations to allow these activities and has defined the Australasian region for this purpose as "the continent of Australia, the Australian Territory of Papua and New Guinea, Dutch New Guinea, territory not previously mentioned which lies in an area bounded by the equator on the north, the 60th south parallel of latitude in the south, the 140th meridian of east longitude on the west and the 160th meridian east longitude on the east, but including the Solomon Islands. This area is extended to include material of New Zealand and Pacific Islands which has already been processed in the laboratory of Dr. J.A.R. Miles, Department of Microbiology, University of Otago." In addition to the University of Otago and the Australian National University, there are active arthropod-borne virus research centres within the Australasian region at the Queensland Institute of Medical Research (Dr. Ralph Doherty), and at the University of Western Australia (Professor Neville Stanley).

With regard to independent research, it is planned to commence field investigations at Maprik in New Guinea at the end of October, 1961. This area is in the Sepik River region, about forty miles inland from Wewak on the north coast of New Guinea. It was selected as the first field study area after consultation with Dr. F.S. Schofield, the Director of Medical Research in New Guinea, and after a recent reconnaissance of the Territories. Dr. Schofield has considerable clinical and serological evidence of the presence of unidentified arthropod-borne encephalitis viruses in the area, associated with mortality in native children. We have been offered facilities at the laboratory of the Malarial Control Unit stationed at Maprik. Dr. Schofield, and subsequently Dr. McLennan, will be handling the clinical aspects of the investigations, and will be conducting human serum surveys in conjunction with Dr. Charles Wisseman of the University of Maryland. The Australian National University unit will be attempting to recover viruses from pathological material from human cases, and from arthropods, birds and animals likely to be concerned in the primary virus cycles. At present it is planned

to spend periods of three to four weeks collecting field material every few months, and all processing of the material for virus recovery will be carried out at our laboratories in Canberra between field trips.

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

Recent work at the Queensland Institute of Medical Research has been concerned with the epidemiology of the three arbovirus diseases known to occur in Australia (dengue, Murray Valley encephalitis (MVE), and epidemic polyarthritis), and has led to the isolation of a number of other arboviruses previously unrecognized.

Antibody surveys already published showed that human dengue immunity is common along the east coast of Queensland. MVE immunity is frequent in the aboriginal population bordering the Gulf of Carpentaria, and serial bleedings of children and chickens gave evidence of group B infection in that area in the wet seasons of early 1959, 1960, and 1961. Neutralizing antibody to AMM2354, thought to represent previous infection with the virus of epidemic polyarthritis, is widespread in Queensland, occurring in man, horses, cattle, pigs, and kangaroos in all the areas tested.

Mosquito collections were made at Mitchell River mission in Cape York peninsula in March-April 1960. Thirty-five virus strains (two of which proved to be mixtures of two viruses) were isolated from 11, 595 mosquitoes, and were found to belong to eight antigenic groups.

1. MVE: four strains, three from C. annulirostris, one from Aedes normanensis, were found to be identical with MVE.

2. Kunjin (MRM 16 prototype), nine strains from C. annulirostris, of a group B virus distinct from MVE. The prototype strain was submitted to the Rockefeller Foundation Virus Laboratories, New York, where it was tested against the world range of group B viruses. It was found to be a distinct virus.

3. Kokobera (MRM 32 prototype): four strains from C. annulirostris, of another group B virus, also considered by the RFVL to be distinct from any previously described.

4. Sindbis (MRM 39 prototype): one strain from C. annulirostris, and one from A. normanensis of a group A virus identified by the RFVL as Sindbis virus.

5. Koongol (MRM31 prototype): five strains from C. annulirostris, one from Anopheles farauti, and one from Anopheles bancroftii of a virus found by RFVL to belong to none of the accepted groups and to be unrelated to any of the ungrouped viruses against which it could be tested.

6. MRM 168: three strains from C. annulirostris of a virus related to but distinct from Koongol. It has also been tested at RFVL by HI technique against a range of grouped and ungrouped viruses and no relationship found.

7. MRM 186: two strains from Anopheles meraukensis of a virus which has failed to yield haemagglutinin, but which by CF test has shown no relationship to any arbovirus found in Australia. It has also been tested at RFVL and no relation found to a wide range of grouped and ungrouped viruses.

8. MRM 1: Six strains from C. annulirostris of a virus which is not sensitive to sodium desoxycholate or ether and has not been further identified.

Mosquito collections were made in April 1961 at Mitchell River mission, Cairns, and Normanton. Five thousand two hundred forty-seven mosquitoes were collected in Cairns during an epidemic of polyarthritis. Nine viruses were isolated, all clearly distinct from the (as yet unisolated) polyarthritis agent. Four strains were identified as Sindbis and four as group B virus distinct from those previously found in Australia; one strain proved to be a mixture of the two viruses. The new group B virus has tentatively been named "Edge Hill." It has not yet been fully studied at RFVL. Studies on mosquitoes collected at the other centers have not yet been completed, but strains of MVE were again isolated at Mitchell River mission.

Antibody studies with the new viruses are still at an early stage. It is evident that both Kunjin and MVE infection have contributed to the group B antibody previously demonstrated in man in the Gulf of Carpentaria area. There is as yet only equivocal evidence for the occurrence of Kokobera infection. Antibodies to Sindbis have been found in man at Mitchell River mission, and in wild and domestic birds there and along the east coast of Queensland.

Further studies are planned to determine the geographical and species distribution of the new viruses, and to determine their importance in human and veterinary medicine.

Applications are invited for the position of Entomologist. Duties in general the study of blood-sucking arthropods, as part of a comprehensive investigation being carried out at the Institute into Arthropod-borne viruses. Salary for an experienced entomologist who has shown ability to conduct independent research: 2889-2959 pounds. Alternatively, an appointment may be made at a lower salary, the amount of which will depend on qualifications and experience. Salary for a recent graduate, male: 1575-1834 pounds; female, 1279-1649 pounds. Tenure of appointment (subject to good conduct and efficient service) five years. Applications close on December 31, 1961, with the Director, Queensland Institute of Medical Research, Herston Road, Herston, N9, Brisbane, Queensland, Australia, from whom further particulars are available. Copies of not more than three testimonials may be attached to application and names of two referees, to whom direct enquiries may be made, should be given.

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

Arbor Virus Studies in Western Australia, 1960-61:

These studies represent the first attempt to obtain an overall serological pattern of arbor viruses in the western half of Australia. Neutralization and haemagglutination-inhibition tests were carried out initially with representative strains of group A and group B. Approximately 800 specimens of sera from white people and natives covering an area of almost 1 million square miles were tested. The table summarizes the results. There is an obvious difference in distribution of group A and group B antibody. The high percentage of Central Australian natives with AMM2354 antibody and the virtual absence of group B antibody indicate that this area should be particularly suitable for the investigation of possible vertebrate hosts and mosquitoes for AMM2354 antibody or virus.

HI and Neutralization* Tests with MVE and AMM2354 Arbor Viruses

(673 specimens of human serum)

Positive sera recorded as those with (titres 1/10 or > (Neut.)
(titres >1/10 (HI)

Sera	Locality	Percentage of sera with antibodies to HI MVE (B)	HI AMM2354 (A)	Neut. MVE (B)	Neut. AMM2354 (A)
White (207)	Southwest (1958)	34	28	3	10**
White (50)	Kimberley (1960)	60	25	18	4
Aboriginal (20)	Central Aust. (1958)	0	100	0	45
Aboriginal (285)	Kimberley (1960)	92***	50	(50)	(68)
Aboriginal (86)	Leonora (1958)	4	22	0	28
Aboriginal (25)	Carnarvon (1958)	17	8	11	8

*Only 420 sera tested for neutralizing antibody.
**In the Busselton area this rose as high as 38%.
***30% >1/320.

The results suggest the presence of more than one group B arbor virus in the Kimberley area. This has just been confirmed with neutralization tests carried out with MRM16 and MRM32 strains isolated by Dr. Doherty in Queensland.

REPORT FROM DR. ERIC L. FRENCH, VIROLOGY SECTION OF THE DIVISION OF ANIMAL HEALTH, C.S.I.R.O. PARKVILLE, N. 2, VICTORIA, AUSTRALIA

After several years spent at the Walter & Eliza Hall Institute, Royal Melbourne Hospital, engaged in research on virus diseases of man, an opportunity presented to set up a virus unit within the Division of Animal Health of C.S.I.R.O. The section has been built up slowly both in equipment and staff, and a survey made of the possible virus diseases of importance to the livestock industry in Victoria and Tasmania.

Australia, fortunately, is free from many of the more serious virus diseases of livestock which cause trouble in other parts of the world, but one virus disease which is believed to be arthropod-borne and which has reached epizootic proportions on several occasions is ephemeral fever or 3-day sickness of cattle. This disease has been studied in Australia by I. M. and M. J. Mackerras, who published their findings in Bulletin No. 136 (1940) of the Council for Scientific and Industrial Research. They concluded that ephemeral fever of cattle is closely related to human dengue fever, that it is almost certainly insect-borne, that transmission is most probably cyclical, that the vector is probably a sandfly. In collaboration with F. M. Burnet, they showed that the aetiological agent was filtrable. The virus was also shown to be associated with the platelet and leucocyte elements of the blood.

Some experiments have been undertaken with this virus during the colder months of the past two years and it is planned to extend this work further when suitable insect-proof accommodation for cattle becomes available. Up to the present we have found infected blood collected in 1956 and stored as citrated whole blood at -20° C. for 4 years to be still infective when inoculated intravenously (10 ml.) into a susceptible bovine. Association of the virus with the white cell layer of the infective whole blood has been confirmed as has also the solid immunity which follows an active infection in the bovine.

The finding of viable virus in blood stored at -20° C. for 4 years suggested that the agent was somewhat more resistant than most of the recognized arthropod-borne viruses. It was therefore of interest to determine the effect of desoxycholate on the agent (Theiler, Proc. Soc. exp. Biol., Vol 96, page 380, 1957). Treatment of a sample of known infective blood with 1/1000 sodium desoxycholate in broth at pH 7.4 for 1 hour at 37° C. was found to completely inactivate the infectivity of the blood. The treated blood failed to cause any thermal or other clinical reaction in the inoculated cattle and these animals gave a typical response when challenged with a small dose of the virus 14 days later.

It is planned to carry out some work in an effort to grow this virus on tissue culture. Results of this work will be reported as they come to hand.

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

A virus isolated from the liver of a cuckoo (Centropus Toulou) taken near Jesselton North Borneo in September 1960 appears to be West Nile virus or very closely related to it. It is related in lesser degree to Murray Valley virus.

In tests for Japanese encephalitis virus hemagglutinin inhibiting antibodies, about 20 per cent of a sample of 261 men in a Marine battalion on Okinawa changed from negative to positive during the summer of 1960. This is a much higher rate of conversion than has been observed previously. No illness could be attributed to Japanese encephalitis virus infection in any of these men. There was one case of overt encephalitis in the same battalion but not in the study group.

There was a relatively severe epidemic of encephalitis, presumably JE, in Taiwan during July 1961, involving the younger age groups as is usual in this area.

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

This report reviews field and laboratory studies on arthropod-borne viruses during the period May 1, 1960, through April 30, 1961.

Investigations have been continued on the wintertime persistence of WEE and SLE viruses. Chronic latent infection of birds with WEE virus was confirmed. No further isolations have been made from naturally infected birds, mammals, or mosquitoes collected during the winter. However, a very large bank of specimens from this time period must be tested.

Biological and morphological studies of the overwintering population of female Culex tarsalis were extended. Spermathecae were dissected and nearly 100 per cent of females were found to be inseminated. The average volume of fat in the abdomen per female increased sharply with the onset of winter, reached a maximum in late December, than steadily decreased, reaching minimal or undetectable levels by mid-February. A source of fluids was essential to survival through the winter.

The addition of a source of sugar increased the likelihood of survival through the winter. A second technique is under evaluation to determine the history of prior ovarian activity of female C. tarsalis. This technique depends on the observation of separate dilations formed on the stalk of an ovariole following each ovarian cycle. It appears that C. tarsalis does not develop a separate dilation as is reported for other mosquitoes.

Culiseta inornata reached a peak of reproductive activity and maximum population density during the winter and spring in the valley portion of Kern County. Extensive collections of adults were made but no virus isolations could be made. A laboratory colony was established.

The project to evaluate the influence of intensive C. tarsalis control on the levels of WEE and SLE virus transmission is now in its second year. C. tarsalis populations were markedly reduced in two areas, largely by control measures, whereas the population remained relatively high in a comparison area where there was minimal control. There was circumstantial evidence of infiltration of female C. tarsalis from uncontrolled into controlled areas. Virus transmission was at a very low level throughout Kern County in 1960 and the data on vector and avian host infection rates did not indicate that control efforts resulted in significant changes in this already low level of virus activity. An extensive flight range study of C. tarsalis marked with fluorescent dusts during the summer of 1961 has led to evidence of frequent flights as far as three to nine miles. This represents a significant increase over earlier flight range studies and emphasizes the problems in protecting an area from massive infiltration by this vector.

There were no confirmed clinical cases of WEE or SLE in man or horse in Kern County in 1960. There was an acute shortage of water for agricultural use. The C. tarsalis population was very small throughout the summer in most areas of the County. Vector infection and transmission rates were so low that it is suspected they were approaching a level where transmission would be insufficient for virus maintenance. There was no evidence of virus activity until August, a delay of almost two months from the normal for WEE virus. Temperatures were unusually high during the summer so the delay in virus activity was not a reflection of insufficient heat for completion of extrinsic incubation.

A serological survey of human and animal bloods from the region of Hermosillo, Mexico, provided evidence of the previous activity of at least five viruses: WEE, SLE, EEE, Powassan, and either Dengue I or II.

HAI antibodies for SLE and Powassan viruses are commonly found in rodents collected in Kern County, but not WEE antibodies.

The duration of WEE and SLE antibodies in persons who have had inapparent infections is now under study. CF and HAI antibodies were no longer detectable in a high proportion of persons who were positive two years previously. A small proportion of individuals converted from negative to positive or had a rise in titer during the two-year period. HAI antibodies to both WEE and SLE viruses persisted about three times as long as CF antibodies. Neutralizing antibodies were more persistent than HAI antibodies. HAI and CF antibody titers to SLE virus appeared to be longer lasting than were WEE antibodies by several methods of analysis.

Snakes, lizards, frogs, and toads were not as attractive to, or fed on as frequently, by C. tarsalis as were rodents or birds. Virus was not recovered from a fairly extensive collection of cold-blooded vertebrates.

Techniques are sufficiently advanced to permit the development of Order and Species specific precipitin antisera for identification of bird bloods.

Different strains of WEE virus have marked differences in the size of plaques they produce on chick embryo fibroblasts. All freshly isolated strains produced predominately large plaques. The large plaque characteristic was maintained during serial passage in embryonated eggs. However, a virus population characterized by predominately small plaques was selected by five passages in chick embryo tissue culture or adult mouse brain.

Hamster kidney tissue culture was equally or more sensitive than the 21 day old mouse for primary isolation of WEE or SLE viruses from mosquito pools, when equal volumes of test suspensions were inoculated into the two systems and on reisolation attempts after prolonged storage of triturated mosquito pools.

No evidence of antigenic drift was found in a study of seven strains of SLE virus isolated in the period 1933 through 1959.

A wide range of HAI cross reactions to Group B antigens was found in sera from persons who probably had been infected with only SLE virus. Chickens infected with Modoc virus developed low titers of homologous HAI antibodies but no heterologous Powassan antibodies.

Fluorescent conjugates have been prepared with WEE immune rabbit sera and these will specifically stain smears of WEE virus infected chick embryo fibroblasts.

Continued studies on myxomatosis indicated that the brush rabbit (Sylvilagus bachmani) is the primary enzootic host of the virus in California.

WEE virus has maintained a farily high level of activity in Culex tarsalis in Kern County through most of the summer. Tests of 17,512 C. tarsalis yielded 78 isolations of WEE virus and one of the SLE virus. WEE virus appeared in the third week of June, and has continued to be present through August, with a peak in the third and fourth week of July.

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

Only two human cases of arthropod-borne encephalitis have been identified in California during 1961 up to September 15th. An adult male developed Western equine encephalitis in Riverside County on August 8th and a child developed St. Louis encephalitis in Madera County on July 13th. There have been five cases of Western equine encephalitis in horses. Three of these were in July, one each in Fresno, Tulare, and Kern Counties, and two in August in Yuba County.

There have been 8 cases of Colorado tick fever in 1961, 2 in Lassen County, 2 in Modoc County, 1 in Nevada County, and 1 in Inyo County. One case was from tick exposure in Nevada. In one case the tick bite exposure occurred after a visit to the mountains and it is not certain where this individual picked up the tick.

There were no regular collections of mosquitoes in 1961 for surveillance for encephalitis viruses. One isolation of Turlock virus was obtained from a pool of 14 Culex tarsalis mosquitoes collected at Hart Park, Kern County, September 1, 1960.

A tick survey was done by the Vector Control Division and the Viral and Rickettsial Disease Laboratory. Ten strains of Colorado tick fever virus were isolated, 5 from Lassen County, 2 from Modoc County, 2 from Mono County, and 1 from Inyo County. Eight of these

isolations were from Dermacentor andersoni and two from Dermacentor occidentalis. In addition, 11 strains of Colorado tick fever virus were isolated from ticks collected in Modoc County in April as a part of the field study of Colorado tick fever in wildlife. Ten of these were from Dermacentor andersoni and one from Dermacentor occidentalis. Six of the isolations were from ticks collected as fed nymphs from a gound squirrel, Citellus lateralis, whose blood specimen was positive for Colorado tick fever virus. The fed nymphs were reared and tested as adults and all were positive for Colorado tick fever virus.

Three isolations of Colorado tick fever virus were obtained from blood specimens of small mammals trapped at Hackamore Camp, Modoc National Forest, Modoc County, on April 16, 1961. One of these was from a yellow pine chipmunk, Eutamias amoenus, and two from golden-mantled ground squirrels, Citellus lateralis. Colorado tick fever virus was isolated from the liver of one of the ground squirrels that died ten days after capture. It has been found that Colorado tick fever virus can be isolated from the blood cells when the plasma is negative for virus. The virus was isolated from the plasma of two of the animals. The washed blood cells of all three were positive for the virus on the day the animals were captured. The washed blood cells of the chipmunk were positive for the virus at 17 and 38 days after capture, at which time the plasma was negative for virus. In studies of Colorado tick fever virus infection in mice, it has been found that the virus reaches a peak titer in the blood cells at about a week after exposure and persists for at least three weeks. The plasma is positive for virus for only a few days during the first week after exposure.

A skunk, Mephitis mephitis, was fed mice infected with hamster kidney tissue culture passage 5 of a strain of Western equine virus isolated from a wild bird. The mice were inoculated intramuscularly so as to produce a symptomless infection. The skunk developed an asymptomatic infection as shown by the demonstration of viremia.

REPORT FROM DR. HUGH H. SMITH, DEPARTMENT OF BACTERIOLOGY, UNIVERSITY OF ARIZONA, TUCSON, ARIZONA

Investigation of Arbovirus Infections in Arizona:

Knowledge of arbovirus infections in Arizona is in a highly unsatisfactory state. For many years, outbreaks of equine encephalitis have been known to occur. In 1941, Meiklejohn and Hammond, of the Hooper Foundation at the University of California, described a small epidemic of encephalitis that occurred in Pinal County in

the south central part of the state. Eighteen cases among the families of migratory farm workers were recorded. It was thought that most of those cases were due to infection with St. Louis encephalitis virus, but there was evidence also that Western encephalitis virus was active in the same area at that time.

During the past ten years, from 5 to 18 human cases of "Infectious Encephalitis" have been reported to the Arizona State Health Department annually. In most instances, the diagnosis was made on clinical grounds and was not confirmed by the laboratory. Data on equine cases have only been collected for the past five years. From 5 to 50 cases per year have been reported during this period. In some cases, serological tests pointed to Western encephalitis virus as the etiological agent.

Both human and equine cases were reported from widely separated parts of the state and were not confined to the irrigated areas of the hot desert country in the south.

Under the terms of the NIH grant, a virus laboratory is in process of organization at the University of Arizona with the usual facilities. Hugh H. Smith, M.D., formerly with the Rockefeller Foundation, is Director of the project.

Field collections were started in June 1961. Blood specimens and tissue samples from 346 birds and 271 rodents have been collected and 76 lots of mosquitoes have been captured and identified. These materials are now stored in the deep freeze but laboratory tests are to begin on them shortly to demonstrate antibodies and to attempt virus isolations.

Robert J. Janssen, Ph.D., who has been engaged in virological activities for some four years at the Biological Laboratories of Fort Detrick, Frederick, Maryland, joined the staff on September 1. He will be in charge of the technical aspects of the laboratory procedures. The field collections have been carried out by four graduate students of the Department of Zoology.

This study project is designed to define the nature and extent of the viral encephalitis problem in Arizona and some contiguous areas. It is expected that surveys to determine the possible presence of other arbovirus infections will also be undertaken.

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.

Relationships Between Temperatures and Transmission Rates of SLE and WE.

The relationships between temperatures in the Greeley and in other selected areas and the transmission rates of WE and SLE in avian sentinel flocks has received continued study. Use of cumulative degree-days (i.e., the cumulative sum of the degrees by which the daily average temperatures exceed certain threshold temperatures) as the temperature variable has resulted in correlation coefficients greater than .9 in the Greeley area. Positive correlations were obtained with SLE, and negative correlations with WE. Data from sentinel flocks in Washington, North Dakota, Utah, Montana, and other parts of Colorado when plotted on log-log graph paper against the same temperature variables (i.e., for WE the date on which 50/DD over 70° were first accumulated and for SLE the date on which 10/DD over 75° were first accumulated) produced similar distributions. The distributions showed a variation in slope. The SLE transmission rate slope was greatest in the warmest areas and least in the coldest and the reverse was true with the WE transmission rate slope.

Search for Hosts and Reservoirs of WE and SLE.

No serologic or virologic evidence was obtained that snakes collected in Weld County might be involved in the natural history of WE and SLE; however, both antibodies and virus have been detected in several species of small mammals.

Viremia and Antibody Responses of Adult Chickens Infected with WE and SLE Viruses.

Adult chickens, inoculated with small doses of WE or SLE viruses, responded with a brief viremia, neutralizing antibody, and HAI antibody; the latter was consistently present at weekly intervals after inoculation throughout the period of observation. Similar chickens exposed to infection in nature and bled at weekly intervals throughout the year showed much more variability, and certain individuals appeared to lose their detectable HAI antibody for varying periods of time.

Mosquito Population Indices and Virus Activity.

Observations have been continued on the relationships of mosquito populations to the amount of virus activity as measured by antibody conversions in sentinel chicken flocks. Information collected from nine sites in Weld and Boulder counties in Colorado and from two towns in Texas strongly suggest that within observed limits there was no correlation between the size of the total mosquito population or the C. tarsalis population and the antibody rates in the chicken flocks.

Seasonal Sequence of WE and SLE Virus Activity.

An investigation was initiated in 1960 to determine the time of year when WE and SLE viruses are active in various components of the environment in a single study site (St. Vrain). Efforts were made through the spring and summer to isolate virus and detect antibody in mammals, wild birds, chicken flocks, and reptiles, and to isolate virus from mosquitoes. During March, April, and May (prior to the appearance of large mosquito populations), 3 per cent of adult chickens developed HAI antibody to WE but none showed evidence of SLE activity. During this period, high percentages of white-footed deer mice (Peromyscus maniculatus) had HAI antibody to WE virus, but few had SLE antibody. Two virus isolations were made from rodents during this period, but these do not appear to be WE, EE, or SLE.

During June and July no antibody conversions occurred in six chicken flocks, although the four most common mosquito species had now reached near maximum population index levels. The Peromyscus samples still showed evidence of some WE antibody, but only one virus isolation was made, and it was not WE virus. There was no additional evidence of virus activity either in wild birds or mosquitoes. Work is now underway to identify these mammal isolates and to determine whether these viruses might be responsible for immunologic overlap with western.

The period August through October, as in past years, was the time of most WE and SLE virus activity in the mosquitoes and chicken flocks. Antibody conversions in the flocks began in late July or August and continued into October. The mosquito populations were high in August and significantly reduced by mid-September. WE virus was isolated twice from mosquitoes collected on August 3, and SLE on August 8 and 9. SLE was also isolated in August from a pigeon. No other virus isolations were made during this time from wild birds, mammals, snakes, or mosquitoes although a small percentage of the wild birds had HAI antibody. WE virus, apparently active in the small

mammals in early spring, was not in evidence in the small mammals during August and September when the chickens were converting and virus was prevalent in the mosquito population. The small number of isolations from wild birds is also noteworthy, particularly in view of the rather complete sampling of nestling birds in the study area.

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.)

The first active infections of WE virus in Massachusetts were detected by isolations of the virus from 15 pools of unengorged and 11 pools of engorged Culiseta melanura and from 1 pool of engorged Aedes canadensis collected in 1959, and from 3 pools of unengorged C. melanura collected in 1960. WE virus also was isolated from the brain of an infected 3-year-old Impeyan pheasant and from the blood of a 3-week-old chicken in 1959. The widespread nature of WE virus activity in Massachusetts was indicated by the detection of specific antibody among domestic fowl tested in widely separated areas in eastern Massachusetts during 1959.

A survey of migrant birds was conducted during April, May, and June 1961 within a Culiseta melanura-breeding swamp in the vicinity of Taunton. Japanese mist nets were used to capture the birds. The birds were bled, banded, and released. No virus was isolated from 221 blood specimens collected from 79 catbirds, 21 northern yellow-throats, 17 song sparrows, and 104 specimens from 24 other species.

Applications of granular formulations of dieldrin and heptachlor at the rates of 0.5 and 1.0 pound per acre were applied to fresh water swamp study plots for control of C. melanura larvae. Three untreated study plots were established for comparison in the evaluation of the larvicidal effect of the toxicants in the treated areas. Initial kill in the treated areas was good, and a continuing evaluation of treatments is currently in progress.

III. Report from Plainview, Texas - Bruce Francy

During the 1961 season, data were collected to provide the fourth year of background information for carrying out field experiments to determine the effectiveness of mosquito control in reducing transmission rates of WE and SLE. HAI antibody rates in farm and sentinel chicken

flocks will provide transmission indices, and infection rates in humans before and after treatment will also be determined. Information on mosquito populations was obtained by means of larval surveys, light traps, biting counts, and sentinel shed traps, the latter yielding as many as 3,000 mosquitoes in a single night. Approximately 35,000 mosquitoes were frozen for virus tests, over 90% of which were Culex tarsalis, about 4% C. quinquefasciatus, and 4% Aedes vexans.

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

In the spring of 1961, experimental control of mosquitoes by means of residual larvicides was initiated in one of four study areas to determine the effects upon WE and SLE transmission rates in avian sentinel flocks. Difficulty was experienced in keeping adult mosquito populations at low levels in the treated area, and there appeared to be considerable infiltration across the two-mile buffer zone to the sentinel sites. Of 245 pools of mosquitoes collected for virus tests during August, 217 were Culex tarsalis and 28 were Culiseta inornata.

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

Much of the material collected during 1961 remains to be examined and during early 1961 material collected during 1960 was examined.

COLORADO TICK FEVER

Objectives of study:

Through observation of human disease, an attempt is made to get 1) an estimate of the incidence of serious disease syndromes such as central nervous system involvement and hemorrhagic phenomena, 2) through the variation in yearly incidence of human disease, get some measure of variation in the ecological conditions maintaining the virus, and 3) through the geographical distribution of disease an indication of virus distribution.

Through observation of ticks and their animal hosts get data as to geographical distribution of the virus and the ecological conditions which maintain the virus.

1) Human disease (Table 1)

The past spring and summer isolation of virus was made from the blood of 23 patients; none had any but the usual dengue like course. This has been the lowest number of isolations made at this laboratory during the past 10 years. The number of isolations has varied from 23 to 118 per year. This variation in incidence from year to year must reflect variation in ecological conditions from year to year which at present are not understood.

2) Geographical distribution of virus as determined by examination of ticks.

It has been felt that the geographical distribution of CTF virus as now known is too restricted when judged by the known distribution of other arbor viruses and regions other than that occupied by Dermacentor andersoni should be explored. A total of 5117 Dermacentor variabilis collected in Minnesota, Montana, Nebraska, and North Dakota were examined without any evidence of CTF virus.

Table 1
Isolation of CTF Virus from Patients

1952	1953	1954	1955	1956	1957	1958	1959	1960	1961
42	44	80	68	59	118	93	27	47	23

States in which disease has been recognized:

Prior to 1961: California, Colorado, Idaho, Oregon, Montana, Nevada, South Dakota, Washington, Wyoming, Utah.

1961: California, Colorado, Idaho, Oregon, Montana, Nevada, Wyoming.

3) Ecological conditions in Black Hills of South Dakota.

The Black Hills region is the most eastern point where human infection and tick infection has been demonstrated. Here, the golden manteled ground squirrel, which is an important host of larva and nymphs of D. andersoni in the Rocky Mountains is missing.

This summer virus was isolated from 12 pools of adult D. andersoni (322 ticks examined), blood clots from 12 chipmunks, a red squirrel, and 3 Peromyscus. Three isolations of virus were also made from larvae and nymphs of D. andersoni collected from the above animals.

Table 2

Adult D. andersoni

No. pools examined	No. positive
28	12

Animals - Blood Clots

Animal	Number Run	No. positive
Chipmunks	66	12
Peromyscus	53	3
Microtus	12	0
Red Squirrel	1	1
Wood Rat	2	0
Total	134	16

In the Black Hills, the chipmunks were very numerous and appeared to play the same role as the golden manteled ground squirrel in the western mountains.

4) Isolation of virus from HLP ticks.

Isolation of CTF virus from this tick was made by Dr. Clifford from ticks collected from a cottontail rabbit in Wyoming and by Dr. Newhouse from ticks collected from snowshoe hare in the Bitterroot Valley. The significance of HLP ticks in the maintenance of CTF virus remains to be determined.

5) Vaccine against CTF.

Suckling mouse brain is the only material with a high enough concentration of virus to appear to make vaccine production possible. When brain material is used for vaccination of human beings there is always the fear of allergic encephalomyelitis. Previously, Olitsky and co-workers and Russian workers had reported that suckling mouse brain was nonallergenic. At this laboratory, using guinea pigs and

Freund's adjuvant, it has not been possible to produce allergic encephalomyelitis with suckling mouse brain although it can be done readily with adult mouse brain and guinea pig brain. Using a crude 10% saline suspension of infected suckling mouse brain and 1:4000 formalin and a temperature of 4° C., inactivation took approximately 4 weeks and the potency of the vaccine in mice and human beings was very poor. When the 10% suckling mouse brain was treated with $Ca_3(PO_4)_2$ according to Gordon Smith's method to attempt to remove extraneous mouse brain material it was found that the purified material was inactivated within 2 days at 4° C. when 1:6000 formalin was used and the vaccine protected mice against IC challenge of at least 3 logs of virus and in human beings 2 doses of vaccine (0.5 or 1.0 ml) at one month's interval produced antibodies in 90% detectable in an IC neutralization test.

POWASSAN VIRUS

Objective of study:

To find an area where an intensive study of the ecology can be made.

A total of 1058 human sera have been run from North Dakota, Wyoming, and Minnesota in the neutralization test and two sera have been shown to have a neutralization index of 1000 or >, 3 between 50 and 100 and one of 30. Of those with a neutralization index above 1000, one lives in Minnesota and one in North Dakota. Those with a low index lived on an Indian reservation in Wyoming.

One isolation of virus was made from 322 adult D. andersoni collected in the Black Hills of South Dakota. In the same area, antibodies were found in the sera of 4 chipmunks, 3 Microtus pools and 3 Peromyscus pools and from one sample of either chipmunk or Peromyscus serum (mixed label?) (Table 3).

No Powassan virus has been isolated from these animals. Although Kennedy and Clifford have shown that Powassan virus passes from stage to stage in the development of D. andersoni when it is infected as a larva, no evidence has been found to suggest that a cycle of infection is established between larva and nymphs of D. andersoni and its small animal hosts in nature. At present it appears that the chipmunks and mice are being infected by some other arthropod, perhaps an Ixodes species of tick and that D. andersoni occasionally becomes infected from these animals.

Table 3

Animal Sera

	No. sera run	No. with antibodies on screen neut. test
Chipmunks	63	4
Peromyscus	50	3
Microtus	12	3
Red Squirrel	1	
Rat	2	
Mice or Chipmunks*	6	1
	134	11

*label mixup

CALIFORNIA VIRUS

Dr. Newhouse has isolated California virus from Haemaphysalis leporis-palustris collected from a snowshoe hare and from D. andersoni collected from a chipmunk and from a golden manteled ground squirrel. He has also found neutralizing antibodies in these species of animals.

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

Arthropod-borne virus studies are now included in the programming of two Air Force laboratories: The USAF 3790th Epidemiology Laboratory, Lackland AFB, and a virus laboratory recently initiated at the School of Aerospace Medicine, Brooks AFB.

Lackland 3790th Epidemiology Laboratory: A study is now in progress under the guidance of Captain C. J. Hodapp and Major R. A. Crandell and is concerned with occurrence of arthropod-borne encephalitides on and around Air Force installations within the continental United States. A survey of the literature for the years 1955-1959 suggested that several bases in California, Florida, and Texas would be suitable for the collection of appropriate material.

Four pools of mosquitoes were collected from one base during the late season of 1960. Each pool, comprising 50-80 mosquitoes, consisted of the following species: (a) Aedes dorsalis, (b) Aedes nigromaculis,

(c) Aedes vexaus, and Culex tarsalis. Inoculation of suckling mice with the Aedes dorsalis pool resulted in isolation of an agent identified in neutralization tests as western equine encephalitis virus. Immune serum prepared by inoculation of guinea pigs with this agent had a CF titer of greater than 1:512 to WEE.

Continued surveillance of various Air Force installations will be made in order to obtain information regarding the occurrence of the various vectors and viruses in these locations. Consideration will also be given to establishing the capability for HA and HAI testing for these viruses.

School of Aerospace Medicine: One of the Objectives of this laboratory is to ascertain which viruses are encountered by Air Force personnel in their tours of duty in different geographic areas. Special consideration will be given to the viruses responsible for respiratory, central nervous system, and enteric disease.

Multiple serums are being collected on recruits from different geographic areas. The first specimen is taken upon admission to the Lackland AFB reception center and prior to receipt of any immunization. Additional recruits will be bled during the course of the year for indications of antibody development, as influenced by the season. HAI tests are to be used with representative group A, B, and C arthropod-borne virus antigens to indicate incidence of viral infection following relocation in new geographic areas. Specific neutralization tests will be performed as necessary. In addition to collecting epidemiological data, an indication for specific vaccination programming may result. In the course of this study, the practical application of filter paper discs for antibody surveys will be field tested.

As a corollary, serum from various primates collected in different geographic areas will be tested for the presence of antibodies to a number of viruses producing infection in man. When laboratory findings warrant additional study, an attempt will be made to isolate various viruses from patients and vectors.

REPORT FROM DR. W. McD. HAMMON, DEPT. OF EPIDEMIOLOGY & MICROBIOLOGY, GRADUATE SCHOOL OF PUBLIC HEALTH, UNIVERSITY OF PITTSBURGH, PITTSBURGH 13, PA.

Philippine Hemorrhagic Fever:

During a recent visit in Manila, it was learned that this disease has occurred annually in Manila since the study made in 1956. It has

occurred, with two exceptions, always during the rainy season in definite epidemic form in a number of other urban areas. One of these was an outbreak of over 1200 cases involving three cities in Isabela Province in Northern Luzon during May and June of 1961. Entomological observations indicated good association with Aedes aegypti in these cities. Breeding in and about houses occurred intensively in vessels purposefully kept filled with water; therefore, the breeding was not dependent on rains.

Thai Hemorrhagic Fever:

A two-day symposium on this disease was held in Bangkok in August of 1961 under the co-sponsorship of the Thai Ministry of Health and the SEATO Medical Research Project. A number of excellent papers were presented on epidemiology, vectors, etiological agents, clinical aspects and pathology, together with extensive discussion. This symposium with selected portions of the discussion will be published shortly with limited distribution including the recipients of the Arbovirus Newsletter.

Annual epidemics have occurred in Bangkok and neighboring cities, the largest epidemics occurring in 1958 and 1960. Currently, numerous cases were observed in several hospitals. Our laboratory showed by serological tests on about 50 of the 1960 cases from one hospital that the etiology was principally by dengue group viruses with rare participation of chikungunya virus. The several virus isolates have not been completely identified but a number belong in the dengue group. Dr. Ambhan Dasaneyavaja reported isolation of types 3 and 4 dengue viruses from 1960 patients, the latter from the liver of a fatal case.

Dr. Rudnick collected mosquitoes intensively during two weeks in Bangkok. These will be tested for virus in San Francisco and in Pittsburgh. Collections were similar to those of 1958--large numbers of Aedes aegypti and Culex quinquefasciatus in houses. Aedes albopictus was essentially impossible to find in the urban area.

Information on other geographical areas was difficult to obtain since hemorrhagic fever is not reportable. Inquiry of a variety of sources, however, revealed that a similar disease occurs in many other cities from northern Thailand to near the Malayan border on the south but as in the Philippines not in areas where the malaria control program is active which involves residual house spraying.

On the basis of cross comparative tests of TH-36, the prototype of the most common virus isolated in Bangkok in 1958, and TH-Sman isolated once, and dengue types 1 through 4, we are looking more favorably toward considering the Bangkok agents of 1958 as dengue types 5 and 6, respectively, rather than strains of types 1 and 2 as originally suggested because of closest immunologic relationships. Further study is required and we invite others to assist in this comparison. These agents are available in lyophilized form for those with proper government authorization for their transfer. Types 3 and 4 have been deposited with the American Type Culture Collection.

Attenuated Japanese B Encephalitis Virus Derived from OCT-541:

The attenuated strain derived from this mosquito isolate through hamster kidney tissue culture at 24° C and plaque picking was previously reported as of extremely low neurovirulence for mice, weanling and suckling, and for monkeys and burros. It is not infectious for mosquitoes. It has been further selected by plaque passage for low neurovirulence and remains fairly stable in characteristics during several passages at 24°. It can be produced in high titer and large amounts in Parker's 199 medium with human albumin through repeated harvests at 6-10 hour intervals. It has been lyophilized without great loss. But animals without illness have not developed detectable antibodies. Man is yet to be injected; a pool for human tests has been prepared and is undergoing safety tests and characterization in the laboratory. If it is too attenuated to produce antibody in man a less modified passage material may be selected.